EDITORIAL BOARD

EDITOR IN CHIEF
HUSSIEN M. KHairy
(Faculty Dean)

ASSOCIATE EDITORS
ADEL F. RAMZY
NADER A. ABULATTA
MAGD A. KOTB

EDITING SECRETARY
SUZAN EL SHAFEI

EXECUTIVE DIRECTOR
HODA EISSA

ADVISORY BOARD
(Department Chairmen, Cairo University)

AHMED ABOUL NASR (Obst. & Gynec.)
AHMED EL-BELEIDY (Pediatrics)
ALEYA ABDEL FATTAH (Critical Med.)
ALGohary AL-GOHARY (Neurosurgery)
AMAL EL-SAFTY (Industrial Med.)
AZZA ABBAS HELMY (Neurology)
AZZA EZZAT (Anaesthesiology)
AYMAN YOUS SRY (Tropical Med.)
EMAN AL-SEROUGY (Rheumatology)
HAMDY ABDEL-AZEEEM (Radio. Nucl. Med.)
HEBA ARNAOUT (Micro. & Immuno.)
HEND SHAFIQ (Histology)
HISHAM NEGM (OtoRhinolaryngology)
HISHAM BADGY (Urology)
HISHAM TARRAF (Gen. Medicine)
HODA ABOU YOUS SEF (Chest Med.)
HODA RASHEED (Dermatology)
LAMIS AL-RAIE (Psychiatry)

MAGDY KAMAL (Parasitology)
MAHA ASHMAWY (Anatomy)
MAHA GAMAL EL-DIN (Physiology)
MAYSA M. SHAWKY (Community Med.)
MERVAT EL-ANSARI (Clinical & Chemical Path.)
MOHAMED EL-TOUKHY (Radiology)
MOHAMED ABDEL RAOUF (Cardio-Surgery)
MOHAMED RAIA (Andrology)
MOHSEN SALEM (Ophthalmology)
MONA ANWAR (Pathology)
MONA OTHMAN (Pharmacology)
NAGWA EID (Family Medicine)
OSAMA EL-BARANY (Forensic Med.)
SAYED MAREY (Gen. Surgery)
WAFAA EL-AROUSSY (Cardiology)
YASSER NASSAR (Biochemistry)
YOUSSRY EL-HAWARY (Orthopedics)
PEER REVIEWERS

ABDALLAA EL-MUHAMED (Cardio., UK.)
ABDEL MAGID EL-NAHASS (Med., UK.)
ADEL LOTFY (Paed. Surg., Egypt)
AHMED NASSEF (Gen. Surg., UK.)
CHRISTOPHER CHEN (Obst. & Gyn., Singapore)
ESSAM EL-SAHWY (Pediat. Surg., Egypt)
HAMDY BADRAWY (Obst. & Gyn., Egypt)
HANY LASHIN (Obst. & Gyn., UK)
HAYDAR GHALEB (Pharm., Egypt)
HAZEM EL-MEHERY (E.N.T., Egypt)
HOSSAM KAMEL (Oncology, Egypt)
HUSSEIN ABDEL DAYEM (Radio., USA)
HUSSEIN KHALED (Oncology, Egypt)
JOHN PAUL CHIGOT (Endocrine Surg., France)
KAMAL IBRAHIM (Orthop. Surg., USA)
KAMAL KHALIL (Cardiothorac. Surg., USA)
KAMAL MANSOUR (Thoroc. Surg., USA)
KAZOU SHIMIZU (Surg., Japan)
KEICHI S. DOBSON (Psych., Canada)
KOICHI TANAKA (Transplant, Japan)
MAHER HALAWA (Orthop., UK.)
MAHER RAMZY (Nephrology, Egypt)
MAHMoud EL-MENAWY (Obst. & Gyn., Egypt)
MAKRAM MILAD (Pathology, Egypt)
MOHAMED ABOU EL-GHAR (Obst. & Gyn., Egypt)
MOHAMED EL-GUINDY (Gen. Surgery, Egypt)
MOHAMED GAZAYERLI (Laparoscopic Surg., USA)
MOHAMED GHONEIM (Urology, Egypt)
MOHAMED HABIB (Dermat., Egypt)
MONA SALEM (Pediat., Egypt)
MOUNIR AGEEB (Gen. Surg., Egypt)
MOUNIR HANNA (Paed. Urol., USA)
NABIL FAHMY (Anaesth., USA)
NADEY HAKIM (Transplant., UK.)
NAGY HABIB (Hepat. Surg., UK.)
PAULO MICCOLI (End. Surg., Italy)
RAOUF SALLAM (Gen. Surg., Egypt)
RASHAD BARSOUM (Nephrology, Egypt)
ROCCO MARUOTTI (Gen. Surg., Italy)
SHERIF MOKHTAR (Cardio., Egypt)
YEHYA EL-RAKHAWY (Psychiatry, Egypt)
NOTES TO CONTRIBUTORS

The Medical Journal of Cairo University welcomes original papers. Review articles, book reviews, abstracts from current literature and historical notes are also accepted. Particular priority is given to works presented to the Kasr El Aini clinical society by the staff of Kasr El Aini Faculty of Medicine and by its guess speakers.

Papers are accepted on the clear understanding that the subject matter has not and will not be published in any other journal.

Manuscripts should be typewritten in double spacing on one side of the paper only. The title of the paper should be written in block letters. Name of the author or authors should appear as first name, initials for second name followed by the surname (family name) and the initials of the highest university degree. Finally the name and address of the hospital or laboratory where the work was performed.

An abstract should be written at the beginning of the article.

Photographs and photomicrographs should be printed on glossy paper. They should be cellar and unmounted. Captions to the illustrations should be typed on a separate sheet. Drawings and diagrams should be done boldly in black ink. These should be numbered, the top should be indicated and the author’s name, all written on the back. Long tables should be avoided and preferably replaced by graphs. No more than three tables, submitted in a form of photographs, are accepted.

References in the text are to be arranged to the Vancouver style, i.e. references must be indentified by Arabic numerals between parentheses in order of their mention. The list of references is to be typed in numeric order as follows:

For Journals, author’s name, followed by initials, the title of the article, the name of the journal, the volume number in arabic numerals, the numbers of first and last pages of article followed by year of publication.

For books, authors’ name, title, edition, publisher, town, page and year of publication.

Reprints: Thirty reprints will be supplied.

Editorial Address:
Two manuscripts should be handed or sent to Dr. ADEL F. RAMZY, assistant editor, Clinical Society office. Manyal University Hospital, Cairo, Business communications, advertisements, subscriptions should be addressed to Dr. NADER A. ABULATTA, assistant editor.

Exchange of periodicals and book reviews should be addressed to:
Dr. SUSAN EL SHAFEI, editing secretary.

Notice to subscribers:
Annual subscription rate for individuals and institutions in Egypt L.E. 200 payable to:
The Clinical Society Office
Manyal University Hospital
Cairo, Egypt.

Annual subscription rate for all countries overseas is $ 400 payable to:
The Medical Journal of Cairo University through The Bank of Egypt, Kasr El-Aini Branch. Cairo, Egypt.

Address for mail:
The Medical Journal of Cairo University, the Clinical Society,
El-Kasr El-Aini Hospital
Telefax: (202) 236 55 768
www.medicaljournalofcairouniversity.net
info@medicaljournalofcairouniversity.net
CONTENTS

Threshold Detection Using the Auditory Steady-State Response and the Tone Burst Auditory Brain Stem Response, SAL WA M. ABDEL LA TIF, HODA I. ABUMOUssa and SOHA M. HAMADAA ................................................................. 791


The Diagnostic Value of Estimating CA125 in the Differentiation between Tuberculous and Malignant Causes of Serous Pleural Effusion: A Pilot Study, ELSAYED SALEM, AYMAN SALEM and AMAL ABD ELRASHEED ........................................................................ 801

Hyaluronic Acid (HA) Level in Ascitic Fluid of Cirrhotic Patients with Spontaneous Bacterial Peritonitis (SBP), ZAIN E.A. SA YED, EMAD F.M. KHOLEF, MOHAMED O. ABDELMaLEK and KHALEd M. A TTALLAH .................................................................................. 809

Open Reduction and Internal Fixation of Complex Radial Head Fractures, SHERIF M. ABDELGaID, MOHAMED ABOELNASS and ELSAM EYD AL Y .......................................................................................................................... 815

Risk Factors for Ischemic Heart Disease in Rheumatoid Arthritis, EL-BADRY I. ABO-ELNOR, SALAH A.A.S. ARGOON, OMNIYA ABD ELMONEIM, AYyEN M. SELIM and NORa HASHEM ........................................................................ 823

Intrathecal Dexmedetomidine Enhances Intrathecal Combination of Magnesium Sulphate and Bupivacaine Quality of Spinal Anesthesia and Postoperative Analgesia, SAMY A. AMR, MONTAser A. MOHAMAD, MUSTAFA THABAT and FAISAL F. ADAM .......................................................................................................................... 831

Cardiac CT Coronary Angiography Screening of Coronary Artery Disease in Diabetic Patients Presented with Low to Intermediate Risk Chest Pain, TAMIR A. HASSAN, NESREEN MOHEY, HITAHM DA WO UD, MOHAMED ABDALAAAL and ABDELFA TAH ELASFAr ........................................................................................................ 839

Factors Predicting Fulminant Course of Acute Hepatitis A with Special Emphasis on Predictors of Mortality in Egyptian Children, WESAM S. MORAD, ALIF A. ALLAM and YASSER KAMAL .............................................................................................................. 845

Evaluation of Interleukin-8 and HCV RNA in Chronic Hepatitis C Patients as Predictors of Response to Pegylated Interferon/Ribavirin Therapy at 12 and 24 Weeks, ADEL A. HASSAN, NADER A. NEMRI, KHALL A. KHALIL, AMANY M. HASSEN, WAHEED F. HESSAM and TAMER M. A TTIA .................................................................................................................... 853

Effects of Melatonin Premedication on the Hemodynamic Responses and Perfusion Index During Laryngoscopy and Endotracheal Intubation, AHMED A. MOHAMED, HOsAM M. A TEF, ALAA EL-DIN M. EL KASSABY, SALAH A.M. ISMAIL and AMR M. HELMY ..................................................................................................................... 859

Polymorphism of CAG Repeat in Androgen Receptor Gene in Egyptian Women with Polycystic Ovary Syndrome, DOAA SHAHIN and SHERIN M. SOBH ....................................................................................................................... 869

Comparative Study between Right Liver Lobe Diameter/Albumin Ratio and Platelet Count/Spleen Diameter Ratio as a Non-Invasive Predictor of Oesophageal Varices in Patients with Liver Cirrhosis, HAMDY M. MOSTAFA, KHALED A. EID, MONA M. ABDEL MEGUID and SAFIA A. MOHAMED .................................................................................................................... 875

Neoadjuvant Docetaxel (Taxotere) Plus Cisplatin and 5-Flourouracil Followed by Concomitant Chemoradiotherapy in Patients with Locally Advanced Squamous Cell Carcinoma of the Head and Neck, INAS I. ABDELBHALIM, NA WAL M. ELSAID, ELSAID M. ALI and BASHEER S. A TA .......................................................................................................................... 887

High-Risk Pregnancy and its Outcome among Women Attending Antenatal Clinics in Abha, Saudi Arabia, AESHA FARHEEN ......................................................................................................................... 895

Effect of Resisted Exercise Training on Interleukin-6 in Patients with Chronic Heart Failure, ZEINAB M. HELMY, SHERIN H. MOHAMMED and GABER S.A. SOLIMAN ..................................................................................................................... 899

Imaging Findings of Interstitial Lung Disease Using High Resolution Multi-Detector CT, AHMED A. ISMAIL and EMAN SHABL .......................................................................................................................... 907
<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinduction Ultrasonographic Measurements as a Predictor of Successful Induction of Labour in Prolonged Pregnancy in Primigravidas</td>
<td>AHMED S.A. ASHOUR, RANA M.A. ABDELLA, HASSAN O. GHAREEB and FOUAD A. ABO-HAMILA</td>
<td>915</td>
</tr>
<tr>
<td>Immunohistochemical Expression of Toll-Like Receptor 3 in Verruca Vulgaris</td>
<td>AHMED A. SALEH, FA TMA M. EL-ESA WY and TAGHREED A. ABDEL AZIZ</td>
<td>923</td>
</tr>
<tr>
<td>Study on the Management of Pregancies Complicated by Late Preterm Prelabour Rupture of Membranes between 34 and 37 Weeks of Gestation in Woman's Health Centre-Assiut University: A Prospective Study</td>
<td>HAZEM S. MOHAMAD</td>
<td>929</td>
</tr>
<tr>
<td>Effect of Endurance Exercise and/or Diet Restriction on Mitochondrial Bioenergetics Function in Skeletal Muscle of Diabetic Male Albino Rats</td>
<td>NASHWA ELTABLAWY and EMAN F. KHALEEL</td>
<td>935</td>
</tr>
<tr>
<td>The Role of Multislice CT in Imaging of Different Tracheal Lesions</td>
<td>YOUSRIAH Y. SABRI, MARIAN F. FARID, HEBA H. A SSAL and MAR WAN M. EL TOHY</td>
<td>949</td>
</tr>
<tr>
<td>Combined Tailored Lateral Internal Sphincterotomy with V-Y Advancement Flap Versus Lateral Internal Sphincterotomy Alone in Treatment of Chronic Anal Fissure</td>
<td>TAREK HEGAZI and SALAH S. SOLIMAN</td>
<td>959</td>
</tr>
<tr>
<td>Salivary Gland Neoplasms A Histopathological and Statistical Study</td>
<td>GINA A. NAKHLA, HALA N. HOSNI, MOHAMMED F. DAR WEECH, AHMED A. SOLIMAN and MAR WA A.M. HASSANIN</td>
<td>967</td>
</tr>
<tr>
<td>Preemptive Use of Intravenous Acetaminophen, Ketamine or Their Combination in Patients Undergoing Elective Open Abdominal and Urological Surgeries: Effects on Intraoperative and Postoperative Analgesic Requirements</td>
<td>MAHMoud M. AMER, DOAA A. RASHWAN and DOAA M. SAYEM</td>
<td>975</td>
</tr>
<tr>
<td>Impact of Obesity on Ovulatory Functions in Polycystic Ovarian Syndrome</td>
<td>AHMED AL-SA WAF and EMAN A. HUSSEIN</td>
<td>983</td>
</tr>
<tr>
<td>Congenital Inguinal Hernia: Results of 2207 Procedures</td>
<td>MOHAMED KORANY, MOHAMED A. OSMAN, GAMAL MAKLLOUF and MAHMoud A. MAHMoud</td>
<td>989</td>
</tr>
<tr>
<td>Cardiac Resynchronization Therapy May Avoid Dilated Rather Than Ischemic Cardiomyopathy Patients the Need for Primary Prevention Defibrillator Implantation</td>
<td>SALAH ATTA, MOHAMED BASHAND Y and SHERIF ZAKI</td>
<td>993</td>
</tr>
<tr>
<td>A Structured Review of Outcome Measures Post Aerobic Training for Chronic Obstructive Pulmonary Disease (COPD) Patients</td>
<td>AMANY R. MOHAMED and MARWA M. SHABAN</td>
<td>1001</td>
</tr>
<tr>
<td>Upper Gastrointestinal Mucosal Changes in Patients with Congestive Heart Failure</td>
<td>ZAIN E.A. SA YED, MOHAMMAD ABDEL-GHANY, LOBNA ABDEL- WAHID, ELHAM A. HASSAN and KAILED M. A TTALLAH</td>
<td>1009</td>
</tr>
<tr>
<td>The Effect of Early Versus Late Start of Minimal Enteral Nutrition on Clinical Outcomes of Parenterally Fed Preterm/Very Low Birth Weight Infants</td>
<td>RABAB E.H. EL-SA YED</td>
<td>1015</td>
</tr>
<tr>
<td>The Protective Role of a Flavonoid “Morin” on the Liver of Streptozotocin-Induced Diabetic Rats</td>
<td>ABEER M. EL-MALAHAWAY, OLA A. EL-GOHARY, KHALED ABDULQAWI and ODETTE WAHBA</td>
<td>1025</td>
</tr>
<tr>
<td>Diagnostic Value of CK19 and HMWCK 34BE12 in Differentiation between Selected Thyroid Neoplasms</td>
<td>TA GHREED ABD EL-SAMEE, RANIA GALAL, NIVEEN TAHOON, MA GDA H. BAKR and HALA A. A GINA</td>
<td>1035</td>
</tr>
<tr>
<td>Effect of Mechanical Measures on Prevention of Deep Vein Thrombosis among General Surgical Patients</td>
<td>SHA WKEY S. GAD and AMAL A. EL-SHEIRH</td>
<td>1043</td>
</tr>
<tr>
<td>Effect of Vitamin D Supplementation and/or Physical Training on Cigarette Smoke Induced COPD in Rats</td>
<td>NASHWA EL TABLA WY, SAMAH EL TTAR and ZIENAB ABDEL WAHAB</td>
<td>1053</td>
</tr>
<tr>
<td>Title</td>
<td>Authors</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Association of Serum Fetuin-A with Insulin Resistance in Type 2 Diabetic Patients</td>
<td>MOHSEN KHALID, GHADA HUSSEIN, A YA T I. GHANEM and GHADA A. OMAR</td>
<td>1067</td>
</tr>
<tr>
<td>The Possible Protective Effect of Ginger Against Intestinal Damage Induced by Methotrexate in Rats</td>
<td>OMAIMA M. ABD-ALLAH and ABEER A.I. SHARAF EL-DIN</td>
<td>1073</td>
</tr>
<tr>
<td>Role of Islet Cell Antibodies in the Pathophysiology of Diabetes in HCV Infected Patients</td>
<td>TAREQ HUSIN, AREJ GARABAWY, HATEM DARWISH, MARIAN VICTOR and HANY EL SEBAEE</td>
<td>1085</td>
</tr>
<tr>
<td>Synovial Sarcoma of Extremities; Evaluation of Prognostic Factors and Clinical Outcomes</td>
<td>SEHAM E. A BDELKHALEK and RA SHA HAMDY</td>
<td>1093</td>
</tr>
<tr>
<td>Stressors Facing Mothers of Children with Cerebral Palsy</td>
<td>MAGDA M. EL-SAYED YOUSSEF, FA TEN F. AHMED and SHAIMAA M. MAHMO UD</td>
<td>1099</td>
</tr>
<tr>
<td>Role of Inflammatory Markers on Left Ventricular Functions in Vitamin D Deficiency Rickets</td>
<td>WAFAA S. MOHAMMED and KOTB A. METWALLEY</td>
<td>1105</td>
</tr>
<tr>
<td>Is there Still a Place for Laparotomy in the Management of Tubal Ectopic Pregnancy?</td>
<td>AHMED M.M.K. NOOH</td>
<td>1113</td>
</tr>
<tr>
<td>Nurses’ Knowledge about Physiological and Behavioral Pain Indicators of Newborn in Port-Said</td>
<td>MAGDA M. YOUSSEF, FA TEN F. MAHFOUZ and HALA S. EL-HUSSEINY</td>
<td>1117</td>
</tr>
<tr>
<td>Review Article: Diabetic Foot in the Arab World: An Update</td>
<td>ELSA YED ALSALAMONY</td>
<td>1125</td>
</tr>
<tr>
<td>Case Report: Vaginal Neurofibroma</td>
<td>WAEL S. NOSSAIR and MOHAMED S. FARAG</td>
<td>1133</td>
</tr>
</tbody>
</table>
Threshold Detection Using the Auditory Steady-State Response and the Tone Burst Auditory Brain Stem Response

SALWA M. ABDEL LATIF, M.D.; HODA I. ABUMOUSSA, M.D. and SOHA M. HAMADAA, M.D.
The Department of Audiology, Hearing & Speech Institute, Giza, Egypt

Abstract

Background: Although objective diagnostic methods tend to dominate modern medical science, behavioral pure-tone audiometry (PTA) remains the golden standard for identifying hearing threshold levels. The tone burst auditory brain stem response (TB ABR) is a series of electrical potentials that are recordable from the scalp to give frequency-specific estimates of hearing level. A more recent method, the auditory steady-state response (ASSR) has become more and more important as an alternative to objective audiometry. Both the ABR and the ASSR provided reasonably accurate predictions of behavioral threshold across hearing loss subjects.

Objectives: Comparing the thresholds of tone burst auditory brainstem response (TB-ABR) and auditory steady-state response (ASSR) in adult in relation to pure tone thresholds.

Methodology: Forty adult subjects divided into three groups, normal hearing, moderate SNHL and severe SNHL. All subjects in this research were submitted to the following: Full history taking. Otologic examination. Basic audiological evaluations (Pure tone audiometry, Speech audiometry & Immittacemetry). Tone burst ABR recorded using 500Hz and 4000Hz stimulus and ASSR stimulus using carrier frequencies 500Hz and 4000Hz.

Results: In normal hearing group, ASSR thresholds are closer to PTA thresholds than TB thresholds, 500Hz results are better than 4000Hz results. In moderate hearing loss group ASSR and TB thresholds are approximated to PTA thresholds but still the ASSR thresholds are closer to PTA thresholds than TB thresholds. In severe hearing loss group, TB and ASSR thresholds show the best level of prediction of PTA thresholds. In normal hearing group, correlation between PTA and ASSR thresholds showed a statistically significant correlation at 500Hz only while correlation between PTA and Tone Burst thresholds showed a statistically significant correlation only at 4000Hz. A statistically significant correlation was found at 500Hz and 4000Hz between PTA and ASSR thresholds, between PTA and Tone Burst thresholds and between ASSR and Tone Burst thresholds in severe hearing loss group.

Conclusion: In normal hearing both TB ABR and AS SR testing show poor prediction of PTA thresholds. ASSR thresholds are better to estimate PTA thresholds than TB ABR thresholds in normal and moderate SNHL. Both ASSR and TB ABR thresholds showed a significant correlation with PTA that increases with the increasing severity of hearing loss.

Key Words: ASSR – ABR TB – Sensori-neural hearing loss.

Introduction

ALTHOUGH objective diagnostic methods tend to dominate modern medical science as many of the medico-legal patients who claimed compensation may exaggerate hearing loss that varies in degree, nature, and laterality. Behavioral pure-tone audiometry (PTA) remains the golden standard for identifying hearing threshold levels [7].

The tone burst auditory brain stem response (TB ABR) is a series of electrical potentials that are recordable from the scalp to give frequency-specific estimates of hearing level. Definitive results through TB ABR may require several test sessions for audiometric completeness as a result of the procedure’s long testing time [6].

A more recent method, the auditory steady-state response (ASSR) has become more and more important as an alternative to objective audiometry. In contrast to ABR in which stimuli are broadband clicks or tone bursts, the ASSR is evoked by using continuous amplitude modulated and frequency modulated tone (AM/FM). During the last couple of years, several studies investigated clinical application of ASSR as an objective audiometry method. Most studies agree that there is a significant correlation between hearing thresholds determined by the ASSR and the PTA, but the difference was found to be between 4-34 decibel (dB). The greatest difference in hearing thresholds is found in patients
with normal hearing, while it was significantly smaller in patients with sensori-neural hearing loss [4].

Both the ABR and the ASSR provided reasonably accurate predictions of behavioral threshold across hearing loss subjects. There was no evidence that the predictive accuracy of the AM ASSR differed from the AM/FM ASSR. In general, ABR thresholds were recorded at levels closer to behavioral threshold than the ASSR. For certain individuals with steeply sloping hearing losses, the ASSR may be a more accurate predictor of behavioral thresholds; however, the ABR may be a more appropriate choice when predicting behavioral thresholds in a population where the incidence of normal hearing is expected to be high [3].

The Tb-ABR thresholds were significantly higher than AS SR, when the degree of hearing loss increased the mean difference between the Tb-ABR and ASSR thresholds decreased. The mean difference in normal group was always higher than in patients group [1].

Aim of the work:

To compare the thresholds of tone burst auditory brainstem response (TB-ABR) and auditory steady-state responses (ASSR) in adult in relation to pure tone thresholds.

Subjects and Methods

Forty adult subjects ranging from 20 to 60 years (80 ears) divided into three groups, normal (15 subjects) their mean pure-tone hearing thresholds at 500Hz was 18.6dBHL and mean pure tone hearing thresholds at 4000Hz was 22.7dBHL, moderate SNHL (13 subjects) their mean pure-tone hearing thresholds at 500Hz was 49.5dBHL and mean pure tone hearing thresholds at 4000Hz was 49. 1 dBHL and severe SNHL groups (12 subjects) mean pure-tone hearing thresholds at 500Hz was 80.9dBHL and mean pure tone hearing thresholds at 4000Hz was 83.6dBHL, they were selected randomly from outpatient clinic of hearing and speech institute after exclusion of patients complicated with element of conductive hearing loss.

Equipment:

• Clinical Audiometer Interacoustics model AC40.
• Immittancemeter Ineracoustics model AZ 26.
• ERA GSI Audera model using both the tone burst stimulus at 500 and 4000Hz and ASSR stimulus at carrier frequencies CF at 500 and 4000Hz.

• Ethical aspects: A written consent were signed by all subjects showing their acceptance regarding participation in this study. Each subject was informed about all steps and any possible side effects.

All subjects in this research were submitted to the following:

• Full history taking.
• Otologic examination.
• Basic audiological evaluations (Pure tone audiometry, Speech audiometry & Immittancemetry).
• Tone burst ABR was recorded using 500Hz and 4000Hz stimulus, Blackman gated with 2-0-2 cycles using a rate of 41.9 stimuli per second. Threshold was identified as the lowest stimulus level that wave v can be identified.
• ASSR stimulus using carrier frequency at 500Hz and 4000Hz with modulation percentage, (AM 100% and FM 10%) modulated at rates of 87.3 and 9 1. 1 Hz, threshold was identified as the lowest stimulation level that shows a statistically significant response (phase locked).
• Statistical analysis of results were carried out using SPSS system (Statistical package for social sciences) (version 16), IBM Corporation, USA.

Results

In this research we compared the hearing threshold of the pure tone audiometry (PTA), auditory steady state response (ASSR) and tone burst (TB) tests in normal hearing and in moderate and severe SNHLs. Two frequencies were tested, 500 and 4000Hz.

It was assumed that responses from each ear of a subject could be treated independently, so the results from right and left ears were pooled.

Mean and standard deviation of hearing thresholds of PTA, TB and ASSR in normal hearing group are shown in (Table 1 and Fig. 1), they revealed that ASSR thresholds are closer to PTA thresholds than TB thresholds, 500Hz results are better than 4000Hz results.

Mean and standard deviation of hearing thresholds of PTA, TB and ASSR in moderate hearing loss group are shown in (Table 2 and Fig. 1), they revealed that ASSR thresholds are closer to PTA thresholds than TB thresholds, 500Hz results are better than 4000Hz results.

Mean and standard deviation of hearing thresholds of PTA, TB and ASSR in severe hearing loss group are shown in (Table 3 & Fig. 3) with the
best approximation of thresholds of TB and ASSR to PTA results and even the AS SR thresholds became better than PTA thresholds at 500Hz.

In normal hearing group: Correlation between PTA and ASSR thresholds showed a statistically significant correlation at 500Hz, correlation between PTA and Tone Burst thresholds showed a statistically significant correlation only at 4000Hz and a non significant correlation was found between ASSR and Tone Burst thresholds (Table 4).

In moderate hearing loss group, a statistically significant correlation was found at 500Hz in moderate hearing loss group between PTA and ASSR thresholds and between ASSR and Tone Burst thresholds, but a non significant correlation was found between PTA and Tone burst thresholds (Table 5).

A statistically significant correlation was found at 500 and 4000Hz between the three groups; a- between PTA and ASSR thresholds, b- between PTA and Tone Burst thresholds and c- between ASSR and Tone Burst thresholds in severe hearing loss group (Table 6).

Table (1): Mean and standard deviation (SD) of hearing threshold of PTA, TB and ASSR in normal hearing group.

<table>
<thead>
<tr>
<th>Test</th>
<th>500Hz Mean</th>
<th>SD</th>
<th>4000Hz Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTA</td>
<td>18.6667</td>
<td>4.41858</td>
<td>22.7273</td>
<td>4.41858</td>
</tr>
<tr>
<td>TB</td>
<td>40.0000</td>
<td>9.25820</td>
<td>40.9091</td>
<td>10.44466</td>
</tr>
<tr>
<td>ASSR</td>
<td>22.6667</td>
<td>16.78293</td>
<td>38.1818</td>
<td>16.01136</td>
</tr>
</tbody>
</table>

Fig. (1): Mean and standard deviation (SD) of hearing threshold of PTA, TB and AS SR in normal hearing group.

Table (2): Mean and standard deviation (SD) of hearing threshold of PTA, TB and ASSR in moderate hearing loss group.

<table>
<thead>
<tr>
<th>Test</th>
<th>500Hz Mean</th>
<th>SD</th>
<th>4000Hz Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTA</td>
<td>49.5833</td>
<td>8.90820</td>
<td>49.1667</td>
<td>8.48350</td>
</tr>
<tr>
<td>TB</td>
<td>61.2500</td>
<td>14.79020</td>
<td>58.3333</td>
<td>13.87171</td>
</tr>
<tr>
<td>ASSR</td>
<td>45.0000</td>
<td>18.21588</td>
<td>53.7500</td>
<td>13.33570</td>
</tr>
</tbody>
</table>

Fig. (2): Mean and standard deviation (SD) of hearing threshold of PTA, TB and AS SR in moderate hearing loss group.

Table (3): Mean and standard deviation (SD) of hearing threshold of PTA, TB and ASSR in severe hearing loss group.

<table>
<thead>
<tr>
<th>Test</th>
<th>500Hz Mean</th>
<th>SD</th>
<th>4000Hz Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTA</td>
<td>80.9091</td>
<td>9.17011</td>
<td>83.6667</td>
<td>12.74288</td>
</tr>
<tr>
<td>TB</td>
<td>82.2727</td>
<td>10.33529</td>
<td>84.0000</td>
<td>10.88905</td>
</tr>
<tr>
<td>ASSR</td>
<td>77.2727</td>
<td>13.10794</td>
<td>85.0000</td>
<td>14.88048</td>
</tr>
</tbody>
</table>

Fig. (3): Mean and standard deviation (SD) of hearing threshold of PTA, TB and AS SR in severe hearing loss group.
In this research we found that, in normal hearing individuals ASSR and TB showed poorer prediction of normal thresholds, but ASSR thresholds were closer to PTA thresholds than TB, that is better seen at 500Hz than at 4000Hz. In moderate hearing loss patients, ASSR and TB thresholds became closer to PTA thresholds with ASSR threshold better than PTA thresholds at 500Hz.

In severe hearing loss ASSR and TB becomes closer to PTA, even ASSR thresholds became better than PTA thresholds at 500Hz.

A recent study done by El Moazen and Sobhy [2], that agrees with our results, they found a poor response of ASSR and TB in confirmation of thresholds in normal hearing individuals, and ASSR was superior to TB ABR at 500Hz stimulus.

Another research that disagrees with our results, they compared the hearing thresholds of Pure tone audiometry, Auditory steady state response and Tone burst ABR, they found that Tb-ABR thresholds were recorded much closer to behavioral threshold than ASSR thresholds [7].

Another study done by Dalati [1] that evaluated three frequencies (500, 1000, 2000Hz). All subjects tested for AS SR, and Tb-ABR for threshold measurement. Mean Tb-ABR thresholds were significantly higher than ASSR. He found that, when the degree of hearing loss increased the mean difference between the Tb-ABR and ASSR thresholds decreased, that is matching with our results.

Another research, where they found that, ASSR thresholds exceeded Tb-ABR thresholds by 12.1 dB on average with the test difference greatest at 4000Hz. Results of the two techniques were more similar at 500Hz, with ASSR thresholds being significantly higher than TB-ABR thresholds at birth only. They found that there was no significant difference in mean thresholds with age for either test [5].

Another study done by Johnson and Brown 2005, they found that TB ABR thresholds were recorded 3dB closer to behavioral threshold than ASSR thresholds. However, in the subjects with the most steeply sloping hearing losses, TB ABR thresholds were recorded as much as 25dB below behavioral threshold, whereas ASSR thresholds were never recorded more than 5dB below behavioral threshold. In contrast, the AS SR overestimated behavioral threshold in two subjects with normal hearing, where the ABR provided a more accurate prediction of behavioral threshold.

### Table (4): Pearson correlation coefficient in normal hearing group.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>500</th>
<th>4000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between PTA threshold and ASSR threshold</td>
<td>r = 0.750*</td>
<td>0.006</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.986</td>
</tr>
<tr>
<td>Tone Burst threshold</td>
<td>Sig.</td>
<td>$S$</td>
</tr>
</tbody>
</table>

A statistically significant correlation was found at 500Hz between PTA threshold and ASSR threshold and between Tone Burst threshold at 4000Hz.

### Table (5): Pearson correlation coefficient in moderate SNHL group.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>500</th>
<th>4000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between PTA threshold and ASSR threshold</td>
<td>r = 0.714**</td>
<td>0.372</td>
</tr>
<tr>
<td>p-value</td>
<td>0.009</td>
<td>0.234</td>
</tr>
<tr>
<td>Tone Burst threshold</td>
<td>Sig.</td>
<td>$S$</td>
</tr>
</tbody>
</table>

A statistically significant correlation was found at 500Hz between PTA threshold and ASSR threshold and between Tone Burst threshold.

### Table (6): Pearson correlation coefficient in severe SNHL group.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>500</th>
<th>4000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between PTA threshold and ASSR threshold</td>
<td>r = 0.688*</td>
<td>0.631*</td>
</tr>
<tr>
<td>p-value</td>
<td>0.019</td>
<td>0.012</td>
</tr>
<tr>
<td>Tone Burst threshold</td>
<td>Sig.</td>
<td>$S$</td>
</tr>
</tbody>
</table>

A statistically significant correlation was found at 500 and 4000Hz.

### Discussion

This study compared the hearing thresholds of Pure tone audiometry, Auditory steady state response and Tone burst ABR in normal hearing, moderate SNHL and severe SNHL patients at 500 and 4000Hz, aiming to find the best threshold prediction method in relation to PTA threshold.
In our research we found that in normal hearing persons, there was a significant correlation between PTA and ASSR at 500Hz, and between PTA and TB at 4000Hz. In moderate SNHL there was a significant correlation between PTA and ASSR and between AS SR and TB at 500Hz only. In severe SNHL patients, there was a significant correlation at 500Hz and 4000Hz between the three groups a-PTA & ASSR, b- PTA & TB and c- ASSR & TB.

Our results agree with El Moazen and Sobhy (2010) work, they compared thresholds of PTA, ASSR and TB ABR in SNHL patients, they found a high correlations between AS SR and PTA thresholds and between ASSR and TB ABR thresholds.

A recent study that evaluated the accuracy of auditory steady-state response (ASSR) and tone burst auditory brainstem response (Tb-ABR) in relation to pure tone audiometry in SNHL patients, they found that both AS SR and Tb-ABR thresholds had high correlations to pure-tone thresholds [7].

Dalati 2012, found that there were positive correlation between ASSR and Tb-ABR thresholds in SNHL patients that matches with our results.

Conclusions:
• In normal hearing both TB ABR and AS SR testing show poor prediction of PTA thresholds.
• ASSR thresholds are better to estimate PTA thresholds than TB ABR thresholds in normal and moderate SNHL.
• Both ASSR and TB ABR thresholds show a significant correlation with PTA that increases with the increasing severity of hearing loss.

Recommendations:
• Using AS SR for more accurate prediction of PTA thresholds as frequency specific method in difficult to test patients.

References
Alteration of Hematological and Plasma Physiological Parameters Following a Standard Protocol of General Anesthesia: A Pilot Study in Assiut University Hospitals

NERMIEN WALY, M.D., Ph.D.*; WALEED S.H. FARRAG, M.D., Ph.D.** and ASHRAF HASABALLA, M.D., Ph.D.***

The Departments of Physiology*, Anesthesiology** and Clinical Pathology***, Faculty of Medicine, Assiut University

Abstract

The objective of this study was to evaluate the effects of a standard anesthetic protocol using Propofol, fentanyl, and Sevoflurane, on hematological and plasma physiological variables in humans. Blood samples were collected from fourteen (14) individuals undergoing surgery for a complete blood count (CBC) before and after the induction of anesthesia. Plasma physiological parameters as creatinine, urea, and blood glucose level were also measured.

Hemoglobin concentration, showed a significant decrease after anesthesia induction (p<0.0001). There was also a significant decrease in the red blood cell (RBC’s) count and hematocrit values (p<0.001 & p<0.05 respectively). Total leucocytic count (TLC) and platelets exhibited a non-significant reduction. Serum creatinine significantly reduced (p<0.0001) after the induction of anesthesia while blood urea significantly increased (p<0.001). There was a non-significant increase in blood glucose level.

This study suggests that a careful monitoring during anesthesia is advisable to avoid possible postoperative life threatening complications.


Introduction

POSTOPERATIVE impact of anesthesia on hematological as well as plasma biochemical parameters have been documented in animals [1]. In human, anesthesia has been reported to alter cardiovascular as well as renal and hepatic homeostasis [2]. Cardiovascular and hemodynamic effects are due to the effects of anesthetics on the heart and blood vessels [3,4]. Anesthesia has also been implicated in altering stress response [5].

Sevoflurane and Desflurane anesthesia has been shown to affect leucocytic counts [6]. Alteration of the mechanical properties of the RBC’s was observed under the influence of Halothane and Isoflurane anesthesia [7].

In our institute no study has been performed to evaluate the effect of anaesthesia on haematological and plasma biochemical parameters. Clinical observation shows that there are often some unexplained haematological changes following surgery (authors’ observation). It is important to anticipate this potential anaesthesia induced clinical effects to avoid any possible postoperative complications.

The aim of this study is to evaluate the effect of a standard protocol of anaesthesia using Propofol-Fentanyl and Sevoflurane on haematological parameters and some plasma biochemical parameters in our institute.

Patients and Methods

The study protocol was conducted at anesthesia department of Assiut University Hospital between January 2012 and January 2013, after approval by our ethical committee board, informed written consent was obtained from each patient.

We enrolled 14 patients between 20 and 60 years of age (ASA I-II) scheduled for elective urological procedures as follow: 5 patients undergo varicocelectomy, 5 patients hydrocelectomy and 4 patients for DJ insertion by endoscopy. Patients with expected lengthy operations and operations with major fluid shift are exclude from the study. All patients were premedicated with atropine 0.01mg/kg and midazolam 0.04mg/kg injected intramuscular 30min before induction. On arrival into the operating room, 20G venous line was inserted through which normal saline solution (0.9% sodium chloride) was infused at a rate of
100ml/hr. After establishing standard patient monitoring, noninvasive blood pressure, pulse oximetry and electrocardiogram 5 leads, all the patients received fentanyl 1 µg/kg and Propofol 2-3mg/kg, and anesthesia mask was applied with oxygen sevoflurane 4-5%, then after patient was deeply anesthetized laryngeal mask 3 was inserted. Maintenance of anesthesia would be with oxygen and Sevoflurane 2% till the end of procedure. Blood samples were collected after 5 minutes of induction from the other hand of venous line.

**Laboratory investigations:**

Blood samples for complete blood count (CBC) and biochemical analysis was withdrawn preoperative as a part of pre-anesthetic evaluation and preparation of the patients for surgery. The postoperative samples were withdrawn after the induction of anesthesia. The CBC and biochemical analysis was done in the central laboratories of the Assiut University hospitals using CELL-DYN 3700 automated hematology analyzer (Abbott Diagnostics, Abbott Park, IL, USA) and the Dimension EXL 200 integrated chemistry system (Siemens, Erlangen, Germany) respectively.

**Statistical analysis:** Was performed using Graphpad® software, paired t-test was done, p-value was set at 0.05, 0.001, and 0.0001.

**Table (1): The effect of sevoflurane-propofol anesthesia on various hematological parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-induction (g/dL)</th>
<th>Post-induction (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs</td>
<td>12.36±1.49</td>
<td>10.97±1.37</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>36.7±0.05</td>
<td>33.5±0.04</td>
</tr>
<tr>
<td>WBCs (x 10^9/l)</td>
<td>9.17±0.43</td>
<td>8.64±0.22</td>
</tr>
<tr>
<td>PLT (10^9/l)</td>
<td>297.8±130.1</td>
<td>284.9±133.4</td>
</tr>
</tbody>
</table>

RBC = Red blood cell. WBC = Total white blood cell. Hb = Hemoglobin concentration. PLT = Platelet.

**Results**

In this study all hematological variables as well as plasma biochemical parameters measured, exhibited variations following induction of Sevoflurane-Propofol anesthesia as follows:

**I- Hematological parameters:**

Hemoglobin was reduced significantly (p<0.0001) from an average of 12.36±1.49g/dL to 10.97±1.37g/dL. Red blood cells (RBCs) count was also significantly reduced from 4.75±0.52x 10^12/l to 4.19±0.42x 10^12/l (p<0.001). On the other hand platelet count and total leucocytic counts exhibited a statistically non-significant reduction. There was a significant reduction in Hematocrit value as well (p<0.05).

Data are presented in Table (1), Figs. (1,2).

**II- Plasma biochemical parameters:**

Serum creatinine decreased significantly from 102.9±90.55 µmol/l to 98.26±70.90 µmol/l (p<0.0001) following our anesthetic protocol. Glucose increase from 6.07±1.64mmol/l to 6.95±1.35mmol/l was statistically non-significant. Blood urea also increased significantly from 5.63±4.43mmol/l to 7.36±5.50mmol/l (p<0.001). Results are presented in Table (2), Fig. (3).

**Table (2): Variation of serum biochemical parameters following induction of Sevoflurane-Propofol anesthesia.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-induction (µmol/l)</th>
<th>Post-induction (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>6.07±1.64</td>
<td>6.95±1.35</td>
</tr>
<tr>
<td>Urea</td>
<td>5.63±4.43</td>
<td>7.36±5.50</td>
</tr>
<tr>
<td>Creatinine</td>
<td>102.9±90.55</td>
<td>98.26±70.90</td>
</tr>
</tbody>
</table>

**Fig. (1): Sevoflurane-propofol anesthesia induced a significant reduction in hemoglobin, RBCs, and HT level (p<0.0001, p<0.001, and p<0.05 respectively).**
Discussion

This study showed significant variation in the haematological as well as plasma biochemical variables before and after induction of a standard protocol of anaesthesia of Propofol and Sevoflurane.

To our knowledge this is the first study at our institute to evaluate the effect of our standard protocol of anaesthesia on haematological and plasma biochemical parameters.

We have found a significant reduction in haemoglobin concentration following induction of anaesthesia as well as a decrease in red blood cells counts (RBCs). Splenic sequestration of blood elements could explain this result. The spleen as an organ is capable of sequestering the RBC’s through splenic vascular relaxation. Many anaesthetic drugs can induce splenic vascular relaxation and causes a decrease in the circulating erythrocytes [8]. Blood sequestration could also occur at different organs as skin, and skeletal muscles [9].

On the other hand, there was a non-significant increase in blood glucose that could be attributed to the surgical neuroendocrine stress response [10]. Hyperglycaemia is associated with poor surgical outcomes especially in critically ill patients [11]. It is very important to be aware of this fact to avoid unnecessary post-operative complications.

Anaesthesia is known to reduce blood pressure [12,13]. In addition Propofol anaesthesia can also cause hypotension [14]. This hypotensive effect can reduce renal perfusion and result in the observed increase blood urea in this study. The fact that there was no concomitant increase in serum creatinine could be attributed to the fact that serum creatinine does not change until more the 50% of kidney function is lost [15]. This finding is similar to our findings in cats [1].

The observed finding cannot be attributed to dilutional effect because we withdrew our sample five minutes after the induction of general anaesthesia and we withdrew the sample from the other...
hand away from the line. Moreover, if this additional effect, it will be universal affecting all the parameter we measured. In fact, there is increase in some parameters and there is a decrease in others.

In conclusion, we have found a significant reduction in hemoglobin concentration as well as a significant increase of both blood urea and serum glucose following the induction our standard protocol of anesthesia. This is the first study in our institute to evaluate the effect of anesthesia on hematological and plasma biochemical parameters. Careful and standardization of preoperative evaluation as well as postoperative follow up is strongly recommended to avoid potential complications as well as to enhance recovery.

References


The Diagnostic Value of Estimating CA125 in the Differentiation between Tuberculous and Malignant Causes of Serous Pleural Effusion: A Pilot Study

ELSAYED SALEM, M.D., FCCP*; AYMAN SALEM, M.D., FCCP* and AMAL ABD ELRASHEED, M.D.**
The Departments of Chest*, Faculty of Medicine, Cairo University* and Microbiology**, Faculty of Medicine, Tanta University**

Abstract

CA 125 was first introduced as a tumor marker in ovarian malignancy, which was extended to other tumors. Later it was introduced in the diagnosis of abdominal tuberculosis. Recently it was studied in chest diseases with variable results. Such data are evident in the text of the present study, which was aiming at its possible use in differentiating tuberculous from malignant cases of serous pleural effusion. This was done in 31 such cases, in whom the etiologic diagnosis was definitely known by other authenticated means. The fluid was transudate in 10 cases and exudate in 21 cases. The tuberculosis or malignant etiology in these cases was proved by other means, in order to test the validity of estimating CA125 for the differentiation between such definite cases. CA125 was estimated in both the serum and pleural fluid in these patients. Its value was significantly higher in malignancy than in tuberculosis in both examined samples; figures being 48.4U/ml, 606U/ml in tuberculous cases as compared to 202U/ml and 1308U/ml in malignancy, either in the serum or pleural fluid, respectively. A cut-off point was calculated as 130U/ml in the serum and 750U/ml in the pleural fluid, to differentiate between the tuberculous and malignant etiology in such cases. CA125 was estimated in both the serum and pleural fluid in these patients. Its value was significantly higher in malignancy than in tuberculosis in both examined samples; figures being 48.4U/ml, 606U/ml in tuberculous cases as compared to 202U/ml and 1308U/ml in malignancy, either in the serum or pleural fluid, respectively. A cut-off point was calculated as 130U/ml in the serum and 750U/ml in the pleural fluid, to differentiate between the tuberculous and malignant etiology in such cases. It is recommended to apply the results of the present pilot study on a bigger number of patients and also in multiple centers. Another study of its validity when the etiology is disputed, in the so called idiopathic cases, the same as it was validated in already proved cases. Another recommendation is to evaluate its level in the follow-up of cases under specific therapy, to assess their future prognosis.

Key Words: CA 125 – Malignant – Tuberculous – Effusion.

Introduction

AMONG other causes of pleural effusion, tuberculosis and malignancy figure as salient etiologic factors. The aspect of the fluid, among other features, is a possible differentiating factor between these two cases; being essentially serous exudate in tuberculosis and sanguinous in malignancy. But, not infrequently, the effusion is serous in malig-

Patients and Methods

31 patients having serous pleural effusion were included in the present study, during the year 2012. They were collected from the Chest Departments
of the Cairo University Hospitals and the Italian Hospital in Cairo; where the senior authors work. Inclusion criteria of all cases comprised that the etiologic diagnosis of the effusion is definitely proved by other appropriate investigations, in order to test the validity of the new parameter in this study, namely the estimation of CA125 in both serum and effusion in the diagnosis of the etiology of fluid collecting in the pleura in these proven cases. The effusion was of the exudative type in 21 cases and of the transudate type in another 10 cases, according to the authenticated Light's criteria [4] of the serous exudate type. 10 cases were definitely of tuberculous etiology and another 10 cases were having proved malignancy in the lung or pleura; namely 6 cases of bronchial carcinoma and 4 cases of mesothelioma. The last case of the serous exudate was having Parapneumonic effusion. The serous transudate pleural effusion cases were due to mediastinal invasion by inoperable bronchial carcinoma proved by endoscopie biopsy in 5 cases. In another 4 cases the pleural transudation of fluid was due to obvious heart failure. The last single case of serous transudate was due to proved tuberculous mediastinal lymphadenopathy, again proved by histopathologic evidence of the granuloma. Of the whole group 21 were males and 10 were females. Their age ranged from 16 to 48 years. All cases were subjected to full clinical assessment and radiology of the chest including C.T. and relevant routine labs in order to reach the etiologic diagnosis of the effusion: e.g CBC, ESR, tuberculin test which was highly positive in tuberculous cases. Echocardiography, and also endoscopy was resorted to in particular situations; specially in malignant cases. Pleural aspiration was done in all cases, to assure that it was clear serous fluid and was examined both physically and chemically particularly for its protein, glucose content. Lactic dehydrogenase (LDH) and adenosine deaminase content (ADA) was also estimated. Microbiologic and cytologic examination of the fluid were carried out. When the etiology of the fluid collection was not definitely reached, after all the relevant investigations, the case was excluded from the study.

The level of CA125 was measured in the serum and pleural fluid in all cases in units/ml using the commercially available ELISA kit (Phoenix pharmaceuticals, catalogue number: E.K-310-13).

Collection and storage of samples:
- **Serum samples:**
  Three centimeters of blood were withdrawn by venipuncture. Centrifugation was done for the clotted blood and the sera removed from the clots and transferred to clean storage tubes by sterile pipette and was immediately stored frozen at –20 °C.

Estimation of serum CA-125:
**Principle of the test:**
The CA125 ELISA test is based on the principle of a solid phase enzymelinked immunosobent assay; the assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule.

List of copmonents:
**Materials provided with the kit:**
- Murine Mono clonal anti CA125 coated micro titer plate with 96 wells.
- Enzyme conjugate reagent, 13ml.
- CA125 reference standards containing; 0,15, 50,100,200,400 unit and above/ml of CA125, 1 ml each, ready to use.
- Wash buffer Concentrate (20X), 50ml.
- TMB Reagent (one-step) 1 1 ml.
- Stop Solution (1N HCl), 1 lml.

Reagent preparation:
- All reagents should be brought to room temperature (18-25 °C) before use.
- To prepare Wash Buffer (1X): Add 50ml of wash buffer (20X) to 950ml of DI water. The diluted wash buffer is stable at 2-8 °C for 30 days. Mix well before use. Note: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

Assay procedure:
- Secure the desired number of coated wells in the holder. Dispense 100ml of CA125 standards, specimens, and controls into the appropriate wells.
- Dispense 100ml Enzyme Conjugate Reagent into each well.
- Mix gently for 30sec. It is very important to have a complete mixing in this set up.
- Incubate at 37 °C for 90min.
- Remove the incubation mixture by emptying the plate content into a waste container.
- Rinse and empty the microtiter plate 5 times with wash buffer (1 X).
- Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
Dispense 100ml of TMB reagent by precision pipettes into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20min, resulting in the development of a blue color.

Stop the reaction by adding 100ml of Stop Solution to each well.

Gently mix by vortex mixer for 30sec. It is important to make sure that all blue color changes to yellow color completely.

Read the optical density, spectro photometricaly, at 450nm with a micro platereader within 15min.

The concentration of CA125 is directly proportional to the color intensity of the test sample and is determined from the standard curve.

Calculation of the results:

- Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
- Construct standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis.
- Using the mean absorbance value of each sample, determine the corresponding concentration of CA125 in U/ml, from the standard curve.

Expected values and sensitivity:

Healthy individuals are expected to have CA125 assay values below 35U/ml. The minimum detectable concentration of CA125 in this assay is estimated to be 5U/ml.

Pleural fluid samples:

All pleural fluid samples for biochemical analysis were immediately centrifuged for 5min., at 3000rpm, to remove cells and then stored at 70°C (Burits and Ashwood, 1994) and then manipulated as in serum samples.

Results

The age and the sex distribution is shown in Table (1).

Table (2) shows the type and etiology of serous pleural effusion and effusions other than serous type were exudate.

The result of estimation of CA125 are shown in Tables (3,4), both with serum and pleural fluid. There is a definite statistically significant increase in both serum and pleural fluid levels of CA125, more in favor of malignant cases when compared to tuberculous cases.

There is statistically significant higher levels of serum CA125 in malignant more than tuberculous cases.

There is statistically significant higher pleural levels of CA125 in malignant more than tuberculous exudate.

Table (1): Age and sex distribution (n = 31).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>20-40</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>&gt;40</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>10</td>
<td>31</td>
</tr>
</tbody>
</table>

Table (2): Type and etiology of pleural effusion (n = 31).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exudate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>10</td>
<td>32.25</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>10</td>
<td>32.25</td>
<td></td>
</tr>
<tr>
<td>Parapneumonic</td>
<td>1</td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td>Transudate:</td>
<td>10</td>
<td>32.25</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>5</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Heart failure</td>
<td>4</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>1</td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Mean and SD± of CA125 in serum (n = 31).

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean (U/ml)</th>
<th>SD±</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exudate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>48.4*</td>
<td>17</td>
<td>32.7-65.5</td>
</tr>
<tr>
<td>Malignant</td>
<td>202*</td>
<td>64</td>
<td>151.1-291</td>
</tr>
<tr>
<td>Parapneumonic</td>
<td>36</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Transudate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>39.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Malignant</td>
<td>55.2</td>
<td>16</td>
<td>39.4-72.3</td>
</tr>
<tr>
<td>Heart failure</td>
<td>40.0</td>
<td>13</td>
<td>29.1-55.6</td>
</tr>
</tbody>
</table>

*<0.05.

Table (4): Mean and S.D.± of pleural fluid (U/ml) (n = 31).

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean (U/ml)</th>
<th>SD±</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exudate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>606*</td>
<td>120</td>
<td>480-740</td>
</tr>
<tr>
<td>Malignant</td>
<td>1308*</td>
<td>480</td>
<td>970-1880</td>
</tr>
<tr>
<td>Parapneumonic</td>
<td>38</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Transudate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>574</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Malignant</td>
<td>73.2</td>
<td>16.8</td>
<td>57.4-86.2</td>
</tr>
<tr>
<td>Heart failure</td>
<td>45.7</td>
<td>12.1</td>
<td>36.7-56.1</td>
</tr>
</tbody>
</table>

*<0.05.
Discussion

Serous pleural effusion is either exudate or transudate. Their differentiation is not difficult when Light’s criteria [4] are adopted. This was applied in the present study: Exudates have a pleural/serum protein ratio >0.5. The lactic dehydrogenase (LDH) concentration is >200IU/L and pleural/protein serum ratio >0.6. Otherwise it is a transudate. The cause, in particular, in either situations, is however rather difficult to disclose, concerning the etiology of the fluid collection. It is more so, for the differentiation between a tuberculous and a malignant cause, although in the malignant cases the fluid is usually serosanguinous and rapidly accumulating, besides other clinical and investigational criteria. But not uncommonly it can be serous, as it is the usual situation in the tuberculous etiology. In tuberculosis, in particular, the tuberculin test is usually highly positive and the glucose content of the fluid is below 60mg/dl. The adenosine deaminase (ADA) usually exceeds 45U/ml and the cytology is usually lymphocytic. In malignancy the positive cytology or biopsy is conclusive and the tumor markers are usually helpful. One of such biomarkers, CA1 25 in particular, is the topic of the current study. This presentation is not concerned with other etiologies of serous pleural effusion. However the etiology of nearly 15% of such cases remains unexplained (Maskell and Butland 2003) [5]. These are designated as idiopathic and in the current presentation are recommended to be the topic in a future study. More elaborate manipulations were not needed in the present study as it included only cases of definite proved etiology, to evaluate the agent under study; CA125, which can be a possible differentiating investigation in this respect.

CA125 (Cancer Antigen 125 or Carbohydrate antigen 125) is also known as mucin 16 or mucame 16 and is encoded by the M.UC 16 gene (Min Loyd, 2001) [6], although it was originally discovered by Basteral 1981 [7]. It is a high mass glycol protein and is the most frequently used biomarker for ovarian marker detection (Suh et al., 2010) [8]. This tumour marker is also elevated in pleural effusion Meigs’ syndrome, secondary to ovarian fibroma (Timmerman et al., 1995) [9]. Although the tumour is definitely benign. This syndrome is also associated with ascites and pleural effusion, which might suggest that the peritoneum and pleura might secrete CA125. The peritoneal level of CA125 is higher with ascitic fluid than in the pleural fluid, suggesting that the peritoneal epithelium has a higher capacity to produce CA125, than the pleural mesothelium (Hussain and Camilleri, 2007) [10]. It is also known that the removal of the ovarian fibroma in Meigs’ syndrome, results in the disappearance of the ascetic and pleural fluid. This benign condition hence may represent just injury or insult, ending in mere proliferation, rather than actual malignant invasion of the mesothelial cells hence leading to serosal effusion. Hence irritation and not only tumours can raise CA125 in inflammatory conditions. This idea was reported by the Kumar et al., 2001 [11], who mentioned that abdominal and peritoneal tuberculosis can result in increase in the amount of serum CA125. The same was reported as early as 2000, by Mass et al., [12]. Similarly Dagr et al., 2008 [13] reported the same finding. This was the issue of the present communication which aimed at estimating the level of CA1 25, in both serum and fluid in cases of serous effusion, in proved case of tuberculous and malignant etiologies, in a trial to evaluate its validity in the differentiation between either conditions. The results proved the rise of CA125 in both conditions either in the serum or pleural fluid. But it was only statistically significant in the exudate, rather the transudate type, which does not directly insult the pleura; fluid figures being 1308 and 606U/ml for malignancy and tuberculosis respectively as mean values (Table 4). The rise is more in the malignant than the tuberculous cases. Table 3 also documents the rise of CA125 in the serum of both entities, figures being, 202 and 48.4U/ml in either malignancy or tuberculosis, as mean values, respectively. This was statistically significant as differentiating item only in cases with serous exudate type of the effusion: The number of cases of serous transudate was too small to suggest any statistical significance. Malignancy however was showing the more rise in CA125 level. It seems that the inflammatory insult in the pleura is less operating in the production of CA125. The shorter duration of the insult may be also operating in this respect as shown by the relatively low figure in the parapneumonic case of effusion, of much shorter duration than the analogous period in the tuberculous case: figures being 88 and 606U/ml CA125, respectively in pleural fluid (Table 4). It is not possible to draw any conclusion in this aspect in particular, as it was only a single case of non-specific parapneumonic infection. It also seems that the transudate type of serous pleural effusion does not share in the risk of raising CA125 levels, although the number of cases is also too small to draw conclusions (Tables 3,4). This, however, may be due to the fact that there is no direct injury or insult to the serous membrane in such cases.
It is thus proved, from the presented data in the current study, that both malignancy and tuberculosis do raise CA125 levels, to different degrees, with only statistical significance in the exudative type of pleural fluid. The differentiation in this rise between both conditions, thus needs to calculate a cut-off point for their discrimination: To start with, no statistical different values as regards CA125 levels were reported as regards to either sex or age of the patients. This was reported by Fortun and Mendez, 2009 [15]. Hence all patients could be gathered in a common pool to fetch for a cut-off level. Such cut off level was suggested by (Abd Elsamad et al., 2013) [14] in a master thesis on the evaluation of the diagnostic study the role of CA125 in patients with pleural effusion [14]. In malignancy in particular, they gave the cut-off level as 150U/ml in serum and 700U/ml in pleural fluid: Its sensitivity was 79% and 94% in either the serum and pleural fluid respectively. Their analogous figures for specificity were 86% and 87% respectively for malignant cases. Their figures for tuberculosis and other pathologies were 1 00U/ml for the serum and 600U/ml for the fluid (Abd elsamad et al., 2013) [14]. Their sensitivity figures were 84% and 99% for the serum and pleural fluid respectively in tuberculous cases. The analogous specificity figures were 96% and 87% respectively (Abd elsamad et al., 2013) [14].

In the present study a trial for the detection of a cut-off level in cases of exudate serum effusion between the tuberculous and malignant cases was done; based on calculations from the standard deviation (SD±) around the mean figures and also from the range values actually measured in the two etiologies: As shown in Table (5) comparing mean values of serum and pleural fluid level of CA125 in patients with serous exudative effusion, the difference was statistically highly significant <0.001, figures being 48.4U/ml, 606 for tuberculosis, both in the serum and pleural fluid respectively. The analogous figures were 202U/ml and 1308U/ml for malignancy and both in the serum and pleural fluid respectively.

There is statistically highly significant difference between serum and pleural fluid in both situations.

This proves, beyond doubt, that the pleura is a productive structure for CA125 as already reported by Jamaldin et al., 2006 [3] and Hussein and Camilleri, 2007 [10]. Table (5) is also helpful in calculating the cut-off level between the two etiologic entities: Namely malignancy and tuberculosis in the two measured CA125 levels namely in serum and pleural fluid in patients with serous exudate. Thus four situation are encountered as shown in Tables (6,7), by taking in consideration both calculation of the mean values ±S.D and also considering the highest and lowest range values: Table (6) in particular attends to such calculations in the 4 situations in serum of patients.

Hence, under any circumstances for calculation, there is no overlap between the lowest figure in malignancy 138 and the highest figure in tuberculosis 65.4 according to S.D± calculations. Also there is no overlap between the lowest in malignancy 15 1.1 and the highest figure in tuberculosis 65.5. Eventuaty the figures are almost double in the cited comparative calculation.

Table (7), on the other hand, applies the same calculation rules to the pleural fluid in the same serous exudate pleural effusion cases in both malignancy and tuberculosis.

Hence under, any circumstances for calculation, there is no overlap between the lowest figure in malignancy 828 and the highest figure in tuberculosis 726 according to S.D calculations. Also there is no overlap between the lowest figure in malignancy 970 and tuberculosis 740. As the calculation continues table 8 is formulated by creating hypothetical levels of CA125 which could be encountered even when malignancy attains its lowest levels or tuberculosis attains its highest levels than those already mentioned and underlined in Tables (6,7). This is achieved by calculating the difference between the highest calculated level in tuberculosis and the lowest calculated level in malignancy as appearing in serum in Table (6) and in pleural fluid in Table (7). This difference is then added to the highest figure calculated in serum of tuberculosis patient and also subtracted from the lowest figure calculated in pleural fluid of malignant cases. Thus we get a maximum situation of a cut-off level which tuberculosis can never exceed and also malignancy can never get lower. Round figures are then postulated for simplicity of application. These final cut-off levels appear in the bottom line of Table (8) at figures 130U/ml for the serum and 750U/ml for the pleural fluid.

Table (5): Comparison between serum and pleural fluid levels of CA125 (mean levels) in patients with serous pleural effusion (n=20).

<table>
<thead>
<tr>
<th>Item</th>
<th>Serum U/ml</th>
<th>Pleural fluid U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis exudate (10 pts)</td>
<td>48.4*</td>
<td>606*</td>
</tr>
<tr>
<td>Malignant exudate (10 pts)</td>
<td>202**</td>
<td>1308**</td>
</tr>
</tbody>
</table>

* p<0.001  **p<0.001
Table (6): Calculations of highest and lowest possible figures of CA125 in serum of patients with malignant and tuberculous pleural exudate (n 20).

<table>
<thead>
<tr>
<th>Item</th>
<th>MeanU/ml</th>
<th>S.D±</th>
<th>Highest possible figures</th>
<th>Lowest possible figures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>According to S.D±</td>
<td>According to range (Table 3)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>202</td>
<td>64</td>
<td>266</td>
<td>291</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>48.4</td>
<td>17</td>
<td>65.4</td>
<td>65.5</td>
</tr>
</tbody>
</table>

Table (7): Calculation of highest and lowest possible figures of CA125 in pleural fluid of patients with malignant and tuberculous pleural exudate (n 20).

<table>
<thead>
<tr>
<th>Item</th>
<th>MeanU/ml</th>
<th>S.D±</th>
<th>Highest possible figures</th>
<th>Lowest possible figures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>According to S.D±</td>
<td>According to range (Table 4)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1308</td>
<td>480</td>
<td>1788</td>
<td>1880</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>606</td>
<td>120</td>
<td>726</td>
<td>740</td>
</tr>
</tbody>
</table>

Table (8): Suggested cut-off levels of CA125 in serum and pleural fluid.

<table>
<thead>
<tr>
<th>Item</th>
<th>Serum figures (Table 6) U/ml</th>
<th>Pleural fluid figures (Table 7) U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest level in malignancy</td>
<td>138</td>
<td>828</td>
</tr>
<tr>
<td>Highest level in tuberculosis</td>
<td>65.5</td>
<td>720</td>
</tr>
<tr>
<td>Difference</td>
<td>62.5</td>
<td>88</td>
</tr>
<tr>
<td>Hypothetical addition of difference to highest T.B level</td>
<td>128</td>
<td>_</td>
</tr>
<tr>
<td>Hypothetical subtraction of difference from lowest malignant level</td>
<td>_</td>
<td>740</td>
</tr>
<tr>
<td>Suggested round figure for cut-off level</td>
<td>130</td>
<td>750</td>
</tr>
</tbody>
</table>

Hence, as far as this study is concerned, the etiology in patients with serous pleural effusion below this cut-off levels in serum and pleural fluid is most probably tuberculosis and if higher, the most feasible etiology is malignancy.

The presented cut-off figures are higher than those given by Abdelsamad et al., 2013 [14] as 100 in serum and 600U/ml for pleural fluid. Our calculations are more scrutinizing which is considered a more safe-guard for the patients’ sake. It remains however essential to put such cut-off levels to more meticulous testifying studies on a bigger number of patients to reach definite conclusions. Also it should be done in multiple centers. It is also a must that such studies should always be correlated with other parameters on the subject as, for example, the parameter forwarded by the same authors; Salem et al., 2010 [16] concerning quantiferon gold test in the diagnosis of idiopathic pleural effusion; an entity in which such cases are also recommended for future studies, concerning CA125 assays. All efforts should be directed to disclose the etiology in these obscure cases, specially that the presented parameter in this study is a non-invasive approach.

Conclusions and Recommendations:

1- The estimation of CA125 levels proved helpful in the differentiation between exudate and transudate types of serous pleural effusion, figures being much higher in the exudate type, more so in the pleural fluid.

2- It also proved of value to differentiate between the tuberculous and malignant etiology in cases with serous exudate pleural effusion which show a definite rise in CA125 level both in serum and in the pleural fluid at a cut-off point of 130U/ml in serum and 750U/ml in the pleural fluid; the figures are lower in tuberculous and higher in malignancy than the cut-off level.

3- It is recommended to carry out similar studies, in the light of the present pilot study, on bigger number of patients, and also in multiple centers, for further wider evaluation and application.

4- Another recommendation is to apply the test, being non-invasive, in cases of idiopathic pleural effusion when the etiology is obscure, before resorting to more invasive investigations.
References


Hyaluronic Acid (HA) Level in Ascitic Fluid of Cirrhotic Patients with Spontaneous Bacterial Peritonitis (SBP)

ZAIN E.A. SAYED, M.D. 1; EMAD F.M. KHOLEF, M.D. 2; MOHAMED O. ABDELMALEK, M.D. 3 and KHALED M. ATTALLAH, M.D. 4

The Departments of Internal Medicine, Gastroenterology Unit 1, Clinical Pathology 2, Tropical Medicine 3, Faculty of Medicine, Assiut University and Hepatology & Gastroenterology 4 Departments, National Liver Institute, Menoufiya University

Abstract

Introduction: Spontaneous bacterial peritonitis (SBP) is a common problem that affects liver cirrhotic patients. It is also, a major contributor to the deterioration and aggravation of liver failure complications. Complement deficiency considered as a major complication of liver cirrhosis and bacterial overgrowth in the intestine is the major source of bacterial peritonitis. Hyaluronan or hyaluronic acid (HA) is a connective tissue polysaccharide, synthesized by many cell types, although mesenchymal cells are believed to be predominant. Serum level of HA is regulated by the influx from the tissues via lymphatic system and its receptor-mediated clearance by liver endothelial cells. So, marked increase in serum levels are noted in liver diseases, especially in patients with cirrhosis, when the clearance is impaired. Hyaluronic acids have an important role in controlling tissue permeation, bacterial invasiveness and macromolecular transport between cells. HA was observed to enhance cellular infiltration and migration by facilitating cell detachment. It also, increases the proinflammatory cytokines TNF-α and IL-8 production. It is interesting to note that HA not only can promote the inflammation, but can also moderate the inflammatory response. This may contribute to the stabilization of granulation tissue matrix.

Aims and Methods: To measure the level of complement-3 (C3) and hyaluronic acid in ascitic fluid of liver cirrhosis patients with and without spontaneous bacterial peritonitis.

Results: In our study we found that there was a significant decrease in C3 level in ascitic fluid of cirrhotic patients in comparison to ascitic fluid of patients with other causes (i.e. Nephrotic syndrome) (p<0.05). Also, HA level was found to be highly significantly lower in ascitic fluid of cirrhotic patients in comparison to ascitic fluid of patient with other causes (i.e. Nephrotic syndrome) (p<0.001). HA level in serum of liver cirrhosis patients was significantly higher than the contro group (p<0.001). There was a highly significant decrease in HA level in ascitic fluid of cirrhotic patients with SBP in comparison to HA level in ascitic fluid of cirrhotic patients without SBP (p<0.001).

Conclusion: C3 and HA are significantly decreased in ascitic fluid of cirrhotic patients. HA significantly decreased in ascitic fluid of cirrhotic patients with SBP in comparison to patients without SBP.

Key Words: Hyaluronic acid (HA) – Spontaneous bacterial peritonitis (SBP).

Introduction

According to some recent data [1], hepatic cirrhosis represents the tenth major cause of death world wide. Among the major complications of cirrhosis, ascites seems to be the most frequent one (85%), along with hepatic encephalopathy and the hemorrhage caused by the rupture of the esophageal varices. Patients with cirrhosis and ascites show a higher susceptibility to bacterial infections mainly because of the inadequate defense mechanisms. In these patients, the most frequent infectious complication that occurs (25% of the cases), and at the same time the most severe one is spontaneous bacterial peritonitis (SBP), followed by urinary infections (about 20%), pneumonia (about 15%) and bacteremia (12%) [2]. For SBP diagnosis, the number of polymorphonuclear leucocytes (PMN) from the ascetic fluid obtained by paracentesis must exceed 250 cells/mm 3 and from bacteriological cultures only one germ must be isolated [3]. Spontaneous bacterial peritonitis is probably related to several impaired defense mechanisms, such as depressed reticuloendothelial system phagocytic activity, leukocyte dysfunction, reduced serum complement, and low bacterial activity of ascitic fluid. Infection of ascitic fluid is related to its antimicrobial activity. In cirrhotic patients, the
bactericidal and opsonic activity of the ascitic fluid is lower than that observed in noncirrhotic ascites or in normal peritoneal exudate \[4\]. Patients with liver cirrhosis have a proposed three possible mechanisms for the development of SBP: Intestinal bacterial overgrowth, the alterations (structural and functional) of the intestinal mucosal barrier and the deficiencies of the local immune response \[4\]. In cirrhotic patient there is a local and systemic immune deficiencies, so bacteremia and ascitic fluid inoculation is a leading result. If the ascitic fluid complement level is low, this will determine a low bactericidal activity and thus a higher risk of SBP \[5\]. The hyaluronic acids (HA) are a class of macromolecular proteoglycans characterized by a highly polymerized chain of glucuronic acid and N-acetylglucosamine units bonded to protein. Due to its high water binding capacity hyaluronic acid is responsible for the hydration and osmotic balance of tissues. Hyaluronic acid network maintains matrix homeostasis by establishing spaces for fluid flow and diffusion barriers which regulate the distribution of proteins. It also influences cell proliferation and differentiation as well as the migration of cells and contributes to pericellular matrix formation by binding to the cell surface receptor CD44. Additionally, it is involved in tissue repair, so that large amounts of hyaluronic acid were observed in granulation tissue \[6\]. Occurring in intercellular ground substance of connecting tissue \[7\], they have an important role in controlling tissue permeation, bacterial invasiveness and macromolecular transport between cells \[8\]. HA acts as a promoter of early inflammation, HA was observed to enhance cellular infiltration \[9,10\]. Kobayashi and colleagues \[9,11\] showed a dose-dependent increase of the proinflammatory cytokines TNF-\(\alpha\) and IL-8 production by human uterine fibroblasts at HA concentrations of 10 \(\mu\)g/ml to 1 mg/ml via a CD44 mediated mechanism. Endothelial cells, in response to inflammatory cytokines such as TNF-\(\alpha\), and bacterial lipopolysaccharide, also synthesize HA, which has been shown to facilitate primary adhesion of cytokine-activated lymphocytes expressing the HA-binding variants of CD44 under laminar and static flow conditions \[12\]. It is interesting to note that HA has contradictory dual functions in the inflammatory process. It not only can promote the inflammation, but also can moderate the inflammatory response, which may contribute to the stabilization of granulation tissue matrix. In chronic liver disease permanent inflammation might cause fibrosis or even cirrhosis of the liver at last. Liver fibrosis/cirrhosis is characterized by an enhanced extracellular matrix synthesis by hepatic stellate cells leading to progressive induration of the entire organ. The formation of scar tissue causes the progressive loss of liver function and also decreases the capacity of the liver sinusoidal endothelial cells for the clearance of hyaluronic acid. So, HA increases in liver cirrhosis both due to the loss of degradation capacity and an increased synthesis of hyaluronic acid \[5\].

### Material and Methods

Thirty nine patients and 9 healthy subjects (group D) have been included in this study. Patients were divided into three groups: Group (A) included 16 patients with liver cirrhosis and ascites which develop spontaneous bacterial peritonitis (SBP) diagnosed according to the polymorphonuclear leukocytic cout in ascitic fluid (>250/mm\(^3\)) and +ve culture (with only one organism). Group (B) included 12 patients with liver cirrhosis and ascites without spontaneous bacterial peritonitis and group (C) included 11 patients with nephrotic syndrome and ascites.

Patients with pancreatitis, tuberculosis, peritoneal carcinomatosis and secondary peritoneal infection are excluded. Also patients with renal impairment are excluded from this study. All groups were subjected to the following investigations:

- Complete blood picture, liver function (Bilirubin, total and direct, total proteins and albumin, AST, ALT, ALP, GGt), Serum Urea and creatinine, Ascitic fluid examination, Complement-3 level in ascitic fluid of group A, B and C and serum level in the four groups by ELISA technique (using Wkea Med Supplies Elisa kits, New York, USA).

Statistical analysis was performed using the SPSS 21.0 statistical software package. Continuous variables were expressed as means±SD. \(p<0.05\) was considered stastically insignificant, \(p<0.05\) was considered stastically significant while \(p<0.00\) was considered stastically highly significant. Student t-tests were used to compare variables between patients groups.

### Results

Table (1) demonstrates the sex distribution and mean±SD for age in all groups; while Table (2) illustrates the levels of AST, ALT, serum HA, ascetic HA and ascetic C3 for different groups.
The statistical analysis of these data shows that there was no significant statistical difference for age and sex in different groups. Also AST levels and ALT level showed no significant statistical difference between the four groups.

The ascitic fluid complement-3 (C3) level shows a significant decrease in group A in comparison to group C ($p<0.05$). Also, there was a significant decrease in C3 in group B in comparison to group C ($p<0.05$) Fig. (1).

There was a highly significant increase in serum HA in group A in comparison to group C and group D ($p<0.001$). Also, there was a highly significant increase in serum HA level in group B in comparison to group C and group D ($p<0.001$) Fig. (2).

Hyaluronic acid level in the ascitic fluid shows a highly significant decrease in group A (liver cirrhosis with ascites and SBP) in comparison to group C (nephrotic syndrome with ascites) ($p<0.001$). Also, there was a significant decrease in ascetic HA in group B (liver cirrhosis with ascities) in comparison to group C ($p<0.05$). Fig. (3).

There was a highly significant decrease in HA level in ascetic fluid of cirrhotic patients with SBP in comparison to HA level in ascetic fluid of cirrhotic patients without SBP ($p<0.001$).

Table (1): Demographic data of patients in all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>16</td>
<td>12</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>(Age in years) Mean±SD</td>
<td>43±9.13</td>
<td>45.75±3.5</td>
<td>34.27±5.34</td>
<td>45±11.0</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 males</td>
<td>(75%)</td>
<td>7 males</td>
<td>(58.3%)</td>
<td>7 males</td>
</tr>
<tr>
<td>4 females</td>
<td>(25%)</td>
<td>5 females</td>
<td>(41.7%)</td>
<td>4 females</td>
</tr>
</tbody>
</table>

Table (2): AST, ALT, serum HA, and Ascitic HA and C3 levels in all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L) Mean±SD</td>
<td>30±10</td>
<td>30.16±6</td>
<td>26±6.22</td>
<td>24.55±7.68</td>
</tr>
<tr>
<td>ALT (U/L) Mean±SD</td>
<td>39.5±5.80</td>
<td>38.33±0.5</td>
<td>36.0±3.54</td>
<td>39.88±5.89</td>
</tr>
<tr>
<td>Serum HA (ng/dl) Mean±SD</td>
<td>110.5±2.96</td>
<td>108.8±3.78</td>
<td>69.9±15</td>
<td>66.44±7.31</td>
</tr>
<tr>
<td>Ascitic HA (ng/dl) Mean±SD</td>
<td>16.94±1.32</td>
<td>38.05±0.98</td>
<td>41.73±0.79</td>
<td>–</td>
</tr>
<tr>
<td>Ascitic C3 (ng/dl) Mean±SD</td>
<td>25.36±6.40</td>
<td>37.36±3.67</td>
<td>52.02±1.08</td>
<td>–</td>
</tr>
</tbody>
</table>

Table (3): Significant difference between different groups.

<table>
<thead>
<tr>
<th>Paramete</th>
<th>Group A Vs B</th>
<th>Group A Vs C</th>
<th>Group A Vs D</th>
<th>Group B Vs C</th>
<th>Group B Vs D</th>
<th>Group C Vs D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALT</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ascitic C3</td>
<td>NS</td>
<td>$p&lt;0.05$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Serum HA</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>NS</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td>Ascitic HA</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>NS</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

– NS: Not significant.
Discussion

Patients with severe acute or chronic liver disease are often deficient in complement and may also have malfunctioning of the neutrophilic and reticuloendothelial systems [18]. In these patients, the most frequent infectious complication that occurs (25% of the cases), and at the same time the most severe one is spontaneous bacterial peritonitis possibly due to three mechanisms; intestinal bacterial overgrowth, the alterations (structural and functional) of the intestinal mucosal barrier and the deficiencies of the local immune response [12]. In cirrhotic patient there is a local and systemic immune deficiencies, so bacteraemia and ascitic fluid inoculation is a leading result. If the ascitic fluid complement level is low, this will determine a low bactericidal activity and thus a higher risk for SBP [14]. In our work we tested the complement-3 level in ascitic fluid as the first defense against the infiltrating gut organism (source of SBP). We found that the C3 level is significantly decreased in ascitic fluid of liver cirrhosis patient in comparison to ascites of other causes mainly Nephrotic syndrome patients. This may explain the high susceptibility of these patients to develop SBP. In patients with hepatic cirrhosis, because of intra- and extrahepatic shunts (due to portal hypertension), circulating bacteria do not come in contact with Kupffer cells (have a special role in preventing Infections), the result being bacteremia and high possibility of ascitic fluid inoculation of organism with development of SBP [14]. Hyaluronic acid is essential for the structure and organization of extracellular matrices since it forms a network interacting with proteins, receptors and cell surfaces. HA is synthesized at the plasma membrane and released directly into the extracellular environment [13]. This may contribute to the hydrated microenvironment at sites of synthesis, and is essential for cell migration by facilitating cell detachment. Most of HA are removed and terminally degraded by liver endothelial cells. A minor part (about 10%) is metabolized by the kidneys and the spleen. HA is closely associated with the cell migration process, and studies show that cell movement can be inhibited, at least partially, by HA degradation or blocking HA receptor occupancy [14]. In sepsis increased levels of hyaluronic acid were found to correlate with disease severity and prognosis with high values of hyaluronic acid associated with a poorer survival rate [12]. Our results show an increase in the mean value of serum hyaluronic acid in patients with ascites due to liver cirrhosis. The ascitic fluid level of hyaluronic acid shows a highly significant decrease in patients with ascites due to liver cirrhosis with or without spontaneous bacterial peritonitis (SBP) in comparison to ascitic patients due to nephrotic syndrome. From our results, and from the role of hyaluronic acid in inflammatory cell migration and activation and also its role in cytokine secretion, we conclude that the defense mechanisms are impaired in patients with liver cirrhosis even without SBP and patients with liver cirrhosis are more vulnerable to the dangerous attack of the gut organism (due to portosystemic shunt) and development of SBP with the bad prognostic results and rapid deterioration of cirrhotic patients. So, improvement of ascitic defense contents and even external supplementation of HA in those patients may impair the development of ascitic fluid infection and rapid deterioration of cirrhotic patients.

References

11- ARUFFO A., STAMENKOVIC I., MELNICK M., UNDERHILL C.B. and SEED B.: CD44 is the principal cell


Open Reduction and Internal Fixation of Complex Radial Head Fractures

SHERIF M. ABDELGAID, M.D.; MOHEMED ABOELNASS, M.D. and ESLAM ELSAYED ALY, M.Sc.
The Department of Orthopedic Surgery, Al-Razi Orthopedic Hospital, Kuwait

Abstract

Fractures of the radial head have an incidence of about 1.7% to 5.4% of all adult fractures. These fractures are classified as simple or complex, depending upon whether there is an associated fracture or a ligamentous injury. Thorough clinical & radiological assessment should be done to differentiate simple fractures from complex fractures with concomitant elbow injury. The management of complex radial head fractures remains controversial including radial head excision, open reduction & internal fixation and radial head replacement. The aim of this study is to assess the possibility and review the results of open reduction and internal fixation of complex radial head fractures. Twelve patients with complex radial head fractures underwent open reduction and internal fixation during the period from 2006 to 2009. The overall outcome was rated using Mayo Elbow Performance Score. Excellent results were achieved in 5 cases 41.5%; good in 6 cases 50%; % fair in one case 8.5% and no poor results. No infection, skin complications or heterotropic ossification in all cases. We conclude that, in such complex injuries, preservation of radial head by open reduction and internal fixation is mandatory together with appropriate treatment of concomitant injuries to regain the elbow stability for better outcomes.

Key Words: Radial head fractures – Mason classification – Essex-lopresti lesion.

Introduction

Fractures of the radial head have an incidence of about 1.7% to 5.4% of all adult fractures [1]. 85% of these fractures occur in young and active people [2].

According to the Mason classification, type I fracture is a fissure or marginal fracture without displacement; type II fracture is a marginal sector fracture with displacement; and type III fractures are comminuted fractures with the head is completely detached from the neck. Johnston added a fourth type in which radial head is fractured with dislocation of the elbow Joint [3,4] Fig. (1). Displacement of 30% of the articular surface or step off >2mm should be considered as a Mason type II fracture, opposed to the nondisplaced type I fracture [5] Mason type-III classified into three subtypes, A: Fracture of the entire radial neck, with the head completely displaced from the shaft. B: Fracture involving the entire head, which consists of more than two large fragments completely displaced from the shaft. C: Fracture with a tilted and impacted articular segment, and some articular fragments displaced from the shaft [6]. Fig. (2).

Radial head fractures are further classified as simple or complex, depending upon whether there is an associated fracture or a ligamentous injury [7]. There is a strong correlation between the likelihood of associated injury and the severity of the radial head fracture: The incidence can increase from 20% in Mason type I fractures to 80% in type III fractures. LCL lesions are found in 11% of cases, MCL lesions in 1.5% and a combination of both MCL and LCL lesions in 6% [8]. Another type of complex radial head fracture is that associated with acute longitudinal radioulnar dissociation (ALRUD) or Essex-Lopresti lesion with rupture of the interosseous membrane (IOM) and triangular fibrocartilage complex. Partial ruptures of the IOM diagnosed with MRI in nine of 14 patients with a Mason type I fracture, suggesting that injuries of the IOM are more frequent than generally expected [9].

5% to 10% of fractures of radial head are associated with elbow dislocation. The combination of an elbow dislocation, radial head fracture and coronoid fracture is called' the terrible triad of the elbow’ as it can result in severe joint instability and many post-traumatic complications [10]. Fractures of the coronoid process classified into three types. Type I is a small fleck of bone, type II
Aim of the study:

Clinically: Tenderness or ecchymosis at the medial joint line may represent a medial collateral ligament injury, whereas tenderness at the DRUJ could reflect longitudinal instability of the elbow. All 3 major nerves in the arm are examined, with particular attention to the posterior interosseous nerve (PIN). Forearm rotation is assessed to see if a mechanical block to motion exists. Indications for operative treatment of radial head fractures are with intra-articular step-off >2mm of articular or that create a mechanical block to motion [12].

The management of complex radial head fractures with associated ligament disruption remains controversial. Several surgical options have been advocated for these complex injuries, including ORIF, excision of the radial head, and arthroplasty. Biomechanically, the radial head is the secondary constraint of the ulnohumeral joint in resisting valgus stress [13]. The radial head becomes the main stabilizer if the coronoid process is fractured, the medial collateral ligament is incompetent, or the lateral ulnar collateral ligament is disrupted [14]. Accordingly, simple radial head excision is contraindicated in these injuries as it yields poor results with a lot of complications including, valgus elbow instability, elbow stiffness and proximal migration of the radius [15]. Radial head arthroplasty is indicated for complex radial head fractures that cannot be managed reliably with ORIF [16].

Aim of the study:

To assess the possibility and review the results of open reduction and internal fixation of complex radial head fractures.

Material and Methods

Twelve patients (seven males & five females) with complex radial head fractures underwent open reduction and internal fixation during the period from 2006 to 2009. All patients are young active with the average age was 28 years (range eighteen to thirty seven years). There were two cases with Mason type-II fractures, seven cases Masson type III and three Mason type-IV variations (posterior elbow dislocation).

Associated medial collateral ligament disruption was detected in two patients (one with Mason type-II & one Mason type III). Lateral collateral ligament disruption was found in three cases with Mason type-III. Interosseous membrane disruption was detected in three patients (one with Mason type-II & two with Mason type-III). Patients with Mason type-IV (posterior dislocation) had concomitant fracture tip of the coronoid process in two cases and fracture olecranon in one case. One case with Mason type III fracture head had concomitant fracture olecranon without dislocation (Table 1).

Surgical technique:

Our technique is quite similar to those reported by Capo et al., [12], Nirmal et al., [17] and Kaas et al., [18].

Under general or infraclavicular block anesthesia the elbow is first examined for 1) Axial migration: The elbow is stabilized on the hand table with the forearm in neutral rotation, and a load placed by the surgeon on the fisted hand. Proximal migration of the radius into the capitellum is observed. Alternatively, the radial shaft can be grasped with a tenaculum and pulled proximally and radial shortening can be observed at the DRUJ on fluoroscopy. 2) Medial collateral ligament: With the forearm pronated and the elbow flexed to 30°, valgus stress is applied, the degree of opening, the feel of the end point, and radiographic appearance are observed. 3) Anteroposterior stability: Evaluated in progressive extension. If posterior subluxation of the elbow is seen at more than 30° of flexion, then the radial head should be repaired or replaced.

Then the patient is turned to lateral position with the arm rested over arm support, the forearm hanged off the support and the elbow flexed 90°. The surgical exposure depends on the associated lesions.

Mason type II fractures need exposure of radial head only and approached through Kocher posterolateral approach. Skin incision extends from the posterior surface of the lateral epicondyle and continuing downward and medially 6cm distal to the tip of the olecranon over the posterior border of the ulna. The interval between the anconeus and extensor carpi ulnaris (Kocher interval) is identified and opened to expose the joint capsule which is incised longitudinally exposing the radial head and capitellum. Pronation of the forearm will keep the PIN away from the field. Longitudinal traction of forearm will distract the joint. This distraction together with increasing the forearm flexion will bring the head more superficial in the wound and facilitate fracture inspection and reduction. The articular fragment is reduced to the remaining part of the head using spatula or K wire. The reduction is hold by pointing reduction clamp and temporary...
fixed with K-wire. In situ definitive fixation is performed using mini screws (2.4mm).

In cases with Mason type III direct lateral surgical approach is used because it allows enough proximal radial shaft exposure needed for plate fixation. The skin incision is centered over the lateral epicondyle and extends distally over the radial head and neck, anterior to the posterolateral collateral ligament complex. The muscle interval between the extensor carpi ulnaris and extensor digitorum communis is identified and opened. Then the fibers of the supinator will be seen run obliquely at approximately a 45° angle from the muscle fibers of the extensor mass. The supinator is divided for a distance of approximately 3cm from the articular surface of the radial head with the forearm in pronation to protect PIN. However, further distal dissection requires identification of the PIN. Two small blunt bone spikes are inserted subperiosteally around the neck to facilitate proximal radius exposure. The head fragments are assembled to each other with small K wires. If in situ reduction of is difficult, the entire head is removed and reassembled on the side table. The head is fixed with screws to the transverse limb of mini fragment T plate. Then the head-plate construct returned back inside the wound and fixed to the shaft with screws through the longitudinal limb of T plate. The head is compressed to the shaft using spatula during plate fixation. Plates and screws are fixed within the nonarticular "safe zone" of the radius to prevent impingement of the hardware with the proximal radioulnar joint which limits the forearm rotation. This zone comprises approximately 100° and is on the dorsal aspect of the radius, in line with the Lister tubercle of the wrist. With the forearm in neutral rotation, the direct lateral surface of radial neck is the center of the safe zone [19].

In cases with torn lateral collateral ligament, the ligament is repaired using non absorbable sutures through drill-holes into lateral epicondyle. Cases with torn medial collateral ligament, repair is not required since there was no elbow instability persists after radial head fixation. In cases with torn interosseous membrane, the distal radioulnar joint is fixed with K wire which removed after 4 weeks.

In cases with fractures coronoid or olecranon process, a posterior midline skin incision is used. 15-cm skin incision is centered on the olecranon just lateral to its tip. Full thickness fasciocutaneous flaps are elevated to expose the lateral and medial side of the elbow. Radial head fracture exposed through Kocher interval between anconeus and extensor carpi radialis muscles. Coronoid fracture is approached by muscle elevation from the lateral side of proximal ulna. When it is part of a terrible triad of the elbow, the coronoid process exposure is done through the olecranon fracture. Two pull out non absorbable sutures are passed over the top of the small coronoid fragment & through the capsular attachments. The sutures are pulled out through drill-holes in the ulna, and tied over the bone. Check X-ray then taken to assess the reduction & fixation. The wound is closed on layers over redivac which is removed after 48hrs.

**Examples of cases:**

*Figures (3,4,5).*

**Follow-up:**

Postoperatively, Indomethacin has been for 6 weeks as analgesia and to reduce the prevalence of heterotopic ossification unless there were contraindications for its use. Above elbow posterior slab is applied with elbow flexed 90° and forearm in neutral rotation for two weeks. At the end of 2nd week the stitches were removed and a removable posterior above elbow splint is worn for eight weeks with early gradual range of motion within a safe arc program is started in the 3rd week.

Patient was discharged from the hospital in 3rd postoperative day and followed-up in outpatient clinic with two weeks interval for 2 months, then monthly for six months and lastly every three months. The overall outcome was rated with the Mayo Elbow Performance Score (MEPS), (Table 2) [20]. Elbow arthritis was rated according to the system of Broberg and Morrey as grade 0 (normal joint), grade 1 (slight joint space narrowing with minimum osteophyte formation), grade 2 (moderate joint-space narrowing with moderate osteophyte formation), or grade 3 (severe degenerative changes with gross destruction of the joint) [21].

**Results**

The follow-up duration ranged from 32 to 44 months (mean, 38 months). According to Mayo Elbow Performance Score, excellent results were obtained in 5 cases 41.5%; results were good in 6 cases 50%; fair results obtained in one case 8.5% and no poor results (Table 3).

Fell down on outstretched hand were the most common mechanism of injury (8 patients) followed by fall from height in three patients and R.TA in one patient (Table 3). Eleven of twelve patients included in this study regained functional arc of elbow motion allowing comfortable daily living activities (Table 4).
Patient who had fair result reduced his activity level, due to continued pain and swelling and radiologic signs of secondary osteoarthritis (Brob-erg and Morrey grade 3) with pre-existing primary synovial chondromatosis. No infection, skin complications or heterotropic ossification in all cases.

Table (1): Data of the patients.

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>Age</th>
<th>Side</th>
<th>Mechanism</th>
<th>Associated injuries</th>
<th>Masson type</th>
<th>MEPS Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>F</td>
<td>R</td>
<td>Fell down</td>
<td>MCL</td>
<td>III</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>M</td>
<td>R</td>
<td>R.T.A</td>
<td>Coronoid fracture</td>
<td>IV</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>M</td>
<td>R</td>
<td>Fell down</td>
<td>LCL</td>
<td>III</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>F</td>
<td>L</td>
<td>Fell down</td>
<td>Interosseous lig</td>
<td>II</td>
<td>Excellent</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>F</td>
<td>L</td>
<td>Fell down</td>
<td>Interosseous lig</td>
<td>III</td>
<td>Good</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>M</td>
<td>R</td>
<td>F.F.H</td>
<td>Olecranon fracture</td>
<td>IV</td>
<td>Fair</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>R</td>
<td>F.F.H</td>
<td>LCL</td>
<td>III</td>
<td>Excellent</td>
</tr>
<tr>
<td>8</td>
<td>37</td>
<td>F</td>
<td>L</td>
<td>Fell down</td>
<td>Interosseous lig</td>
<td>III</td>
<td>Good</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>M</td>
<td>L</td>
<td>Fell down</td>
<td>Olecranon fracture</td>
<td>IV</td>
<td>Good</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>M</td>
<td>R</td>
<td>F.F.H</td>
<td>MCL</td>
<td>II</td>
<td>Good</td>
</tr>
<tr>
<td>11</td>
<td>31</td>
<td>F</td>
<td>L</td>
<td>Fell down</td>
<td>Coronoid fracture</td>
<td>III</td>
<td>Excellent</td>
</tr>
<tr>
<td>12</td>
<td>29</td>
<td>M</td>
<td>R</td>
<td>Fell down</td>
<td>LCL</td>
<td>III</td>
<td>Excellent</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Function Definition</th>
<th>Points</th>
<th>Score classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>45</td>
<td>Excellent &gt;90</td>
</tr>
<tr>
<td>Mild</td>
<td>30</td>
<td>Good 75-80</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>Fair 60-74</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>Poor &lt;60</td>
</tr>
<tr>
<td>Motion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arc &gt;100</td>
<td>20</td>
<td>Excellent &gt;90</td>
</tr>
<tr>
<td>Arc 50-100</td>
<td>15</td>
<td>Good 75-80</td>
</tr>
<tr>
<td>Arc &lt; 50</td>
<td>5</td>
<td>Fair 60-74</td>
</tr>
<tr>
<td>Stability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable</td>
<td>10</td>
<td>Excellent &gt;90</td>
</tr>
<tr>
<td>Moderate instability</td>
<td>5</td>
<td>Good 75-80</td>
</tr>
<tr>
<td>Chronic instability</td>
<td>0</td>
<td>Fair 60-74</td>
</tr>
<tr>
<td>Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comb hair</td>
<td>5</td>
<td>Poor &lt;60</td>
</tr>
<tr>
<td>Feed</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hygiene</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Shirt</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Shoe</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Results according to MEPS score [18].

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>MEPS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>5</td>
</tr>
<tr>
<td>Good</td>
<td>6</td>
</tr>
<tr>
<td>Fair</td>
<td>1</td>
</tr>
<tr>
<td>Poor</td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Elbow & forearm ROM in last follow-up. Biomechanical studies showed that activities of daily living can be accomplished without discomfort within a functional arc of motion of elbow flexion extension of 100°, and forearm rotation of about 100° (pronation 50° to supination 50°) [13].

<table>
<thead>
<tr>
<th>No</th>
<th>Flexion Extension Arc</th>
<th>Pronation Supination Arc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110°</td>
<td>110°</td>
</tr>
<tr>
<td>2</td>
<td>130°</td>
<td>140°</td>
</tr>
<tr>
<td>3</td>
<td>110°</td>
<td>130°</td>
</tr>
<tr>
<td>4</td>
<td>110°</td>
<td>145°</td>
</tr>
<tr>
<td>5</td>
<td>115°</td>
<td>120°</td>
</tr>
<tr>
<td>6</td>
<td>80°</td>
<td>70°</td>
</tr>
<tr>
<td>7</td>
<td>110°</td>
<td>130°</td>
</tr>
<tr>
<td>8</td>
<td>120°</td>
<td>110°</td>
</tr>
<tr>
<td>9</td>
<td>130°</td>
<td>150°</td>
</tr>
<tr>
<td>10</td>
<td>125°</td>
<td>140°</td>
</tr>
<tr>
<td>11</td>
<td>110°</td>
<td>130°</td>
</tr>
<tr>
<td>12</td>
<td>120°</td>
<td>120°</td>
</tr>
</tbody>
</table>

Fig. (1): Mason Johnston's Classification (Types I-IV).

Fig. (2): Mason type-III radial head fracture patterns. A: A fracture of the entire radial neck, with the head completely displaced from the shaft. B: An articular fracture involving the entire head, which consists of more than two large fragments. Each fragment is completely displaced from the shaft. C: A fracture with a tilted and impacted articular segment, which must be reduced, and some articular fragments displaced from the shaft [6].
Fig. (3): Case No. 11; A & B: A.P & lateral views showed fracture head radius type III a & coronoid process. C & D: A.P & lateral views fixation of fractures. E,F,G & H: Nearly full R.O.M of injured elbow comparing with contra-lateral elbow.

Fig. (4): Case No. 7; A: Lateral view showed posterior dislocation & fracture head radius type IV. B & C: A.P & lateral views after reduction of the dislocation. D & E: A.P & lateral view after fixation of head radius fracture.
Discussion

Treatment of complex radial head fractures with associated elbow instability due to concomitant ligamentous or bony injuries, remains controversial. 50% to 75% of patients with type-II and III fractures have concomitant pathology and it is quite possible to be missed. In such injuries, internal fixation or arthroplasty, rather than excision, should be considered for the healthy, active patient to avoid delayed complications of radial head excision such as pain, instability, proximal radial translation, decreased strength, osteoarthritis, or cubitus valgus.

When there is acute instability or concern about proximal migration of the radius and injury to the interosseous ligament of the forearm, preservation of the radial head offers the best solution. So, open reduction and internal fixation should be pursued in the treatment of comminuted fractures of the radial head unless extenuating factors, such as poor general health or advanced age, prevent the patient from participating in the postoperative rehabilitation protocol. Internal fixation of displaced fractures of the radial head requires careful preoperative planning and a clear understanding of elbow anatomy. Implants should be appropriate for the size of the fragments and must be placed in a manner that does not restrict forearm rotation which is imperative for an acceptable result.

The overall results of this study according to Mayo Elbow Performance Score showed satisfactory results (excellent & good) in 91.5% of cases with no poor results. Only one case achieved fair result (case No 6). This patient was suffering primary synovial chondromatosis affecting both elbows and knees. He sustained comminuted fractures radial head and olecranon of right elbow. Radial head fracture was fixed with plate & screws and olecranon fracture was fixed by tension band. The preoperative right elbow restricted R.O.M became much worse postoperatively with flexion-extension arc 60° and supination & pronation, 30° and 40° respectively.

The current literature on complex radial head and neck fractures is sparse and provides limited guidance for choosing the optimal treatment method. Our results are similar to those obtained by Ikeda et al., 2003, they operated 10 patients with Mason type III and IV fractures using low-profile plates. The average follow-up was 28 months. 90% good to excellent results were achieved. Our results are nearly the same to those obtained by Ring et al., 2002. They operated 12 patients with type III fracture radial head, none had early failure, 1 had nonunion, and all had an arc of forearm rotation > 100°. They recommended that fixation be reserved for fractures with three or fewer articular fragments. However King et al., 1991, reported 100% excellent results after fixation of Mason type II fractures but only 33% excellent results for Mason type III injuries, suggesting that...
comminuted fractures should be managed with arthroplasty for better outcomes [26].

Conclusion:
Radial head fracture could be a serious, complex injury rather than a simple fracture. This necessitates thorough clinical & radiological patient assessment to detect concomitant bony or ligamentous injury. In such complex injuries, preservation of radial head by ORIF is mandatory together with appropriate treatment of concomitant injuries to regain the elbow stability for better outcomes. Radial head arthroplasty should be only done in cases with comminuted irreparable radial head fracture.

References
Risk Factors for Ischemic Heart Disease in Rheumatoid Arthritis

EL-BADRY I. ABO-ELNOR, M.D.*; SALAH A.A.S. ARGOON, M.D.*; OMNIYA ABD ELMONEIM, M.D.**; AYMEN M. SELIM, M.D.*** and NORA HASHEM, M.D.*

The Departments of Internal Medicine*, Clinical Pathology** and Radiology***, Faculty of Medicine, Assiut University

Abstract

Objective: The study aims to evaluate some cardiovascular risk factors in patients with rheumatoid arthritis (RA) and their relationship to ischemic heart disease (IHD) and atherosclerosis.

Patients and Methods: The study included fifty patients with RA and twenty-five healthy controls. All patients and controls were subjected to full history taking, clinical examination and the following investigations: ECG, echocardiography, carotid Doppler ultrasonography, complete blood count, rheumatoid factor (RF), C-reactive protein (CRP), serum homocysteine, serum prolactin, serum uric acid, and lipid profile (total cholesterol, triglycerides (TG), HDL-C, LDL-C).

Results: The group of RA patients had significant higher number of patients with positive CRP, with high serum uric acid, higher mean serum homocysteine, higher mean serum prolactin, higher mean total cholesterol, higher mean TG, higher mean LDL-C and lower mean HDL-C levels than the control group. Also, the group of RA patients had significant higher number of patients with ischemic changes in both ECG and echocardiography, with carotid atheromatous plaques, and significant higher mean right and left carotid intima-media thickness (IMT) than the control group. In RA patients, there were significant positive correlations between the ischemic changes in both ECG and echocardiography, carotid IMT and carotid atheromatous plaques and all the following risk factors; serum CRP, serum uric acid, serum homocysteine, serum prolactin, serum total cholesterol, serum TG, serum LDL-C levels and prolonged disease duration.

Conclusion: IHD and atherosclerosis are common in RA patients. The risk factors for IHD are hyperlipidemia, hyperhomocysteinemia, hyperprolactinemia, hyperuricemia, elevated inflammatory markers such as CRP, and prolonged disease duration. Targeting these risk factors in RA patients could help in lowering incidence of IHD and its sequelae. We recommend ECG, echocardiography and carotid Doppler ultrasonography as non-invasive screening tests for early detection of IHD and atherosclerosis in RA patients.

Key Words: Rheumatoid arthritis – Heart disease – Ischemic.

Introduction

RHEUMATOID arthritis (RA) is one of the most common autoimmune diseases which cause chronic inflammation of the joints, the tissues around the joints, as well as other organs in the body. The cardiovascular disease (CVD) is the main cause of mortality in RA patients, accounting for as much as 50% of all reported deaths [1]. The risk for CVD in RA is significantly increased versus the general population and often in undertreated patients, leading to a decrease in life expectancy [2]. Traditional risk factors for CVD, such as smoking, hypertension, diabetes and hyperlipidemia, do not fully account for the increased risk of CVD in patients with RA [3]. In patients with RA, traditional and non-traditional risk factors play a role in the development and exacerbation of CVD [4]. Non-traditional risk factors, such as serum prolactin, C-reactive protein, serum homocysteine and serum uric acid have been implicated as factors that cause endothelial dysfunction and exacerbation of CVD [5].

Prolactin has a role in immunomodulation and it has been proposed that prolactin is a risk factor for development of autoimmunity. There is increased risk of developing RA postpartum and this further increases five-fold in breastfeeding and also, disease activity in RA improves with dopamine agonists. This suggests that prolactin may have role in the pathogenesis, or at least modulation of disease activity in RA [6]. Homocysteine as well as mediators of inflammation are considered to play role in increased cardiovascular morbidity and mortality [7]. Higher serum homocysteine concentrations are found in patients with RA than in normal controls [8]. Some studies have shown that methotrexate therapy in RA could raise serum homocysteine levels [9]. C-reactive protein (CRP) is an acute phase reactant produced by hepatocytes. In RA, although CRP has been shown to be a poor predictor of RA incidence, it is central in the evaluation of disease progression and response to therapeutic intervention, and increasingly suspected as a pro-atherogenic agent in affected patients [10]. Uric acid is a waste product normally presents in
Raised serum uric acid concentration is a powerful predictor of cardiovascular risk and poor outcome. Studies have demonstrated mechanisms by which uric acid could be directly injurious to the endothelium and to cardiovascular function, paradoxically, uric acid elevation could be expected to confer protective antioxidant effect in cardiovascular system, but these potential benefits may be obscured by detrimental effects elsewhere [11]. Dyslipidemia observed in RA appears to be dependent on disease activity i.e. a higher disease activity is associated with lower total cholesterol levels and even more depressed high density lipoprotein levels, leading to a higher atherogenic index [12].

Dyslipidemia observed in RA appears to be dependent on disease activity i.e. a higher disease activity is associated with lower total cholesterol levels and even more depressed high density lipoprotein levels, leading to a higher atherogenic index [12].

Atherosclerosis is the most common pathologic process leading to CVD including myocardial infarction and stroke. RA by itself, represents a significant risk factor for early atherosclerosis and the development of CVD [13]. Many methods are used now for detection of subclinical atherosclerosis in RA patients. Carotid Doppler ultrasonography has been found to be a reliable non-invasive method of detecting atherosclerosis which correlates strongly with the presence of coronary artery disease [14].

Aim of work:
This study aims to evaluate some cardiovascular risk factors and their relationship to ischemic heart disease (IHD) and carotid atherosclerosis in patients with RA.

Patients and Methods
Fifty patients (aged 20-60 years, 8 males and 42 females) with a diagnosis of RA, attending outpatient clinic of Rheumatology Unit of the Internal Medicine Department at Assiut University Hospitals during the year 2012, were recruited into a study investigating the risk factors for IHD and carotid atherosclerosis. All patients fulfilled the 20 10 American College of Rheumatology criteria for RA and had a disease duration ranging from 3 months to 25 years. Twenty-five healthy controls (aged 21-58 years, 8 males and 17 females) were assembled, they were randomly selected from the populations register the same region. RA patients known to have IHD, diabetes, hypertension, smokers, pregnant and lactating females were excluded from the study. RA patients were then divided into active and remission groups according to disease activity score-28 (DAS-28); 32 patients were in activity (DAS-28 >2.6) and 18 patients were in remission (DAS-28 ≤2.6). Also, RA patients were classified into early and late groups according to disease duration; 20 patients had disease duration less than one year (early group) and 30 patients had disease duration more than one year (late group). All individuals were subjected to full history taking such as age, sex, and disease duration. All individuals were subjected to full clinical examination, including joints examination. The number of swollen and tender joints (28 joints count) and patients’ global assessment were registered and a disease activity score (DAS-28) including ESR calculated (0.56* (tenderjoints) + 0.28* (swollenjoints) + 0.70* (ESR) + 0.014* visual analog score). All individuals were subjected to the following investigations:

1- Twelve-lead ECG recordings and echocardiography were done to detect the ischemic changes which include; ST segment depression and T-wave changes (inversion or flat) in ECG and diastolic dysfunction and/or segmental or global hypokinesia in echocardiography.

2- Carotid Doppler ultrasonography: Carotid artery studies were performed with the individual in supine position with the neck extended and the chin turned away from the side being examined. Bilateral common carotid arteries, carotid bulbs and extracranial parts of internal carotid arteries were imaged in multiple longitudinal planes for the best resolution of the intima-media thickness (IMT), atheromatous plaques, hemodynamic changes, partially stenotic or occluded segments.

3- Laboratory investigations including:
• Complete blood count (CBC).
• Erythrocyte sedimentation rate (ESR).
• Rheumatoid factor (RF).
• C-reactive protein (CRP).
• Serum homocysteine level: Performed by Glory Science Co, ltd kit and using Enzyme Linked Immunosorbent Assay technique.
• Serum prolactin level: Preformed by biocheck Inc and using Enzyme Linked Immunossorbent Assay technique.
• Serum uric acid.
• Lipid profile (total cholesterol, TG, HDL-C, LDL-C).
• Kidney function tests.
• Liver function tests.
• Fasting and two-hour postprandial blood glucose.

Each of lipogram, serum uric acid, kidney and liver function tests and blood glucose were performed on Modular.
Statistical analysis:

The collected data were analysed by using the statistical package for Social Sciences (SPSS/ PC/ version 17). Discrete variables were presented as numbers and percentages and continuous variables were presented as mean ± standard deviation (SD). Student t-test was used to compare the mean difference between the groups, Chi-square test was used to compare the difference in proportions and Spearman’s rank correlation coefficient was used to detect the association between the risk factors of IHD and ECG, echocardiography and carotid Doppler findings. Significant test results were considered when p-value <0.05.

Results

This study was conducted on 50 patients with RA fulfilled the 2010 American College of Rheumatology criteria for RA, and 25 clinically normal subjects were chosen as control group. The mean age of RA patients was 43.7 ± 11.7 years (ranging from 20 to 60 years) and this group included 8 males (16%) and 42 females (84%). The mean age of control group was 42.1 ± 8.9 years (ranging from 21 to 58 years) and this group included 8 males (32%) and 17 females (68%). The study found that RA patients group had significant lower mean haemoglobin levels (10.7 ± 2.3 g/dl) than the control group (13.3 ± 0.9 g/dl) (p-value<0.001), and also the patients group had significant higher number of anaemic patients (n=39) than the control group (n=0) (p-value<0.001). Also, the study found that the group of cases had significant higher number of individuals with positive RF (n=38) than the control group (n=0) (p-value<0.001) (Table 1).

<table>
<thead>
<tr>
<th>Total (n=75)</th>
<th>Cases (n=50)</th>
<th>Controls (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (Mean±SD)</td>
<td>43.7±11.7</td>
<td>42.1±8.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n=16)</td>
<td>8 (50%)</td>
<td>8 (50%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female (n=59)</td>
<td>42 (71.2%)</td>
<td>17 (28.8%)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl) (Mean±SD)</td>
<td>10.7±2.3</td>
<td>13.3±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anaemia:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=36)</td>
<td>11 (30.6%)</td>
<td>25 (69.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes (n=39)</td>
<td>39 (100%)</td>
<td>0 (0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rheumatoid factor:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=37)</td>
<td>12 (32.4%)</td>
<td>25 (67.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive (n=38)</td>
<td>38 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

The study revealed that the group of RA patients had significant higher number of patients with positive CRP (n=38) and with high serum uric acid (n=10) than the control group (n=0) (p-value <0.001, p-value<0.01 respectively). Also the study found that the patients group had significant higher mean serum homocysteine and mean serum prolactin levels than the control group (p<0.05 for each). As regard the lipogram, the group of RA patients had significantly higher mean total cholesterol, mean TG and mean LDL-C levels than the control group (p<0.01, p<0.05 and p<0.001 respectively), but the patients group had significant lower mean HDL-C level compared to the control group (p<0.05) (Table 2).

Table (2): Comparative analysis of the studied groups regarding risk factors of ischemic heart disease.

<table>
<thead>
<tr>
<th>Total (n=75)</th>
<th>Cases (n=50)</th>
<th>Controls (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=37)</td>
<td>12 (32.4%)</td>
<td>25 (67.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive (n=38)</td>
<td>38 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Uric Acid:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n=65)</td>
<td>40 (61.5%)</td>
<td>25 (38.5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hyperuricemic (n=10)</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Lipogram (Mean±SD):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>165.5±36.4</td>
<td>139.3±25.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>129.8±38.3</td>
<td>87.7±21.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.1±19.2</td>
<td>50.6±8.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>98.8±30.2</td>
<td>71.1±26.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homocysteine ( μmol/L) (Mean±SD)</td>
<td>6.1±1.6</td>
<td>2.9±1.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Prolactin (ng/ml) (Mean±SD)</td>
<td>24.7±5.4</td>
<td>11.2±3.7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The study showed that the RA patients group had significant higher number of individuals with ischemic changes in both ECG (n=8) and echocardiography (n=14) than the control group (n=0) (p<0.05 and p<0.01 respectively). As regard the carotid Doppler findings, the patients group had significant higher mean right and left carotid IMT than the control group (p<0.001) and the patients group had significant higher number of individuals with carotid atheromatous plaques (n=10) than the control group (n=0) (p<0.05) (Table 3).

Table (3): ECG, Echo and carotid doppler findings in cases and controls.

<table>
<thead>
<tr>
<th>Total (n=75)</th>
<th>Cases (n=50)</th>
<th>Controls (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n=67)</td>
<td>42 (62.7%)</td>
<td>25 (37.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ischemic Changes (n=8)</td>
<td>8 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Echo:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n=61)</td>
<td>36 (59.0%)</td>
<td>25 (41.0%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ischemic Changes (n=14)</td>
<td>14 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Intima-media thickness (IMT):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n=41)</td>
<td>16 (39.0%)</td>
<td>25 (61.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thick (n=34)</td>
<td>34 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>IMT (mm) (Lt.) (Mean±SD)</td>
<td>11.5±3.6</td>
<td>7.1±1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IMT (mm) (Rt.) (Mean±SD)</td>
<td>11.4±3.4</td>
<td>7.1±1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atheromatous Plaques:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=65)</td>
<td>40 (61.5%)</td>
<td>25 (38.5%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Yes (n=10)</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

In RA patients, the study showed that there were significant positive correlations between the ischemic changes in both ECG and echocardiography, carotid IMT and carotid atherosomatic plaques and all the following risk factors; the age of RA patients, the occurrence of anaemia, positivity of RF, serum CRP, serum uric acid, serum homocysteine, serum prolactin, serum total cholesterol, serum triglyceride (TG), and serum LDL-C levels. There was significant negative correlation between the serum HDL-C in RA patients and carotid IMT (p<0.01), but there were no correlations between the serum HDL-C and ischemic changes in ECG, ischemic changes in echocardiography and carotid atheromatous plaques (Table 4).

The study showed that there were no correlations between the ischemic changes in ECG and echocardiography and both the RA disease duration and activity. As regard the carotid Doppler findings, there were significant positive correlations between the carotid IMT, carotid atherosomatic plaques and RA disease duration (p<0.05). Although there was significant positive correlations between RA disease activity and the carotid IMT (p<0.05), there was no correlation with carotid atheromatous plaques (Table 4).

| Table (4): Correlation between ECG, Echo, carotid doppler findings and and risk factors of IHD in RA patients. |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| ECG                                              | Echo                                             | IMT                                              | Plaque                                           |
| Age                                              | 0.20 <0.05                                       | 0.21 <0.05                                       | 0.71 <0.001                                      | 0.43 <0.01                                       |
| Disease Duration                                 | 0.13 >0.05                                       | −0.04 >0.05                                      | 0.22 <0.05                                      | 0.21 <0.05                                       |
| Disease Activity                                 | −0.01 >0.05                                      | 0.19 >0.05                                       | 0.20 <0.05                                      | 0.17 >0.05                                       |
| Anaemia                                          | 0.23 <0.05                                       | 0.33 <0.01                                       | 0.67 <0.001                                      | 0.27 <0.05                                       |
| RF                                               | 0.25 <0.05                                       | 0.35 <0.01                                       | 0.52 <0.001                                      | 0.28 <0.05                                       |
| CRP                                              | 0.25 <0.05                                       | 0.35 <0.01                                       | 0.72 <0.001                                      | 0.28 <0.05                                       |
| Uric Acid                                        | 0.46 <0.001                                      | 0.58 <0.001                                      | 0.34 <0.01                                      | 0.38 <0.01                                       |
| Total Cholesterol                                | 0.51 <0.001                                      | 0.52 <0.001                                      | 0.32 <0.05                                      | 0.53 <0.001                                      |
| TG                                               | 0.27 <0.05                                       | 0.31 <0.05                                       | 0.43 <0.01                                      | 0.36 <0.01                                       |
| HDL-C                                            | −0.18 >0.05                                      | −0.13 >0.05                                      | −0.36 <0.01                                     | −0.02 >0.05                                      |
| LDL-C                                            | 0.55 <0.001                                      | 0.46 <0.001                                      | 0.38 <0.01                                      | 0.48 <0.001                                      |
| Homocysteine                                     | 0.36 <0.01                                       | 0.75 <0.001                                      | 0.32 <0.05                                      | 0.26 <0.05                                       |
| Prolactin                                        | 0.61 <0.001                                      | 0.59 <0.001                                      | 0.31 <0.05                                      | 0.35 <0.01                                       |

**Discussion**

Cardiovascular disease is being recognized as the major cause of excess mortality in rheumatoid arthritis [15], also cardiovascular morbidity is enhanced and there is an increased prevalence at all stages of atherogenesis from endothelial dysfunction to fatal and non-fatal myocardial infarction and stroke [16]. moreover, the excess cardiovascular burden persists after adjustment for traditional cardiovascular risk factors [17]. Non-traditional risk factors, such as serum prolactin, C-reactive protein, serum homocysteine and serum uric acid have been implicated as factors that cause endothelial dysfunction and exacerbation of CVD [5].

The study aimed to evaluate some cardiovascular risk factors and their relationship to ischemic heart disease (IHD) and carotid atherosclerosis in patients with RA. This study showed that the RA patients group had significant higher positive CRP than the control group, this result was in concordance with the results of Molenaar et al., [18] who reported that CRP has a great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA. CRP has been clearly shown to predict further cardiovascular risk [19]. Direct pathogenic role of CRP has been suggested and many studies have shown that CRP may facilitate an increase in cellular adhesion to the endothelium, encourage macrophage and uptake of LDL-C [20]. Also, serum uric acid was found significantly higher in RA patients group compared to the control group, this result was consistent with the results of Agudelo et al., [21] who studied hyperuricemia in rheumatoid arthritis. Raised serum uric acid concentration is a powerful predictor of cardiovascular risk and poor outcome. Studies have demonstrated mechanisms by which uric acid could be directly injurious to the endothelium and to cardiovascular function, paradoxically, uric acid elevation could be expected to confer protective antioxidant effect in cardiovascular system, but these potential benefits may be obscured by detrimental effects elsewhere [11].
In this study, we found that the patients group had significantly higher homocysteine levels compared to the control group, this result was in concordance with the results of Hernanz et al., [22] who reported that increased plasma levels of homocysteine and other thiol components in RA women. It is well known that vitamin deficiency can be derived from cell proliferation. Since inflammation promotes cell proliferation at the expense of excess vitamins leading to hyperhomocysteinemia, therefore, homocysteine can be used as a marker of inflammation [23]. The study showed that the mean prolactin level was significantly higher in RA patients group compared to the control group, this result was consistent with results of Mateol et al., [24] and Liederman et al., [6]. T-lymphocytes infiltrating the synovium produce prolactin and have been shown to induce excess synovial cell function in RA patients; bromocriptine treatment in vitro suppressed lymphocyte prolactin as well as IL-6 and lymphocyte proliferation [25]. In the present study, we found that the RA patients group had significantly higher mean total cholesterol, TG and LDL-C levels and significantly lower HDL-C than the control group, these results were in concordance with the results of Lakatos et al., [26] and Nurmo-hamed [12] who studied the atherogenic lipid profiles and its management in patients with RA.

This study revealed that there was significantly higher number of individuals with ischemic changes in both ECG and echocardiography among RA patients compared to the control group, this agreed with Del Rincon et al., [27] who reported that high incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors, and also with Gerli et al., [28] who showed that T-lymphocytes contribute to early atherosclerotic damage in rheumatoid arthritis patients. Also, the study showed that the RA patients group had significant higher mean carotid IMT and higher carotid atheromatous plaques compared to the control group, and this agreed with Roman et al., [29] who reported that the prevalence of carotid atherosclerosis in RA is at least as high as in diabetes mellitus, and also, agreed with the studies of Ebrahim et al., [30] and Mannami et al., [31] who found that the intima-media thickness of extracranial carotid arteries provides an index of atherosclerosis in other vascular regions and has been shown to be associated with most risk factors for atherosclerosis.

In this study, we found significant positive correlation between the age of RA patients and the ischemic changes in both ECG and echocardiography, carotid IMT and carotid atheromatous plaques. These results were in consistent with results of Cecilia et al., [32]. As regard the disease duration, the study showed significant positive correlation between carotid IMT, carotid atheromatous plaques and RA disease duration and these results agreed with results of González-Juanatey et al., [33]. The present study showed no correlation between RA disease duration and ischemic changes in both ECG and echocardiography, and this goes hand to hand with the study of Galutina and Bychak [34]. On the contrary, Fielta and Delsante [35] reported that atherosclerosis is an early and common finding in RA patients, positively correlating to the disease duration and severity.

We also found that there was significant positive correlation between the ischemic changes in both ECG and echocardiography, increased IMT, carotid atheromatous plaques and elevated markers of inflammation, such as increased CRP, positive RF and this agreed with other studies as that of Chung et al., [36] who reported that the prevalence and severity of coronary calcification is increased in established RA and is related in part to elevated inflammatory markers. Maradit-Kremers et al., [37] confirmed that markers of systemic inflammation confer a statistically significant additional risk for cardiovascular death among patients with RA. Galutina and Bychak [34] also found that myocardial ischaemia in patients with RA was associated with high activity of inflammatory process. The present study showed that there was a significant positive correlation between the occurrence of anaemia in RA patients and ischemic changes in both ECG and echocardiography, carotid IMT and carotid atheromatous plaques, these results agreed with the studies of Varat et al., [38] and Kiechl et al., [39].

It was noticed that there was significant positive correlation between homocysteine levels and ischemic changes in ECG, ischemic changes in echocardiography and carotid IMT, and this agreed with the study of Boushey et al., [40]. The study also showed significant correlation between homocysteine levels and carotid atheromatous plaques and this agreed with the results of Spence et al., [41]. Several mechanisms for homocysteine-induced atherosclerosis have been proposed. These include endothelial dysfunction, enhancement of oxidative stress, reduction in nitric oxide bioavailability, and augmentation of thrombus formation. Folic acid supplementation down-regulates these inflammatory response [42]. As regard the uric acid in RA patients, the study showed significant positive correlation between the serum uric acid and myocardial ischemia, carotid IMT and carotid
atheromatous plaques, and this agreed with Panoulus et al., [43]. The present study showed that there was significant positive correlation between serum prolactin levels in RA patients and myocardial ischemia, carotid IMT and carotid atheromatous plaques, and this agreed with Martinez et al., [44] who reported that prolactin inhibits activation of endothelial nitric oxide synthesis, intracellular calcium mobilization, and endothelium dependent vasorelaxation. As regard the lipogram, this study revealed that there was significant positive correlation between myocardial ischemia, carotid IMT, carotid atheromatous plaques and the levels of total cholesterol, LDL-C and triglycerides, and this agreed with Georadias et al., [45] who reported that atherogenic lipid profile is a feature characteristic of patients with RA. Although the present study showed that there was significantly negative correlation between the serum HDL-C in RA patients and carotid IMT, this correlation was not present with carotid atheromatous plaques. Mathiesen et al., [46] found that low levels of high-density lipoprotein cholesterol are associated with echolucent carotid artery plaques, and also Amarenco et al., [47] found that high-density lipoprotein-cholesterol are associated with the risk of stroke and carotid atherosclerosis.

Traditional cardiovascular risk factors can not fully account for atherosclerosis in patients with RA, therefore, inflammation itself may play a part in the progression of atherosclerosis. Atherosclerosis shares many similarities with inflammatory and autoimmune diseases such as RA [48]. Immunohistochemical studies suggest significant similarities between the mechanisms responsible for chronic synovitis and damage in the rheumatoid joint and the generation and rupture of atherosclerotic plaques. These include cellular infiltrates, adhesion molecule expression, the cytokine milieu and free radicals and degenerative enzymes release [49]. Chronic inflammation and immune dysregulation characterizing RA have a key role in accelerating atherosclerosis. Persistent endothelial dysfunction predisposes to organic damage of the vascular wall, that, in a preclinical stage can be detectable by ultrasound measurement of carotid IMT, carotid atheromatous plaques and hemodynamic changes [50].

In conclusion; IHD and atherosclerosis are common in RA patients. The risk factors for IHD are hyperlipidemia, hyperhomocysteinaemia, hyperprolactinemia, hyperuricemia, elevated inflammatory markers such as CRP, and prolonged disease duration. Targeting these risk factors in RA patients could help in lowering incidence of IHD and its sequelae. We recommend ECG, echocardiography and carotid Doppler ultrasonography as non-invasive screening tests for early detection of IHD and atherosclerosis in RA patients.

References


Intrathecal Dexmedetomidine Enhances Intrathecal Combination of Magnesium Sulphate and Bupivacaine Quality of Spinal Anesthesia and Postoperative Analgesia

SAMY A. AMR, M.D.*; MONTASER A. MOHAMAD, M.D.*; MUSTAFA THABET, M.D.** and FAISAL F. ADAM, M.D.***
The Departments of Anesthesiology, South Egypt Cancer Institute*, General Surgery** and Orthopedic Surgery***, Faculty of Medicine, Assiut University, Egypt

Abstract

Background: No drug, used as adjuvant to spinal bupivacaine has yet been identified that specifically inhibits nociception without its associated side effects. The goal of neuraxial drug combination is to provide better analgesia with reduction in the incidence and severity of side effects.

Objective: This prospective randomized double-blind study was conducted to evaluate the analgesic and adverse effects of intrathecal dexmedetomidine when added to intrathecal magnesium sulphate in patients undergoing lower abdominal surgery under bupivacaine spinal anesthesia.

Methods: Ninety adult patients classified as ASA I and II scheduled for lower abdominal surgery were randomized to one of three groups. Each patients was given 3.6ml spinal injectate that consisted of 3ml 0.5% hyperbaric bupivacaine and 0.6ml containing either, normal saline (group C), 50mg magnesium sulphate (group Mg), or 50mg magnesium sulphate and 5ug dexmedetomidine (group MgD). The onset time to reach peak sensory and motor level, the regression time for sensory and motor block, hemodynamic changes, pain score, time to rescue analgesic, level of sedation and adverse effects were recorded intraoperatively and up to 24 hours after spinal anesthesia.

Results: The onset time to reach peak sensory and motor level was shorter in group MgD as compared with the control group C (p<0.007), and it was significantly prolonged in group Mg (p<0.001). In both Mg group and MgD group when compared with group C, there was a significant prolonged time to two segment regression, sensory regression to S 1, regression to Bromage 0 and time to first rescue analgesic in addition to a significant decreased postoperative pain scores and lower postoperative analgesic requirements. The effects were greater in group MgD than in group Mg. Hemodynamic stability was maintained and other side effects were irrelevant in the three groups.

Conclusion: It was found that adding intrathecal dexmedetomidine 5ug to intrathecal magnesium sulphate 50mg, improves the quality of bupivacaine spinal anesthesia and enhances postoperative analgesia in lower abdominal surgery and there were no significant adverse effects in either of the groups.

Key Words: Bupivacaine– Dexmedetomidine– Magnesium Sulphate– Spinal anesthesia– Lower abdominal surgery.

Introduction

SPINAL anesthesia is the most commonly used technique for lower abdominal surgeries as it is very economical and easy to administer. However, postoperative pain control is a major problem because spinal anesthesia using only local anesthetics is associated with relatively short duration of action, and thus early analgesic intervention is needed in the postoperative period. A number of adjuvants, such as opioids, clonidine, midazolam and others have been studied to prolong the effect of spinal anesthesia [1,2]. The addition of opioids to local anesthetic solution have disadvantages, such as pruritis and respiratory depression.

In previous studies, it was demonstrated that intrathecally administered magnesium (Mg) prolonged spinal opioid analgesia both in rats and humans [3,4]. The addition of Mg to spinal anesthesia improved postoperative analgesia in an orthopedic setting [5,6]. The addition of intrathecal magnesium sulphate (MgSO4) to 1 0mg bupivacaine plus 25ug fentanyl prolonged spinal anesthesia in patients undergoing lower extremity surgery [6]. These effects are primarily based on the regulation of calcium influx into the cell (natural physiological calcium antagonism). Magnesium is a noncompetitive antagonist to NMDA receptors and has the potential to prevent central sensitization from peripheral nociceptive stimulation.
Dexmedetomidine, a new highly selective α2-agonist, is under evaluation as a neuraxial adjuvant as it provides stable hemodynamic conditions, good quality of intraoperative and postoperative analgesia with minimal side effects [7-9]. Kanaz et al., found that 3ug dexmedetomidine and 30ug clonidine are equipotent intrathecally when added to bupivacaine in patients undergoing urology procedures and also reduced the onset time of sensory and motor block while its use significantly prolonged the duration of sensory and motor block without serious side effects [9].

Based on previous animal [10-12], and earlier human [13-15], studies, it is hypothesized that intrathecal dexmedetomidine (5ug to 15ug) would produce more postoperative analgesic effect with hyperbaric bupivacaine in spinal anesthesia with minimal side effects. To our knowledge, the effect of adding dexmedetomidine and magnesium sulphate combined together as an adjuvant to bupivacaine spinal anesthesia have yet to be examined.

Therefore, this study was undertaken to evaluate the analgesic and adverse effects of intrathecal dexmedetomidine when added to magnesium sulphate as a combination in patients undergoing lower abdominal and lower limb surgery under bupivacaine spinal anesthesia.

Material and Methods

This prospective double-blind study was conducted in Department of Anesthesia and Intensive Care, Assiut University Hospital and South Egypt Cancer Institute from December 2011 to December 2012, after approval from the hospital ethical committee, and written informed consent was obtained from all patients. We included 90 ASA physical status I and II patients aged 18-60 years, of either sex, scheduled for lower abdominal and lower limb procedures under spinal anesthesia in this prospective randomized, double-blinded study. Patients with a history of allergy to the study drugs, uncontrolled hypertension, heart block/dysrhythmia or on therapy with adrenergic receptor antagonist, calcium channel blocker, opium addiction, sedative drugs consumption, or have contraindication for spinal anesthesia, failure of spinal block and the need for general anesthesia were excluded from the study. Patients received no premedication and upon arrival of patients into the operating room, ECG, pulse oximetry (SPO2) and noninvasive blood pressure (NIBP) were monitored. The patients were preloaded with Lactated Ringer’s solution 15ml/kg, lumber puncture was performed at the L3-L4 level in the sitting position through a midline approach using a 25G Pencil point spinal needles under complete aseptic precautions. Using computer-generated random numbers, patients were allocated into three groups: (Group C) to receive 3ml volume of 0.5% hyperbaric bupivacaine and 0.6ml of normal saline as a control group, (Group Mg) to receive 3ml volume of 0.5% hyperbaric bupivacaine and 50mg magnesium sulphate (0.5ml magnesium sulphate, ampule 1000mg in 10ml-Epico) plus 0.1ml of normal saline and (Group MgD) to receive 3ml volume of 0.5% hyperbaric bupivacaine and 50mg magnesium sulphate (0.5ml) and 5ug dexmedetomidine in 0.1ml of normal saline (dexmedetomidine 100ug/ml was diluted in preservative-free normal saline). Intrathecal injection was given over approximately 10-15 s. Patients were positioned in supine position immediately after completion of the injection and oxygen 2L/min was given through a face mask if the pulse oximeter reading decreased below 90%. Hypotension, defined as a decrease of systolic pressure by more than 30% from baseline or a fall below 90mmhg, was treated with incremental IV doses of ephedrine 5mg and IV fluid as required. Bradycardia, defined as heart rate <50 bpm, was treated with IV atropine 0.3-0.6mg. The incidence of adverse effects such as nausea, vomiting, shivering, pruritis, respiratory depression, sedation and hypotension were recorded.

The anesthesiologist performing the block was blinded to the study drug and recorded the intraoperative data. Sensory block was assessed bilaterally by using analgesia to pin prick with a short hypodermic needle in the midclavicular line. Motor block was assessed using modified Bromage scale [16], every 2min after the spinal block (0=Able to raise legs above table, 1=Inability to raise extended legs, 2=Inability to flex knees, 3=Inability to flex ankles), and the assessor observed the maximum level of sensory block. The time to reach peak sensory level and Bromage 3 motor block were recorded before surgery. The times to two dermatome sensory regression, sensory regression to S 1 dermatome, and motor block regression to Bromage 0 were recorded. All durations were calculated in relation to the time of spinal injection. Duration of pain relief was defined as the time from spinal injection to the first request for rescue analgesics or when the visual analog scale (VAS) reached 4 or more. Sedation was assessed by a modified Ramsay sedation scale [17], (1=Anxious, agitated, restless, 2=Cooperative, oriented, tranquil, 3=Responds to commands only, 4=Brisk response to light tap or loud noise, 5=Sluggish response to light glabellar tap or loud noise, 6=No response). Pain score was recorded by using (VAS) between
0 and 10 (0=No pain, 10=Most severe pain), every 2 hours postoperatively. The rescue analgesics consisted of intravenous infusion of diclofenac 75mg that could be repeated after 8h if needed when VAS reached 4 or more. Additional analgesia if needed was provided by IV fentanyl 0.5-1μg/kg and that could be repeated if needed and rescue doses of diclofenac and fentanyl were recorded. Patients were discharged from the PACU after sensory regression to S1 dermatome and Bromage 0. After 24 postoperative observation patients were discharged to home. All patients were contacted by telephone for a post-operative follow-up 4 weeks following discharge. A questionnaire aimed at assessing any new onset of back, buttock, leg pain or headache or any neurological impairment related to spinal anesthesia was conducted by a blind researcher.

Statistical analysis:

The required numbers for each group in this study were calculated using power analysis to find a significant difference of $p>0.05$ (=0.05) with a power of 80% (β error=0.05), and this analysis determined 28 patients per group is sufficient. The data collected and entered on Microsoft excel to be analyzed using the Statistical Package for Social Science (SPSS, version 16, Chicago, IL). Analysis of data between the groups was performed using one-way analysis of variance (ANOVA) followed by the Bonferroni test for post hoc analysis for parametric data or Kruskal-Wallis test for non-parametric data. Nominal non-parametric data were analyzed using Chi-Square test. Data were expressed as mean and standard deviation (SD), median and range or numbers, and significance level was considered if $p$-value was 0.05 or less.

Results

The three groups were comparable with respect to age, height, body weight, and ASA physical status. There were no significant difference in the type and duration of surgery (Table 1).

<table>
<thead>
<tr>
<th>Table (1): Demographic and clinical data.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C (n=30)</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>ASA I/II</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Types of surgery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal herniorrhaphy</td>
</tr>
<tr>
<td>Bilateral varicoceleectomy</td>
</tr>
<tr>
<td>Lower limb surgery</td>
</tr>
</tbody>
</table>

Data are presented as Mean±SD or numbers.

The onset time of both peak sensory block and motor block to Bromage 3 scale was rapid in MgD group (4.28±1.5min and 4.59±1.45min) and delayed in Mg group (6.82±1.8min and 7.55±2.1min) in comparison with the control group (5.22±1.04min and 5.55±1.6min). The difference between the groups conducted through one-way ANOVA with post test was statistically significant in both sensory and motor block ($p<0.001$). The median and range of the peak sensory level reached were T6 (T4-T9) in group C, T7 (T5-T8) in group Mg, and T6 (T3-T9) in group MgD, with no statistical differences among the groups (Table 2).

<table>
<thead>
<tr>
<th>Table (2): Onset times of peak sensory and motor block and peak sensory block level.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C (n=30)</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Sensory block (min)</td>
</tr>
<tr>
<td>Motor block (min)</td>
</tr>
<tr>
<td>Peak sensory block level</td>
</tr>
</tbody>
</table>

Data are presented as Mean±SD, or median and range.
Time to S1 regression and motor regression to Bromage 0 were significantly prolonged in group MgD (559±58min and 395±45min) and group Mg (415±45min and 295±32min) when compared with the control group (198±25min and 170±20min), and also more prolonged in MgD group when compared with Mg group. The difference between the groups conducted through one-way ANOVA with post test was statistically significant in both sensory and motor block regression ($p<0.001$) (Table 3).

**Table (3): Characteristics of sensory block, motor block and postoperative analgesia.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group C (n=30)</th>
<th>Group Mg (n=30)</th>
<th>Group MgD (n=30)</th>
<th>$p$-value C vs MG</th>
<th>$p$-value C vs MgD</th>
<th>$p$-value Mg vs MgD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time for sensory regression to S1 (min)</td>
<td>198±25</td>
<td>415±45</td>
<td>559±58</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time for motor regression to Bromage 0 (min)</td>
<td>170±20</td>
<td>295±32</td>
<td>395±45</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to rescue analgesic (hour)</td>
<td>3.2±0.25</td>
<td>8.4±1.25</td>
<td>12.5±1.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diclofenac consumption (mg)</td>
<td>175±50</td>
<td>125±50</td>
<td>75±25</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patients needs fentanyl</td>
<td>12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or number.

**Table (4): VAS score in first 24 hours postoperatively.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group C (n=30)</th>
<th>Group Mg (n=30)</th>
<th>Group MgD (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS 0</td>
<td>0.7 (0-1.5)</td>
<td>0.5 (0-1.2)</td>
<td>0.6 (0-1.3)</td>
</tr>
<tr>
<td>VAS 2</td>
<td>1.6 (1.1-2.8)</td>
<td>1.8 (1.2-2.6)</td>
<td>1.5 (1.1-2.5)</td>
</tr>
<tr>
<td>VAS 4</td>
<td>5.5 (5-6.2)*</td>
<td>2.5 (2-3.1)</td>
<td>2.0 (2-2.9)</td>
</tr>
<tr>
<td>VAS 6</td>
<td>4.2 (3.5-4.8)*</td>
<td>2.7 (2.2-3.4)</td>
<td>2.5 (2.1-3.2)</td>
</tr>
<tr>
<td>VAS 8</td>
<td>4.1 (3.5-4.9)*</td>
<td>3.2 (2.4-3.9)</td>
<td>3.1 (2.3-3.8)</td>
</tr>
<tr>
<td>VAS 10</td>
<td>3.9 (3.2-4.5)*</td>
<td>2.7 (2.2-3.6)</td>
<td>2.6 (2.1-3.3)</td>
</tr>
<tr>
<td>VAS 12</td>
<td>3.0 (2.7-3.5)*</td>
<td>2.2 (1.7-2.8)</td>
<td>1.6 (1.1-2.2)#</td>
</tr>
<tr>
<td>VAS 14</td>
<td>2.3 (1.5-3.0)</td>
<td>2.4 (1.6-3.1)</td>
<td>2.4 (1.7-3.1)</td>
</tr>
<tr>
<td>VAS 24</td>
<td>1.8 (1.4-2.2)</td>
<td>1.7 (1.2-2.2)</td>
<td>1.7 (1.1-2.3)</td>
</tr>
</tbody>
</table>

Data are presented as median(IQR).

* Significantly different from groups Mg and MgD.

# Significantly different from group Mg.

All patients could move their legs easily and reported normal sensation and were able to micturate spontaneously through the period of the study. Hypotension was mild to moderate in the three groups (systolic pressure decrease but less than 30% from base line or still more than 90 mmhg) except one patient in group Mg and 2 patients in group MgD, who had a blood pressure less than 90mmHg and they were successfully treated with a rapid intravenous fluid and 5mg ephedrine. There was an episode of nausea and vomiting in one patient in group C and group MgD and in 2 patients in group Mg, but the difference was irrelevant. No other complications were reported such as shivering, itching, sedation or respiratory depression and the SPO2 was higher than 95% in all patients in the three groups either in the intraoperative or postoperative period of the study. Twenty-four hours and 4 weeks following discharge, follow-up did not show any neurological impairment related to spinal anesthesia such as back, buttock or leg pain, post-dural puncture headache or any transient neurologic symptoms.

**Discussion**

In our study, intrathecal 5ug dexmedetomidine combined with 50mg magnesium sulphate when added to 15mg hyperbaric (3.6ml volume) bupivacaine shows significant rapid onset and prolonged duration of spinal blockade by bupivacaine for patients undergoing lower abdominal surgery. Also, the patients that received dexmedetomidine with magnesium sulphate had reduced postoperative pain scores and a longer postoperative analgesic duration with much lower analgesic requirement than those who received spinal bupivacaine with magnesium sulphate or spinal bupivacaine alone. Intrathecal dexmedetomidine when combined with...
spinal bupivacaine prolongs the sensory block by depressing the release of C-fiber transmitters and by hyperpolarization of post-synaptic dorsal horn neurons [18]. Motor block prolongation by $\beta$-2 adrenoreceptor agonists may result from binding these agonist to motor neurons in the dorsal horn of the spinal cord [19]. Intrathecal $\beta$-2 receptor agonists have antinociceptive action for both somatic and visceral pain [20]. The results of Al-Ghanem et al., [7], Al-Moustafa et al., [8] and Eid et al., [15], when they used different doses of dexmedetomidine (5ug, 10ug and 15ug, respectively) intrathecally, they found that its effect on duration of postoperative analgesia is dose dependent and the onset of sensory block to reach T 10 dermatomes was shorter with the use of dexmedetomidine. We had a similar result in our study when adding only 5ug dexmedetomidine to 50mg magnesium sulphate intrathecally to bupivacaine spinal anesthesia.

A lower dose (2.5ug) of intrathecal dexmedetomidine with 2.5mg hyperbaric bupivacaine was used previously and the patients remained hemodynamically stable [21]. Although, previous studies revealed prolongation of spinal block by intrathecal 5ug and 1 0ug dexmedetomidine with no significant effect on blood pressure or heart rate [7,13,14,22]. It has been shown that dexmedetomidine in relatively high doses can lead to hypotension when administered either neuraxially or intravenously. It is well known that intrathecal administration of local anesthetics reduce blood pressure by decreasing sympathetic outflow. However, $\alpha$-2-agonist, when co-administered with bupivacaine intrathecally, did not show a further decrease in blood pressure presumably because the blockade produced by bupivacaine is nearly maximum [23]. This may explain the observation that 150ug clonidine added to a high dose of bupivacaine (15mg or more) did not decrease blood pressure compared with bupivacaine alone [24], but when added to a small dose of bupivacaine (5mg) [7], or used alone as sole analgesic [25], resulted in a greater reduction in blood pressure in comparison to bupivacaine alone or saline, respectively.

Dexmedetomidine has been found to prolong analgesia when used as an adjuvant to local anesthetics for subarachnoid, epidural, and caudal epidural block. However, there is no proper consensus regarding the dose of drug to be used for neuraxial blocks [22]. Although Fyneface-Ogan et al., [21] in their study used only 2.5ug dexmedetomidine combined with 2.5mg hyperbaric bupivacaine and they concluded that a single shot of this combination significantly prolonged sensory block in laboring women. $\alpha$-2-agonists produce sedative effect by acting on $\alpha$-2-adrenergic receptors in locus ceruleus [26,27]. In our study the lack of increasing the sedation scores observed in group MgD is in agreement with a previous study that used 10ug intrathecal dexmedetomidine in patients undergoing transurethral resection of prostate [8]. The cause of sedation after intrathecal dexmedetomidine may be related to its systemic absorption and vascular redistribution to higher centers or cephalic migration in CSF (which are not found in our study), although delayed onset of sedation is possible, it has not been reported [28]. The lack of sedative effect reported by Strebel and colleagues with doses of clonidine as high as 150ug is likely due to its lower affinity for receptors [24]. Sedation from epidural clonidine represents an $\alpha$-2-adrenergic effect and it has been reversed by yohimbine, a relatively specific $\alpha$-2-antagonist in postoperative patients [29]. In human the largest epidural dose of dexmedetomidine used was 2ug/kg [30], and the largest intrathecal dose used was 10ug [8,14] and 15ug [15]. Although no major neurological complications have been reported so far, larger studies are required to rule out any short term or long term adverse effects.

On the otherhand in our study in Mg group, we found a delayed onset for sensory and motor block which may be explained by Ozalevli et al., who observed a similar delay in onset of spinal anesthesia when adding intrathecal Mg to fentanyl and isobaric bupivacaine (we used hyperbaric bupivacaine in our study) [6]. They suggested that the difference in PH and baricity of the solution containing Mg contributed to the delayed onset. Also in our study, there was a prolonged duration of sensory and motor block as well as the duration of postoperative analgesia, althoughless than that with the MgD group. Arcionietal, also observed that intrathecal and epidural Mg potentiated and prolonged sensory and motor block [5]. These results are consistent with a previous study conducted in patients undergoing lower extremity surgery during spinal anesthesia, in which the addition of intrathecal Mg (50mg) to 15mg bupivacaine plus 5ug fentanyl prolonged the period of spinal anesthesia [4]. The same in our results after the addition of 5ug dexmedetomidine to intrathecal Mg (50mg) and 15mg hyperbaric bupivacaine spinal anesthesia. Intrathecal Mg was used in order to increase the analgesic duration of opioids in humans, and they demonstrated that addition of 50 mgintrathecal Mg to intrathecal fentanyl led to better analgesia during painless delivery [31]. These results were comparable with those of animal
In conclusion: Intrathecal dexmedetomidine and magnesium sulphate combination improves the quality of bupivacaine spinal anesthesia and enhances the postoperative analgesia in lower abdominal and lower limb surgery with minimal adverse effects. Intrathecal Mg also prolongs the duration of spinal analgesia, but this is less than intrathecal dexmedetomidine when added to magnesium sulphate and is with a delayed onset.

This approach to pain therapy may hold the promise that favorable outcomes such as successful analgesia may be achieved without an increase in the occurrence of adverse effects such as pruritus, nausea, urinary retention and respiratory depression. An alpha-2 receptor spinal system may provide a new method of improving and reducing the spinal opioid analgesic techniques and consequently its adverse effects. Further studies are required to determine whether larger doses of intrathecal dexmedetomidine and magnesium sulphate can produce greater potentiation of analgesia and reduce opioid requirements.

References


Cardiac CT Coronary Angiography Screening of Coronary Artery Disease in Diabetic Patients Presented with Low to Intermediate Risk Chest Pain

TAMIR A. HASSAN, M.D.*; NESREEN MOHEY, M.D.*; HITAHM DAWOUD, M.D.*; MOHAMED ABDALAAL, M.D.** and ABDELFATAH ELASFAR, M.D., F.A.C.P.***
The Departments of Radiodignosis, Faculty of Medicine, Zagazig University*, Cardiology, Faculty of Medicine, Tanta University**, Egypt and Prince Salman Heart Center***, King Fahd Medical City, Riyadh, Saudi Arabia

Abstract

Background: Coronary artery disease (CAD) is the leading cause of death in patients with diabetes mellitus (DM). Early detection and management of CAD can decrease morbidity and mortality in such high risk patients.

Methods: 200 patients, 140 (70%) were males and 60 were females with mean age of 57 ±6 years presented with diabetes mellitus (DM) and have no past history of CAD. Patients presented to emergency room (ER) with low to intermediate risk chest pain. All patients were examined with CCTA. Patients with evidence of acute coronary syndrome were excluded.

Results: The study included 200 patients, 140 (70%) were males and 60 patients were females with mean age of 57 ±6 years. The average Duration of diabetes mellitus was 7.7 ±7 years. 136 patients (68%) of the study patients had significant CAD that required interventions. The distribution of CAD in the study group according to coronary artery calcium score (CACS) was as follows: 85 patients with CACS 0, 15 patients with CACS 1, 50 patients with CACS 2, 32 patients with CACS 3, and 18 patients with CACS 4.

Conclusion: About 68% of diabetic patients presented to the ER with low to intermediate risk chest pain were found to have significant CAD that required intervention. CCTA may be a suitable tool for screening of diabetic patients with chest pain presented to ER with non acute coronary syndrome symptoms aiming to reduce mortality and morbidity.

Aim of the Work: To evaluate the role of coronary cardiac CT angiography (CCTA) in detecting the incidence of coronary artery disease in diabetic patients presented to emergency room (ER) with low to intermediate risk chest pain.

Key Words: Coronary artery disease – Diabetes mellitus – Multislice computed tomography.

Introduction

DIABETES mellitus is considered a risk factor equivalent to known coronary artery disease [1]. More than 200 million people worldwide have diabetes. The estimated prevalence for 2025 surpasses the 300 million mark [2]. Therefore, the potential CAD in diabetic patients should be early evaluated and treated. Consistently, coronary angiography is used to evaluate the CAD but could not correctly determine the type of plaque. However, the multi-detector CT (MDCT) has the ability to identify the plaque [3]. The advanced 64-slice MDCT can also assess luminal narrowing accurately [4]. Early detection and management of CAD in diabetic patients will reduce mortality and morbidity in such patients. In the past ED (Emergency Department) triage based on history, serial electrocardiogram (ECG), and biomarkers alone resulted in the discharge of 2% of patients who were later diagnosed with acute coronary syndrome (ACS). Such patients have higher mortality rate [5]. In this study we use 64-MSCT as a non-invasive tool for the detection of CAD in diabetic patients presented to ER with low to intermediate risk chest pain.

Patients and Methods

During the period from November 2011 to April 2013, two hundred diabetic patients were examined with CCTA. Patients presented to ER with chest...
After CCTA, 136 patients of them were sent for PCI (percutaneous coronary intervention). Duration of diabetes mellitus was $7.7 \pm 7$ years. 130 patients (65%) were found to be hypertensive, 150 patients (75%) were having dyslipidemia and 156 patients (78%) were smokers. Blood urea nitrogen (BUN) was $13 \pm 2$ and creatinine was $1.1 \pm 3.160$ patients (80%) were treated with oral hypoglycemic drugs (OHD) while 40 patients (20%) were treated with insulin. Baseline demographic and clinical characteristics are shown in (Table 1).

**Inclusion criteria:**

Two hundred diabetic patients included in the study, 160 were males and 60 were females, aged between 40-75 years old with mean age of $57 \pm 6$ years. Diabetes was defined as having history of diabetes mellitus, and/or receiving anti-diabetic treatment, or meeting WHO criteria [6].

**Exclusion criteria:**  
- History of CAD; previous myocardial infarction, coronary artery bypass graft (CABG) or PCI.  
- Renal impairment with serum creatinine $>1.5$ mg/dl.  
- Uncontrolled arrhythmia.  
- Patients presented with chest pain if the cardiac markers were found positive or the ECG showed ischemic changes.  
- Clinical exclusion criteria for CCTA included severe allergy to iodine containing contrast material, pregnancy, a nonsinus rhythm, severe respiratory or cardiac failure, or a body mass index (BMI) greater than 40.

*All patients were subjected to:* History taking, clinical examination, ECG, testing cardiac markers and 64 MSCT.

**Technique and image analysis:** Patients are oriented at first by the steps of the exam and trained how to hold their breath and the warm sensation of contrast administration. All patients with a heart rate greater than 60 beats per minute received a β-blocker (5-15mg metoprolol, IV) unless their systolic blood pressure was lower than 100mmHg or other contraindications were present.

Selected patients underwent 64-slice CCTA with retrospective ECG gating and tube current modulation as well as ASiR (CT OPTIMA 660, GE Healthcare). At first calcium scoring was done followed by contrast study. Circulation time was determined with a timed bolus of 20ml of omnipaque 350mg Iodine/ml (Iohexol, GE health care Ireland, Cork, Ireland). Contrast enhancement was achieved using a bolus of 80ml of Iohexol followed by 40ml of normal saline injected at 5ml/s using a 18 gauge cannula at the right antecubital fossa. CCTA was acquired with detector collimation of 64 x 0.625mm at 0.625mm increments and with a gantry rotation time of 0.35s. ASiR was used between 40% and 60% in all patients and never exceeds 60%. The mean heart rate was $(62 \pm 13bpm)$ and the mean body mass index was $(28.1 \pm 5.4 \text{ kg/m}^2)$. The mean radiation dose was $(4.9\text{mSv} \pm 1.1\text{ mSv})$ with ASiR for the study group patients (Table 1).

Written consents and approval form medical ethics committee was obtained.

**CCTA Image analysis:**

Scans were analyzed independently by three experienced radiologists, one of them is SCCT verified (accredited to perform and interpret cardiac CT according to ACCF/AHA), on a dedicated workstation (AW 4.6, GE Healthcare).

After the results of the CCTA, a panel of two consultant cardiologists and the three radiologists reviewed the data forms and all medical records pertaining to the hospital admission of enrolled patients. Disagreement was solved by consensus.

We analyzed plaque characteristics on a per-segment basis according to the modified American Heart Association classification [7]. Image quality was evaluated on a per-segment basis and classified as good (no artifact), adequate (defined as the presence of image degrading artifacts but feasible for evaluation with a moderate confidence), or poor (the presence of image-degrading artifacts and feasible for evaluation only with a low confidence). If the Image was adequate or good quality, coronary segments were visually scored for the grading of coronary artery stenosis. The severity of diameter stenosis was graded as normal appearing (0-24%), mild (25-49%), moderate (50-74%), and severe ≥75% [8], respectively. Plaques were defined as structures $>1\text{mm}^2$ within and/or adjacent to the vessel lumen, which could be clearly distinguished from the lumen and surrounding pericardial tissue. Plaques in which calcified tissue represented more than 50% of the plaque area (density $>130 \text{HU}$ in native scans) were classified as calcified, plaques with $<50\%$ calcium as mixed, and plaques without any calcium were classified as non-calcified lesions.
Coronary artery calcium scores (CACS) were measured using the scoring system previously described by Agatston et al. [10].

Participants, based on the CACS, were categorized in the following manner: no: 0, mild: 0.1 -100, moderate: 100.1-400, and severe calcification: >400.

Statistical analysis:
Continuous variables are expressed as Means± Standard deviation (S.D.). Categorical variables are expressed as absolute values and percentage. Difference between continuous were analyzed using student t-test. Difference between categorical variables were analyzed using chi-square test. A p-value of <0.05 is considered statistically significant. All tests were done using SPSS statistical package.

Results
The study included 200 patients, 140 patients (70%) were males. The mean age was 57 ±6 years. All patients underwent CCTA that revealed 136 patients (68%) had significant CAD that required intervention (Table 3). Of them 82 patients (60%) were male. Patients were sent to coronary angiography for confirmation of the result and intervention; either PCI or CABG or medical treatment if patient is not suitable for any of them. Coronary artery disease was classified as obstructive if the degree of stenosis is ≥50% of the lumen of a coronary artery and non obstructive if the stenosis is <50% of the lumen. In 64 patients the degree of stenosis was found to be <50% (Table 3, Fig. 2). In 100 patients the degree of stenosis was found to be from 50 to 74%. In the other 36 patients the degree of stenosis was from 75 to 99% (Table 3, Fig. 1). Sixty eight patients (50%) received medical treatment, 10 patients (7%) treated with CABG and 58 patients (42%) treated with PCI. Total number of diseased segments with ≥50% stenosis were found to be 297 with mean number of diseased segments per patient was 2.18.

The distribution of CAD in the study group according to CACS (Fig. 1) was as follows: The number of patients with CACS 0 was 85, of them only 3 patients had significant CAD. 15 patients with CACS 1, of them only one patients has significant CAD. 50 patients with CACS 2, of them 10 patients had significant CAD. 32 patients with CACS 3, of them only 9 had significant CAD. 18 patients with CACS 4, 10 patients of them had significant CAD as shown in (Table 2).

The mean radiation dose was (4.9mSv ±1.1 mSv) with the application of maximum 60% ASiR.
Fig. (1): A 53 year old male patient with atypical chest pain. (A) Shows large ostial LAD hypodense plaque (arrow) causing 75% stenosis with multiple atheromatous calcifications at the proximal third of the LAD, (B) Tree volume rendering (VR) image shows the ostial stenosis (arrow).

Fig. (2): A 50 year old male patient with atypical chest pain. (A) Small hypodense plaque at proximal RCA causing lumen stenosis about 40%. (B) Tree VR shows the stenotic area of the RCA.

Discussion

The study included 200 patients, 140 patients (70%) were males. The mean age was $57 \pm 6$ years in agreement with [11].

To our knowledge, few studies were done regarding the incidence of ischemic heart disease in diabetic patients presented with chest pain using CCTA. In this study, 200 diabetic patients presented with chest pain were examined with CCTA, 136 patients (68%) showed significant CAD that required intervention. The study also showed that 50 patients had calcium score $\geq 3$. The increase in calcium score increases the incidence of significant CAD in agreement with [12]. In the [12] study, the incidence of significant CAD was 26% which is lower than the incidence of our study. This may be explained by the selected patients in current study presented with chest pain while the former study examined asymptomatic patients. They found that the vast majority of patients with a very high CACS (>400) had significant CAD which is in agreement with our results. Chu Z. et al., [13] did comparative analysis of CAD assessed by 64-MSCT between patients with type 2 DM and non diabetic patients. They found that diabetic patients
had more diseased coronary vessels and segments than non-diabetic patients (2.6 ± 1.1 vs 2.0 ± 1.0). This is different from our results as we found the mean number of diseased segments per patient was 2.18. This may be due to that our patients are better controlled than their patients as glycosylated hemoglobin level in our patients was 7.1 ± 1.2 where it was 7.7 ± 2.3 in their study. Scholte AJ, et al., [14] studied the different manifestations of CAD by stress SPECT myocardial perfusion imaging. CACS and CCTA in asymptomatic patients with type 2 DM, again they found the incidence of significant CAD in patients with DM to be 24% which was lower than the incidence of significant CAD in our study. This may be due to we studied patients with chest pain, where they studied asymptomatic patients. Peled et al., [15] stated that the incidence of multivessel CAD was found to be 54.7% which is slightly different from our results.

The mean radiation dose was 4.9 mSv ± 1.1 mSv with the application of ASiR to offer the CCTA with lowest radiation possible. We never used more than 60% ASiR to overcome plastic quality images in agreement with [11,16].

Conclusion:

About 68% of diabetic patients presented to the emergency room (ER) with low to intermediate risk chest pain were found to have significant CAD that required intervention. CCTA may be a suitable tool for screening of diabetic patients with chest pain presented to ER with non acute coronary syndrome symptoms aiming to reduce mortality and morbidity.

References

Factors Predicting Fulminant Course of Acute Hepatitis A with Special Emphasis on Predictors of Mortality in Egyptian Children

WESAM S. MORAD, M.D.*; ALIF A. ALLAM, M.D.** and YASSER KAMAL, M.D.**
The Departments of Community Medicine & Public Health* and Pediatric Hepatology**, National Liver Institute, Menoufiya University

Abstract

Background/Aims: Hepatitis A virus (HAV), a non enveloped RNA virus, is particularly resistant and contagious. The infection is spread chiefly by feco-oral transmission and is a public health problem throughout the world. The main complication of HAV infection is fulminant hepatitis (FH).

Methods: This study was done on 80 children aged from 1 to 17 years, 50 with acute hepatitis A virus and 30 acute hepatitis A who developed fulminant hepatic failure (FHF), 52 males and 28 females. In fulminant group 12 recovered with normal liver function, but 18 were died (case fatality 18/80 22.5%). Children recovered from fulminant liver failure had encephalopathy grade 1 or 2.

Results: The study showed statistically significant differences between acute hepatitis A virus and low socioeconomic level and bad hygiene (p<0.004), children received anti convulsive therapy p<0.009 and also with diabetic children p<0.004. Total bilirubin >9.56, Direct bilirubin >5.1 1, ALT >1365.7, AST >1635.78, prothrombin time prolonged more than 25.87 seconds are indices for increasing the risk for developing fulminant hepatic failure in children with acute HAV (p<0.000001, p<0.00001, p<0.00001, p<0.0001, p<0.00001 respectively). Mortality rates was statistically significant related to prolonged prothrombin time, decreased ALT and AST, elevation of serum bilirubin and blood urea and serum creatinine and also with high grade of coma (grade 3 and 4).

Conclusion: This study emphasize that early detection of FHF can be detected by simple tests and appropriate medical treatment could block further liver destruction and prevent development of FHF.


Introduction

HEPATITIS A virus (HAV), a non enveloped RNA virus, is particularly resistant and contagious. The infection is spread chiefly by feco-oral transmission and is a public health problem throughout the world. The main complication of HAV infection is fulminant hepatitis (FH) i.e., acute liver failure with encephalopathy, which occurs in less than 1% of cases [1].

In children, hepatitis A virus is considered to be a mild or even inapparent disease in most instances and the proportion of symptomatic infections increases with age [2].

Rationale of study: However the overall mortality rate from acute hepatitis A is estimated at 0.2% to 0.4% of symptomatic cases and age specific rates indicates that mortality is higher in patients 50 years of age and older and in those younger than 5 years of age. Moreover, fulminant liver failure, which accounts for approximately 1 1% to 13% of liver transplantation performed in children in the United States, is related to HAV infection in approximately 10% of the cases [3].

Patients and Methods

This is a case control study which was conducted on children aged from 1 to 17 years, patients from different governorates in Egypt treated in outpatient and inpatient clinic of Pediatric Hepatology Department-National Liver Institute (NLI from May 2011 May 2012) as a referral center to highlight the current risk factors of hepatitis “A” infection with special emphasis on fulminant hepatitis “A” risk factors and its prognosis. Eighty children (50 with acute hepatitis A virus and 30 with fulminant hepatitis as defined before).
Diagnosis of hepatitis A was established by detection of immunoglobulin M anti HAV antibodies in the serum samples of all children; no children had evidence of pre-existing liver disease (Detected by enzyme linked immunosorbent assay technique (ELISA)).

**Principle:** Kits from Dia Sorin Co.

Diagnosis of fulminant hepatic failure A was established by careful medical history including nutritional status, drugs, socioeconomic state and history of chronic diseases, also through clinical examination, complete blood count (CBC), liver function tests including ALT, AST, Total bilirubin, direct bilirubin, prothrombin time and concentration, serum albumin, alkaline phosphatase, gamma glutamyl transpeptidase, kidney function tests including urea and creatinine and blood sugar were performed in addition to the routine investigations for the care of FHF patients.

Infection with other viruses (hepatitis B virus, hepatitis C virus, Epstein Barr virus, cytomegalovirus) and also autoimmune hepatitis and Wilson's disease were excluded by histopathology and laboratory investigations. Doppler ultrasound on the portal vein and hepatic artery was performed to show histopathological changes.

**Signs of liver failure, defined as summation of clinical and biochemical parameters as follow:**

1- The acute onset of liver disease with no known evidence of chronic liver disease.

2- Biochemical and/or clinical evidence of severe liver dysfunction: Hepatic based coagulopathy (PT $\approx 20$ seconds or INR $\approx 2.0$) that is not corrected by parenteral vitamin K and or hepatic encephalopathy (must be present if PT is 15-19.9 seconds or INR is 1.5-1.9 but not if the PT is $\approx 20$ or INR $\approx 2.0$). This definition of acute liver failure by the Pediatric acute liver failure (PALF) study group.

All children who developed encephalopathy were treated in the pediatric intensive care unit—National Liver Institute. The standard classification of encephalopathy was adapted to children according to the following grades of severity: grade 1: Child is confused and has mood changes; grade 2: Child is drowsy and displays inappropriate behavior; grade 3: Child is stuporous but obeys simple commands; grade 4a: Child is comatose but arousal by painful stimuli or 4b: Child is in deep coma and does not respond to any stimuli.

**Ethical points:**

During the interview, the respondent of the children was simply informed about the aims of this study and the fact that it is done to improve the health status of all population. Written consent was taken from the respondent who accompanied the child during attending the mentioned hospitals before participating in the research.

**Sample size:**

80 children (50 with acute hepatitis A virus and 30 with fulminant hepatitis) were recruited based on the following assumptions: $x=0.05$, probability of exposure in controls=91.0%, power=80.0%, ratio of cases to controls=1:1, and Odds ratio of exposure in hepatitis A cases relative to controls =29.0%. The required sample size was determined using PS (power and sample size calculation) software.

**Statistical analysis:**

Statistical analyses were performed using SPSS (SPSS, Inc., Chicago, Illinois), Epi Info (CDC, Atlanta, Georgia), and Log Xact (Cytel Software Corporation, Cambridge, Massachusetts). Differences between hepatitis A and fulminant hepatitis A regarding dichotomous variables were assessed with the chi-square statistic. Chi-square for linear trend was used for associations between categorical variables and hepatitis A status. When the expected number in any cell was less than five, a two-tailed Fisher's exact test was used. Odds ratio (OR) was used to calculate the risk of exposure to these risk factors long with 95% confidence interval and both were done to measure the strength of association.

When comparing two-sample means, Student's $t$-test was used for normally distributed variables.

**Results**

Among 80 children aged from 1 to 17 years, 50 with acute hepatitis A virus and 30 acute hepatitis A who developed fulminant hepatic failure, 52 males and 28 females. Only 31 were on breast feeding and 49 were on artificial feeding when infants. Children lived in bad hygiene were 40, other lived in good hygiene were also 40. Those lived in low socioeconomic level were 52 and the other 28 lived in moderate or high socioeconomic level according to social scale classification. Diabetes mellitus was found in 14 children and 17 children under anti-convulsive therapy. In fulminant group 12 recovered with normal liver function but 18 were died. Children recovered from fulminant liver failure had encephalopathy grade 1 or 2.
Anicteric hepatitis was founded in 12 children with acute hepatitis group, presented with abdominal pain, fatigability, loss of appetite and hepatomegaly, confirmed by positive Anti HAV IgM and elevated ALT and AST. The other 68 presented with icteric hepatitis and hepatomegaly and only 15 with splenomegaly. In fulminant group only 2 were anicteric and hepatomegaly were founded in 15 (all of the recovered) plus 3 of the died but the remaining who died had not hepatomegaly. Ascites was found in only 2 with acute hepatitis and found in 15 of the fulminant group.

In acute hepatitis group 49 returned to normal liver function in a period ranging from 28-100 days and only one developed autoimmune hepatitis several months later. Non of the acute hepatitis group and those who recovered from fulminant hepatic failure showed any changes of the kidney function but all of the children died from fulminant hepatic failure showed impaired kidney function during the course of management.

The study showed statistically significant differences between acute hepatitis A virus and low socioeconomic level and bad hygiene ($p<0.004$). There was statistically significant differences regarding the development of fulminant hepatic failure and previous factors (socioeconomic level) ($p<0.008$) and bad hygiene ($p<0.05$).

The study showed statistically significant differences between development of fulminant hepatic failure and children received anti convulsive therapy $p<0.009$ and also with diabetic children $p<0.004$.

Total bilirubin > 10.26, direct bilirubin >6.21, ALT >1365.7, AST >1635.78, prothrombin time prolonged more than 27.32 seconds are indices for increasing the risk for developing fulminant hepatic failure in children with acute HAV ($p<0.000001$, $p<0.000001$, $p<0.000001$, $p<0.000001$, $p<0.000001$, $p<0.000001$, $p<0.000001$, $p<0.000001$. Also, portal vein diameter <6.97 and hepatic artery resistive index <0.64 are indices for development of fulminant hepatic failure in children with acute HAV.

### Table 1: Study risk factors of fulminant hepatitis infection.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Groups</th>
<th>Fulminant hepatitis (F.H.)</th>
<th>Hepatitis “A” cases (H.A)</th>
<th>Odds ratio</th>
<th>Confidence interval (C.I.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of the child:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 year: 5 years</td>
<td></td>
<td>20</td>
<td>36</td>
<td>0.78</td>
<td>0.26-2.31</td>
<td>0.61</td>
</tr>
<tr>
<td>5 years: &gt;10 years</td>
<td></td>
<td>10</td>
<td>14</td>
<td>0.33</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td><strong>Sex of the child:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>22</td>
<td>30</td>
<td>0.55</td>
<td>0.18-1.62</td>
<td>0.23</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>8</td>
<td>20</td>
<td>0.26</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td><strong>Socioeconomic level:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>25</td>
<td>27</td>
<td>4.26</td>
<td>1.27-15.15</td>
<td>0.008*</td>
</tr>
<tr>
<td>Moderate and high</td>
<td></td>
<td>5</td>
<td>23</td>
<td>16.7</td>
<td>46.0</td>
<td></td>
</tr>
<tr>
<td><strong>Personal hygiene:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad hygiene</td>
<td></td>
<td>28</td>
<td>12</td>
<td>44.3</td>
<td>8.27-316.6</td>
<td>0.05*</td>
</tr>
<tr>
<td>Good hygiene</td>
<td></td>
<td>2</td>
<td>38</td>
<td>76.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Present history of infantile diabetes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>10</td>
<td>4</td>
<td>5.75</td>
<td>1.42-25.12</td>
<td>0.004*</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>20</td>
<td>46</td>
<td>92.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of diuretics intake:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>6</td>
<td>1</td>
<td>12.25</td>
<td>1.3-285.75</td>
<td>0.01*</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>24</td>
<td>49</td>
<td>98.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of anti convulsants:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>11</td>
<td>6</td>
<td>4.25</td>
<td>1.21-15.38</td>
<td>0.009*</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>19</td>
<td>44</td>
<td>88.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (2): Mean and one standard deviation of studied laboratory variables of hepatitis “A” cases (a cutoff point) as an index for risk of developing fulminant hepatitis.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Groups</th>
<th>Fulminant hepatitis (F.H.)</th>
<th>Hepatitis “A” cases (H.A)</th>
<th>Range (F.H.) (H.A)</th>
<th>Mean±SD (F.H.) (H.A)</th>
<th>Odds ratio</th>
<th>Confidence interval (C.I.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>Mean</td>
<td>±SD</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin:</td>
<td>≥10.26</td>
<td>28</td>
<td>93.3</td>
<td>13</td>
<td>26.0</td>
<td>9.1-28.64</td>
<td>19.9±4.6</td>
<td>39.85</td>
</tr>
<tr>
<td></td>
<td>&lt;10.26</td>
<td>2</td>
<td>6.7</td>
<td>37</td>
<td>74.0</td>
<td>0.6-14.2</td>
<td>7.17±2.39</td>
<td></td>
</tr>
<tr>
<td>Direct bilirubin:</td>
<td>≥6.21</td>
<td>27</td>
<td>90.0</td>
<td>8</td>
<td>16.0</td>
<td>4.6-19.64</td>
<td>12.7±4.22</td>
<td>47.25</td>
</tr>
<tr>
<td></td>
<td>&lt;6.21</td>
<td>3</td>
<td>10.0</td>
<td>42</td>
<td>84.0</td>
<td>0.15-8.2</td>
<td>3.89±1.22</td>
<td></td>
</tr>
<tr>
<td>AST:</td>
<td>≥1635.78</td>
<td>24</td>
<td>80.0</td>
<td>7</td>
<td>14.0</td>
<td>406-1450</td>
<td>1817.5±225</td>
<td>24.57</td>
</tr>
<tr>
<td></td>
<td>&lt;1635.78</td>
<td>6</td>
<td>20.0</td>
<td>43</td>
<td>86.0</td>
<td>18.1-740</td>
<td>1478±157.8</td>
<td></td>
</tr>
<tr>
<td>ALT:</td>
<td>≥1365.78</td>
<td>26</td>
<td>86.7</td>
<td>6</td>
<td>12.0</td>
<td>332.3 1988</td>
<td>1832±276.6</td>
<td>47.67</td>
</tr>
<tr>
<td></td>
<td>&lt;1365.78</td>
<td>4</td>
<td>13.3</td>
<td>44</td>
<td>88.0</td>
<td>116.2-667.3</td>
<td>1274.2±91.5</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Mean and one standard deviation of studied variables of hepatitis “A” cases (a cutoff point) as an index for risk of developing fulminant hepatitis.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Groups</th>
<th>Fulminant hepatitis (F.H.)</th>
<th>Hepatitis “A” cases (H.A)</th>
<th>Range (F.H.) (H.A)</th>
<th>Mean±SD (F.H.) (H.A)</th>
<th>Odds ratio</th>
<th>Confidence interval (C.I.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>Mean</td>
<td>±SD</td>
<td></td>
</tr>
<tr>
<td>Prothrombin time:</td>
<td>≥27.32 sec</td>
<td>27</td>
<td>90.0</td>
<td>8</td>
<td>16.0</td>
<td>13-79.6</td>
<td>51.53±18.17</td>
<td>47.25</td>
</tr>
<tr>
<td></td>
<td>&lt;27.32 sec</td>
<td>3</td>
<td>10.0</td>
<td>42</td>
<td>84.0</td>
<td>12-45</td>
<td>18.57±7.3</td>
<td></td>
</tr>
<tr>
<td>Prothrombin conc:</td>
<td>&lt;56.14%</td>
<td>28</td>
<td>93.3</td>
<td>9</td>
<td>18.0</td>
<td>12.35-98</td>
<td>23.67±8</td>
<td>63.78</td>
</tr>
<tr>
<td></td>
<td>&gt;56.14%</td>
<td>2</td>
<td>6.7</td>
<td>41</td>
<td>82.0</td>
<td>24-100</td>
<td>63.6±22.9</td>
<td></td>
</tr>
<tr>
<td>Blood sugar:</td>
<td>(R. B.S. &gt;1 76ng/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>10</td>
<td>33.3</td>
<td>4</td>
<td>8.0</td>
<td>5.75</td>
<td>1.42-25.12</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>20</td>
<td>66.7</td>
<td>46</td>
<td>92.0</td>
<td>4.69</td>
<td>1.42-25.12</td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Mean and one standard deviation of hepatitis “A” cases (cutoff point) as an index for risk of developing fulminant hepatitis.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Groups</th>
<th>Fulminant hepatitis (F.H.)</th>
<th>Hepatitis “A” cases (H.A)</th>
<th>Range (F.H.) (H.A)</th>
<th>Mean±SD (F.H.) (H.A)</th>
<th>Odds ratio</th>
<th>Confidence interval (C.I.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>Mean</td>
<td>±SD</td>
<td></td>
</tr>
<tr>
<td>Portal vein diameter:</td>
<td>≤6.97</td>
<td>9</td>
<td>60.0</td>
<td>4</td>
<td>26.7</td>
<td>4.1-8.4</td>
<td>6.4±1.28</td>
<td>4.13</td>
</tr>
<tr>
<td></td>
<td>&gt;6.97</td>
<td>6</td>
<td>40.0</td>
<td>11</td>
<td>73.3</td>
<td>6.5-8.6</td>
<td>7.39±0.72</td>
<td></td>
</tr>
<tr>
<td>Hepatic artery resistive index:</td>
<td>≤0.64</td>
<td>11</td>
<td>73.3</td>
<td>3</td>
<td>20.0</td>
<td>0.58-0.66</td>
<td>0.62±0.02</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>&gt;0.64</td>
<td>4</td>
<td>26.7</td>
<td>12</td>
<td>80.0</td>
<td>0.62-0.76</td>
<td>0.68±0.05</td>
<td></td>
</tr>
</tbody>
</table>
Table (5): Study risk factors for mortality of fulminant hepatitis “A” cases.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Fulminant Hepatitis</th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Died (18)</td>
<td>Survived (12)</td>
<td>(O.R.)</td>
<td>(C.I.)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td><strong>Encephalopathy grade:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade III and IV</td>
<td>16</td>
<td>88.9</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>Grade I and II</td>
<td>2</td>
<td>11.1</td>
<td>11</td>
<td>91.7</td>
</tr>
<tr>
<td><strong>Ascitis:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>17</td>
<td>94.4</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
<td>5.6</td>
<td>10</td>
<td>83.3</td>
</tr>
</tbody>
</table>

Table (6): Liver functions as predictors of mortality in fulminant hepatitis “A” cases.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Fulminant Hepatitis</th>
<th>Range (F.H.) (H.A)</th>
<th>Mean±SD (F.H.) (H.A)</th>
<th>Odds ratio</th>
<th>Confidence interval (C.I.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Died (18)</td>
<td>Survived (12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total bilirubin:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥37.54</td>
<td>14</td>
<td>77.8</td>
<td>1</td>
<td>8.3</td>
<td>29.7-98.6</td>
<td>49.9±24.3</td>
</tr>
<tr>
<td>&lt;37.54</td>
<td>4</td>
<td>22.2</td>
<td>11</td>
<td>91.7</td>
<td>16.3-26.9</td>
<td>27.24±9.64</td>
</tr>
<tr>
<td><strong>Direct bilirubin:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥13.87</td>
<td>17</td>
<td>94.4</td>
<td>4</td>
<td>33.3</td>
<td>14.6-19.64</td>
<td>12.7±4.22</td>
</tr>
<tr>
<td>&lt;13.87</td>
<td>1</td>
<td>5.6</td>
<td>8</td>
<td>66.7</td>
<td>2.4-10.2</td>
<td>3.89±1.22</td>
</tr>
<tr>
<td><strong>AST:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3239.43</td>
<td>16</td>
<td>88.9</td>
<td>2</td>
<td>16.7</td>
<td>1406-4450</td>
<td>1817.5±4225</td>
</tr>
<tr>
<td>&lt;3239.43</td>
<td>2</td>
<td>11.1</td>
<td>10</td>
<td>83.3</td>
<td>818.1-1740</td>
<td>678±1157.8</td>
</tr>
<tr>
<td><strong>ALT:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2968.42</td>
<td>15</td>
<td>83.3</td>
<td>3</td>
<td>25.0</td>
<td>332.3-1988</td>
<td>1832±4276.6</td>
</tr>
<tr>
<td>&lt;2968.42</td>
<td>16</td>
<td>16.7</td>
<td>9</td>
<td>75.0</td>
<td>116.2-667.3</td>
<td>674.3±1291.5</td>
</tr>
<tr>
<td><strong>Prothrombin time:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥34.82 sec</td>
<td>17</td>
<td>94.4</td>
<td>1</td>
<td>8.3</td>
<td>33-79.6</td>
<td>51.53±18.17</td>
</tr>
<tr>
<td>&lt;34.82 sec</td>
<td>1</td>
<td>5.6</td>
<td>11</td>
<td>91.7</td>
<td>22-45</td>
<td>18.57±7.3</td>
</tr>
<tr>
<td><strong>Prothrombin conc:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30.72%</td>
<td>16</td>
<td>88.9</td>
<td>1</td>
<td>8.3</td>
<td>12.35-98</td>
<td>23.67±8.1</td>
</tr>
<tr>
<td>&gt;30.72%</td>
<td>2</td>
<td>11.1</td>
<td>11</td>
<td>91.7</td>
<td>24-100</td>
<td>63.6±22.9</td>
</tr>
</tbody>
</table>

Discussion

This study was undertaken to characterize the factors predicting fulminant course of acute HAV and the predictors of mortality in fulminant HAV in children from Egypt. Hepatitis A is the common cause of acute hepatitis in children and can produce significant morbidity.

In this study, only 12 children had anicteric hepatitis, this may be due to that anicteric cases passed unnoticed.

This study showed statistically significant differences between acute hepatitis A virus and low socioeconomic level and bad hygiene, this finding in agreement with most studies and also with I.I. Salama et al., [4] who demonstrated that seropositivity to anti HAV antibodies was significantly higher among children with low or very low socioeconomic standard.

Bilirubin level >10.26 was associated with developing fulminant hepatic failure in children with acute HAV in our study, this in agreement with Makoto Y. et al., [5] also found high serum bilirubin is a predictive of the development of FHF in acute viral hepatitis, but in difference with Guilhermo R. et al., [6] who found high bilirubin level were independently associated with both low factor V levels and fulminant hepatitis and also with death or transplantation, this difference may be due to the different age between the two studies.

Also, higher bilirubin level was statistically significant related to patients died from fulminant
HAV, this in agreement with Sema et al., [7] who founded total and indirect bilirubin levels significantly higher in patients who died from fulminant hepatic failure in Turkish children.

This study showed that prothrombin time $>27.32$ seconds was associated with increased risk of developing fulminant hepatic failure in children with acute HAV and also prolonged prothrombin time when associated with decreased ALT and AST, elevation of serum bilirubin, blood urea, serum creatinine and high grade of coma were associated with high mortality. Similar data was reported by Uzma et al., [8] who reported that, the prothrombin time was the most significant predictor of survival. They reported a significant difference between those who survived and those who died on discriminate analysis with respect to age, grade of hepatic encephalopathy, duration of hospitalization, prothrombin time, and duration of jaundice.

The study showed statistically significant differences between development of fulminant hepatic failure and children received anti convulsive therapy this may be due the combined effect of HAV and the hepatotoxic effect of anticonvulsant.

Diabetic children when infected with HAV are at risk of develop FHF, Vesely et al., [9], studied case with hepatitis A that case illustrates that hepatitis A infection may be severe with liver failure, acute renal failure, and permanent diabetes mellitus as a sequel of this infection.

Portal vein diameter $<6.97$ and Hepatic artery resistive index $<0.64$ are indices for development of fulminant hepatic failure in children with acute HAV in our study, Tanaka K. et al., [10] found that the mean resistive index of the hepatic arteries in patients who developed fulminant hepatic failure was significantly larger than that of patients who recovered without developing fulminant hepatic failure ($p<0.01$). When a resistive index cutoff level of 0.74 was used, 84% sensitivity and 94% specificity were obtained for the prediction of fulminant hepatic failure. An elevated resistive index of the hepatic artery may be useful for predicting the patient's clinical outcome and determining the need for a liver transplantation in patients with acute viral hepatitis.

Increasing bilirubin and decreasing ALT, increasing creatinine are associated with poor prognosis of fulminant HAV, this in agreement with Ryan M. et al., [11] they found high serum creatinine, lower ALT and lower alkaline phosphatase are associated with transplant or death. These results may reflect severe necrosis.

Decreasing albumin level, prolonged prothrombin time, presence of ascites and higher grades of encephalopathy are associated with high mortality among fulminant group, this in harmony with Poddar et al., [12] who reported that Total serum bilirubin and grade of encephalopathy were significantly higher, serum albumin was significantly lower, and prothrombin time was significantly prolonged in those who died than the recovered.

Conclusion:
A major benefit of this study is that early detection of FHF can be detected by simple tests and appropriate medical treatment could block further liver destruction and prevent development of FHF. Also, the importance of proper health education and of HAV vaccination could prevent the occurrence of HAV infection in children and its subsequent complications (FHF).

Acknowledgments:
This study was supported by cooperation of all children and their respondents’ who responded to study questionnaire and supplied author with the needed data that were included in this work.

References
7- SEMA AYDOGU, FUNDAY OZGENC, SERAP YURTSCVER, SOZIN ASIK AKMAN, YAMAN TOKAT and RASIT VURAL YAGCI: “Our Experience with Fulmi-


Evaluation of Interleukin-8 and HCV RNA in Chronic Hepatitis C Patients as Predictors of Response to Pegylated Interferon/Ribavirin Therapy at 12 and 24 Weeks

ADEL A. HASSAN, M.D. 1; NADER A. NEMR, M.D. 1; KHALIL A. KHALIL, M.D. 2; AMANY M. HASSEN, M.D. 3; WAHEED F. HESSAM, M.D. 4 and TAMER M. ATTIA, M.Sc. 2

The Departments of Endemic & Infectious Diseases 1, Internal Medicine 2, Clinical & Chemical Pathology 3 and Microbiology & Immunology 4, Faculty of Medicine, Suez Canal University, Egypt

Abstract

Background: Numerous studies suggest that HCV-induced changes in levels of chemokine and cytokine expression may be involved in HCV antiviral resistance, persistence, and pathogenesis. Also found that the HCV NS5A protein induces expression of the proinflammatory chemokine IL-8 to partially inhibit the antiviral actions of IFN in vitro.

Aim: To investigate the impact of serum levels of interleukin-8 in patients with chronic hepatitis C viral infection and baseline HCV RNA levels as predictors for response to Interferon/Ribavirin therapy in patients with chronic HCV infection in the centre for treatment of HCV in Ismailia Fever Hospital-East Egypt.

Methods: This descriptive study was conducted on 42 patients to determine the correlation between levels of interleukin-8 in the serum of patients with chronic hepatitis C viral infection and baseline HCV RNA and response to pegylated Interferon/Ribavirin after 12 and 24 weeks of therapy. All investigations and follow-up had been carried out in the centre according to the national program for treatment of chronic hepatitis C virus.

Results: Among the total studied 42 patients the mean age was 41.5 years old. About half of the studied patients were males (52.4%) and more than half of the patients were from rural areas (61.9%).

A 73.8% of the studied patients were responders to interferon therapy while 26.2% were non responders. Seven (16.7%) of these non responders have shown positive PCR and discontinue treatment at 12 weeks while the remaining 4 patients (9.5%) were recorded as non responders at 24 weeks of therapy. There was no statistically significant difference between responders and non responders regarding age, sex and residence. Responders have significantly lower hemoglobin values, FBS, α-fetoprotein and baseline PCR than non responders. More than half of the whole studied patients have IL-8 value 2-6 (mid range elevation) (64.3%) while 35.7% shows high level of IL-8 (>14). Responders have lower mean of IL-8 with statistically significant difference than non responders (7.9 versus 13.3). Most of responders (74.2%) have IL-8 value ranging from 2-6 while most of non responders (63.6%) have high level of IL-8 (>14). Patients with IL-8 (2-6) are less likely to be non responder with RR=0.3 (95% CI: 0.1-0.9) while patients with IL-8 >14 are at higher risk of being non responder with RR=3.1 (95% CI: 1.09-9.03). This shows the predictive characteristics of low levels of IL-8 pretreatment and low baseline HCV RNA in prediction of response to interferon/ribavirin therapy at 12 and 24 weeks respectively.

Conclusion: Most of responders have low levels of pretreatment IL-8, while most of non responders have high levels of IL-8, and also Low pretreatment levels of HCV RNA were predictive to response.

Key Words: HCV – RNA – Chronic hepatitis C – Pegylated interferon/ribavirin therapy.

Introduction

HEPATITIS C virus (HCV) infection is a major cause of liver diseases and liver cancer [1-3]. Among the six genotypes of HCV, the most common genotypes of HCV in the United States are genotype 1 (approximately 75%), genotype 2 (15%), and genotype 3 (7%) [2-4]. Egypt has the largest epidemic of hepatitis C virus (HCV) in the world. The recently released Egyptian Demographic Health Survey tested a representative sample of the entire country for HCV antibody. The sample included both urban and rural populations and included all governorates of Egypt. Over 11,000 individuals were tested. The overall prevalence (percentage of people) positive for antibody to HCV was 14.7% [8].
Among the six major HCV genotypes found worldwide, genotype 4 is the most predominant in Egypt, with 4a as the dominant subtype [6].

At present, the standard treatment for chronic HCV genotype 4 infections is 48 weeks with a combination of pegylated interferon alfa-2a or alfa-2b plus ribavirin. Outcomes are measured by sustained viral response (SVR), defined as an undetectable viral load 24 weeks after the end of therapy. HCV genotype 1 has been reported to have a 54-56% SVR [2,7-11]. Prior studies have shown better response rates with genotype 2 or 3 with a 24 week-course of therapy [2,10,11]. Response rates have been found higher in Caucasians (52%) compared to African-Americans (28%) [2]. Response rates are reported lower with initial levels of HCV RNA > 600,000 IU/ml, male gender, high body weight, and advanced liver fibrosis [12-18], also shown a poorer response to treatment associated independently with HCV genotype 1 infection but a better response with weight loss [19].

Numerous studies suggest that HCV-induced changes in levels of chemokine and cytokine expression may be involved in HCV antiviral resistance, persistence, and pathogenesis. Also found that the HCV NS5A protein induces expression of the proinflammatory chemokine IL-8 to partially inhibit the antiviral actions of IFN in vitro [20].

NS5A induction of IL-8 expression is associated with inhibition of the antiviral actions of IFN in vitro [21]. This may represent a distinct mechanism by which the NS5A protein circumvents the IFN-induced antiviral response. It was also demonstrated that the HCV core protein could also transactivate the IL-8 promoter [22].

Interferon (IFN) and the guanosine analogue ribavirin are widely used treatments for chronic HCV infection. However, 60% of patients with high-titer HCV genotype 1 infections remain non-responsive to combination therapy [20].

The high prevalence of hepatitis C, and the need to understand its epidemiology, warrants global surveillance of the disease in order to determine specific health care measures for disease prevention and control. Several studies have been carried out to identify factors that facilitate identification of chronic hepatitis C patients who are likely to respond to antiviral treatment. So in this study we investigated pretreatment levels of both IL-8 and HCV RNA as predictors for response to interferon/ribavirin therapy in patients with chronic HCV infection.

### Material and Methods

#### Study population:

This prospective descriptive study enrolled 42 patients with chronic HCV infection suitable for interferon/ribavirin therapy, both genders and all age groups above 18 years old attending to the center for treatment of chronic HCV in Ismailia Fever Hospital, Egypt from May 2011 to February 2012.

#### Data collection:

The clinical data was collected in a pre-organized data sheet which included sociodemographic data regarding age, sex, residence, telephone number and medical history.

Clinical assessment, included, general and regional examination (heart, chest and abdominal examination) and fundus examination.

Investigations included laboratory investigations: Complete blood count, fasting blood sugar, S creatinine, S albumin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), S bilirubin, prothrombin time (INR), HbsAg, anti-HCV Ab, HCV RNA by PCR, Thyroid Stimulating Hormone (TSH), antinuclear antibodies (ANA), alpha-fetoprotein (AFP), pregnancy test for females, urine analysis and liver biopsy. Serum level of interleukin-8 was assessed by ELISA. Follow-up to assess response to interferon therapy within either 12 or 24 weeks of treatment by PCR.

#### Results:

Among the total studied 42 patients a 73.8% of the studied patients were responders while 26.2% were non responders (Table 1).

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>31</td>
</tr>
<tr>
<td>Non responders</td>
<td>11</td>
</tr>
<tr>
<td>At 12w</td>
<td>7</td>
</tr>
<tr>
<td>At 24w</td>
<td>4</td>
</tr>
</tbody>
</table>

More than half of the whole studied patients have IL-8 value 2-6 (mid range elevation) (64.3%) while 35.7% shows high level of IL-8 (≥14). (Table 2).
Table (2): IL-8 level among the studied patients (n=42).

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>9.4±6.9</td>
</tr>
<tr>
<td>Range</td>
<td>2.3-26</td>
</tr>
</tbody>
</table>

Table (4): Difference of IL-8 between responders, non responders and breakthrough.

<table>
<thead>
<tr>
<th></th>
<th>Responders (n=31)</th>
<th>Non-responders (n=7)</th>
<th>Breakthrough (n=4)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>7.9±6.2</td>
<td>12.9±7.9</td>
<td>14.1±8.3</td>
<td>Anova test</td>
</tr>
<tr>
<td>Range</td>
<td>2.3-26</td>
<td>4.23-7.1</td>
<td>3.7-24</td>
<td>0.07 (NS)</td>
</tr>
</tbody>
</table>

Responders have lower mean of IL-8 with statistically significant difference than non responders (7.9 versus 13.3). Most of responders (74.2%) have IL-8 value ranging from 2-6 while most of non responders (63.6%) have high level of IL-8 (≥14). Patients with IL-8 (2-6) are less likely to be non responder with RR=0.3, while patients with IL-8 ≥14 are at higher risk of being non responder with RR=3.1 (Table 3).

Table (3): Comparison between responders and non responders regarding IL-8 value.

<table>
<thead>
<tr>
<th></th>
<th>Non responder (n=11)</th>
<th>Responder (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>13.3±7.6</td>
<td>7.9±6.2</td>
</tr>
<tr>
<td>Range</td>
<td>3.7-24</td>
<td>2.3-26</td>
</tr>
<tr>
<td>Mid range</td>
<td>4 (36.4%)</td>
<td>23 (74.2%)</td>
</tr>
<tr>
<td>High level</td>
<td>7 (63.6%)</td>
<td>8 (25.8%)</td>
</tr>
</tbody>
</table>

*Statistically significant difference (p-value <0.05).

From our results we noticed that there is a statistical significant difference between responders and non-responders regarding baseline HCV RNA by PCR (x10^5) as the mean for responders was 5.9±10.7 and for non-responders was 36.8±60.4 with p-value (0.001).

There was no statistically significant difference between IL-8 level in responders, non responders at 12 weeks and breakthrough at 24 weeks or between any pairs of these subgroups.

Pearson correlation co-efficient = 0.2 and p-value = 0.2 (Not significant) regarding the difference between IL-8 levels between groups according to baseline PCR levels.

ROC curve analysis for predictive characteristics of IL-8 in prediction of response to interferon therapy:

Value of IL-8≤6.7 has sensitivity of 74% (95% CI: 55.4-88.1), specificity of 64% (95% CI: 30.9-88.8), positive predictive value of 85%, negative predictive value of 47% and positive likelihood ratio of 2.04 and negative likelihood ratio of 0.4 in prediction of response (being responder) to interferon therapy.
**Discussion**

Hepatitis C virus (HCV) is an infectious disease which affects about 170 million people worldwide [23]. Approximately 70-80% of infected individuals develop chronic infection and 20% of chronically infected individuals will go on to develop cirrhosis. Among these, 1-5% will develop end-stage liver diseases [24,25]. Currently, patients chronically infected with HCV are mainly treated with combination of pegylated interferon-α (IFN-α) plus ribavirin.

Several studies have been carried out to identify factors that facilitate identification of chronic hepatitis C patients who are likely to respond to antiviral treatment.

The HCV NS5A protein has been implicated in the resistance of HCV to antiviral therapy [26]. It was previously found that NS5A induces the CXC chemokine interleukin 8 (IL-8) to inhibit the antiviral actions of IFN in vitro [21]. To investigate the clinical significance of these results, in this study we investigated the impact of the levels of IL-8 in the serum and response to IFN therapy. The aim of the present study was to determine the correlation between levels of interleukin-8 in serum of patients with chronic hepatitis C infection and response to pegylated interferon/ribavirin after 12 and 24 weeks of therapy respectively.

A total of 42 patients with chronic HCV infection were enrolled in the study. Patients were assessed for baseline laboratory characteristics and side effects to interferon therapy. Baseline interleukin-8 was measured before initiation of therapy. Virological response was assessed after 12 weeks and 24 weeks (as an endpoint).

The whole studied patients were in age group (20-58) years old, with mean age of 41.5 years old. About half of the studied patients were males (52.4%) and 47.6% were females. Most of the studied patients were from rural areas (61.9%).

The results of the present study come in accordance with what was recently reported by the Egypt Demographic and Health Survey. The Egypt Demographic and Health Survey (EDHS) estimated a prevalence of 14.9% for the sampled population of 11,126 aged 15-59 in 2008. Prevalence increased with age, with 55-59 year olds showing a rate of 39.4%. Overall prevalence was 17.4% in males and 12.2% in females [8]. Males had a modestly higher infection rate. Rural areas had a higher prevalence than urban [27].

The present study showed that the most commonly reported side effect of interferon/ribavirin therapy were fatigue (88.1%), headache (66.7%), myalgia and arthralgia (54.8% each).

The most common clinical adverse events in patients on treatment with pegylated interferon plus ribavirin are flu-like symptoms, fatigue, myalgia, sleep disturbances, asthma, gastrointestinal disorders and depressive mood changes [28,29]. These findings are consistent with the findings of the present study.

At the end of 24 weeks of therapy, PCR showed that 73.8% of the studied patients have virologic response with negative PCR while 26.2% were positive (breakthrough), (16.7% at 12 weeks and 9.5% at 24 weeks).

There was no statistically significant difference between responders and non responders as regard to age, sex, residence and almost all of reported side effects to interferon/ribavirin therapy.

Baseline laboratory assessment showed that responders were found to have better controlled FBS, lower α-fetoprotein (2.8 versus 5.8 in non responders; p-value <0.05) and lower quantitative viral load (5.9 versus 36.8 in non responders; p-value <0.05).

Non responders were found to have higher baseline interleukin-8 levels than responders with mean IL-8 13.3±7.6pg/ml versus 7.9±6.2pg/ml respectively. Most of non responders were found to have IL-8 levels 14pg/ml (63.6%) while most of responders have IL-8 levels ranging from 2-6 pg/ml (74.2%).

The results of the current study are consistent with that of a previous study in 2001 by Polyak et al., [21] which was considered the first study to examine serum IL-8 levels and the biochemical response to IFN therapy. They have found that levels of IL-8 were significantly higher in patients who did not respond to IFN therapy than in patients who did respond to therapy.

In 2004, a study by Mihm and Colleagues [30] has investigated serum interleukin-8 in 59 healthy controls and 214 patients with chronic hepatitis C and different outcome to interferon-α-based therapy. In patients with chronic hepatitis C, higher interleukin-8 levels were observed compared with healthy controls (p<0.0001). They have also found that hepatitis C genotype 1-infected patients with early and overall virologic response to interferon-
predictor for response to interferon/ribavirin therapy. Their study demonstrated that IFN-γ has synergistic antiviral effects with IFN-α; whereas IL-8 can attenuate the anti-HCV actions of IFN-α and is associated with HCV resistance to interferon-α therapy [31].

The findings of the current study are also consistent with the findings reported by most recent study by Akbar et al., (2011) [32] they aimed to prospectively utilize the baseline IL-8 levels in the HCV infected serum and predict its role in sustained virological response (SVR) to IFN-α plus ribavirin therapy, in chronic HCV patients in Pakistan. They have found that non responders have higher baseline pretreatment levels of IL-8 than responders. They have concluded that increased levels of IL-8 in HCV infection might be involved in pathogenesis, persistence and resistance to IFN-α plus ribavirin combination therapy.

In our study, baseline (pretreatment) IL-8 levels ≤14pg/ml were found to have relative risk of 3.1 (95% CI: 1.09-9.03) to be non responder while pretreatment IL-8 levels 2-6pg/ml show protective effect against being non responder with relative risk of 0.3 (0.1-0.9). Also, ROC curve analysis has shown that in patients with chronic HCV infection, pretreatment IL-8 value ≤6.7pg/ml have sensitivity of 74%, specificity of 64%, PPV of 85% and NPV of 47% in prediction of good virological response to interferon/ribavirin therapy.

We conclude that, low level IL-8 is a significant predictor for response to interferon/ribavirin therapy in patients with chronic HCV infection. Responders were found to have lower pre treatment serum levels of IL-8 than non responders. Also, responders have lower levels of base-line HCV RNA than non-responders. It is advisable to perform a larger scale study for more conclusive results.

References


Effects of Melatonin Premedication on the Hemodynamic Responses and Perfusion Index During Laryngoscopy and Endotracheal Intubation

AHMED A. MOHAMED, M.Sc.; HOSAM M. ATEF, M.D.; ALAA EL-DIN M. EL KASSABY, M.D.; SALAH A.M. ISMAIL, M.D. and AMR M. HELMY, M.D.

The Department of Anesthesiology & Intensive Care, Faculty of Medicine, Suez Canal University

Abstract

Context Rational: Several techniques have been proposed to prevent or attenuate the hemodynamic responses following laryngoscopy and intubation, preoperative melatonin has a significant analgesic and anxiolytic effect for patients undergoing surgery. Melatonin may play an important role in controlling hypertension also in humans. The current study aimed at assessing the usefulness of melatonin in attenuating the pressor response to direct laryngoscopy and tracheal intubation.

Methods: After approval of the ethics committee and informed patients consent the study was carried out at the routine surgical theatre of the Suez Canal University Hospital, during the period from 2011 – 2012 on 90 patients with ASA physical status I, II scheduled for any elective surgery under general anesthesia with endotracheal intubation. Patients were randomly allocated according to computer-generated randomization into three groups: Group I (control group); Group II (melatonin 6mg tablet group) and Group III (melatonin 9mg tablet group). Primary outcome measures include: Heart rate (HR), Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MBP) and perfusion index were recorded before drug premedication, pre-induction, pre-intubation, 1, 2, 3, 5 and 10 minutes after laryngoscopy and intubation. Moreover perioperative anxiety was evaluated by recording the preoperative and postoperative verbal anxiety score (VAS) of the sample patients.

Results: Significant decrease in blood pressure in group II and group III receiving 6mg and 9mg of oral Melatonin 1 hour preoperative at 1, 2, 3, 5 and 10 minutes after intubation as regard SBP, DBP and MBP compared to group I. As regards to heart rate, no significant difference was found between the three groups throughout different time of measurement except for a significant difference at 1 minute after intubation measures for group II and group III compared to the control group. Moreover postoperative verbal anxiety score (VAS) was decreased significantly group II and group III compared to the control group.

Conclusion: Preoperative administration of melatonin one hour before surgery provided a significant decrease hemodynamic response of direct laryngoscopy and tracheal intubation as regard hemodynamic parameters and perfusion index.

Key Words: Oral melatonin premedication – Tracheal intubation– PI – Hemodynamic responses.

Introduction

ENDOTRACHEAL intubation is one of the most invasive stimuli in anesthesia particularly during induction and after tracheal intubation [1]. It is usually well tolerated by normotensive patients, but even short-lasting stimulation has been associated with increased morbidity and mortality in patients with recent myocardial infarction, hypertension, pre-eclampsia, and cerebro-vascular pathology such as tumors, aneurysms or increased intracranial pressure [1, 2]. Stress response increases both SABP and DABP measurements increase by 36-40% in contrast to control levels. Heart rate levels increase more than 20% with tracheal intubation in contrast to laryngoscopy [3,4]. The pressor (stress) response reaches a peak 1-2min after laryngoscopy and tracheal intubation, and usually subsides within 5-6min, although tachycardia may persist for 10min [5,6]. Studies in humans indicate that 1mg of melatonin decreases arterial pressure and the plasma levels of noradrenaline during standing [7]. Several studies reported that melatonin has analgesic potential in addition to anxiolytic and sedative effects without disturbances of the cognitive and psychomotor skills, and thus improves the quality of recovery [8]. The pineal
hormone melatonin (N-acetyl-5-methoxytryptamine) has several putative functions that may make it an attractive option for premedication, including the regulation of circadian rhythms and sedative, analgesic, anti-inflammatory, and antioxidative effects [9]. Moreover, administration of 1 mg of melatonin during the daytime to healthy young women decreased systolic, diastolic and mean arterial pressure along with the reduction of norepinephrine concentration [9] the same depressant effect on BP and noradrenergic activation was observed in healthy men treated with melatonin [10].

Material and Methods

After approval of the ethics committee an informed written patient consent was taken. On arrival in the operating room following one hour of premedication, crystalloid infusion was started through a 20-gauge intravenous canula inserted in an appropriate vein and the SBP, DBP, MAP and HR was monitored.

Anesthesia was induced with thiopental sodium (5mg/kg), fentanyl 1ug/kg and cisatracurium (0. 1 mg/kg), and maintained with 1.2 MAC isoflurane with a fresh gas flow of 100% O₂. Neuromuscular block was confirmed with a nerve stimulator. Laryngoscopy and tracheal intubation was then performed 3min after loss of verbal contact by the same experienced anesthesiologist using a suitable Macintosh Laryngoscope blade and 7.0-8.0mm endotracheal tube (for women and men, respectively). Melatonin 6mg, 9mg or placebo tablets were administrated 1 hour before surgery. Patients were randomly allocated according to computer-generated randomization. The patients and investigator were not aware about the type of drug used (double-blinded pattern). All patients received their drug one hour pre induction of anesthesia in the pre-anesthetic area. Baseline Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were evaluated in the pre-anesthetic visit. Patients were not premedicated by other drugs. The study drugs were prepared by the pharmacy, and an appropriate code number was assigned. Airway assessment: The patient was assessed preoperatively according to a multivariate risk index (MVRI), [11] in which the total score was determined by the sum of the values of the 7 parameters, score ≥3 predicts easy intubation and score ≥4 predicts difficulty (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 points</th>
<th>1 points</th>
<th>2 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-incisor gap (cm)</td>
<td>&gt;4</td>
<td>2-4</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Mallampati score</td>
<td>Class I</td>
<td>Class II</td>
<td>Class III</td>
</tr>
<tr>
<td>Head/Neck movement</td>
<td>&gt;90</td>
<td>80-90</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Ability to prognath</td>
<td>Can prognath or edentulous</td>
<td>Can approximate teeth</td>
<td>Can’t approximate teeth</td>
</tr>
<tr>
<td>Thyromental distance (cm)</td>
<td>&gt;6.5</td>
<td>6-6.5</td>
<td>&lt;6</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>&lt;90</td>
<td>90-110</td>
<td>&gt;110</td>
</tr>
<tr>
<td>History of DI</td>
<td>Non</td>
<td>Questionable</td>
<td>Definite</td>
</tr>
</tbody>
</table>

Primary outcome measures include; HR, SBP, DBP and MAP measured using Datex Ohmeda S/S monitor® were recorded before drug premedication, pre-induction, pre-intubation, 1, 2, 3, 5 and 10 minutes after laryngoscopy and intubation.

Secondary outcome measures included; anxiety using verbal anxiety score (12) before and after premedication by asking the patient how they would rank their anxiety on a score from 1-10. Other outcomes included the occurrence of any side-effects, such as nausea and vomiting, respiratory depression, dizziness, somnolence, headache and emergence agitation was recorded.

Data management and statistical analysis: Power calculations suggested that a minimum of 30 patients per group would detect a 15% difference in SAP or HR between the groups after intubation (α=0.05, β=0. 2). Data collected was coded, entered and analyzed using Microsoft Excel software. The data was then imported into the Statistical Package for the Social Sciences (SPSS version 11.0) software for analysis. According to the type of data, the following tests were used to test differences for significance; Chi square, paired t-test, and analysis of variance (ANOVA) with at least significance difference. Parametric data were analyzed by multivariate analysis of variance (MANOVA). Differences in hemodynamic data among groups were analyzed with Bonferroni’s test correction for multiple comparisons. Differences from baseline within groups were evaluated using paired-sample t-test. The assessment of Odds ratio and multiple logistic regression analysis was used to test the relationship between different dependent and independent variables. p<0.05 was considered as significant.
Results

There was no significant difference between the three groups as regard age, gender, height and weight. In our study there was highly significant decrease in blood pressure in group II and group III receiving 6mg and 9mg of oral Melatonin consecutively 1 hour preoperative and at 1, 2, 3, 5 and 10 minutes after intubation as regard to the SBP, DBP and MBP compared to group I (p<0.001). (Table 2 & Fig. 1) Moreover there was a significant decrease in blood pressure recorded in group III at 1 minute after intubation as regard to the SBP, DBP and MBP compared to group II (p<0.001).

While the group I which received the placebo showed a significant increase in blood pressure after induction of anesthesia at 1, 2 and 3 minutes after intubation as regard to the SBP, DBP and MBP. Group III which received 9 mg of oral melatonin 1 hour prior the operation, showed significant decrease in SBP and MBP at 1, 2, 3, 5 and 10 minutes after intubation compared to premedication measures (p<0.001). Moreover it showed a significant decrease in DBP at 2, 3, 5 and 10 minutes after intubation compared to premedication measures (p<0.001) (Fig. 2). However no significant difference was found between Group II and Group III as regard to SBP, DBP and MBP at pre-intubation, 5 and 10 minutes after intubation except at 1 minute after intubation was significant (p 0.002). In our study there was a significant difference between both groups II and III perfusion index measures before induction and at 1, 2 and 3 minutes after intubation compared to group I measures (p 0.044-0.001). Moreover there was a significant difference in perfusion index measures for group II before induction and at 1, 2, 3, 5 and 10 minutes after intubation compared to group I (p 0.015-0.001). (Table 3 & Fig. 3).

Table (2): Comparison of SBP changes in mm Hg between the three studied groups.

<table>
<thead>
<tr>
<th>SBP</th>
<th>Control</th>
<th>6mg melatonin</th>
<th>9mg melatonin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Before premed.</td>
<td>107.0-155.0</td>
<td>130.77±15.40</td>
<td>132.47±11.61</td>
<td>134.67±10.91</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>128.0</td>
<td>135.0</td>
<td>139.0</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>0.068</td>
<td>0.241</td>
<td>0.507</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before induction</td>
<td>100.0-147.0</td>
<td>126.57±11.95</td>
<td>130.0</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>128.0</td>
<td>130.0</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>0.340</td>
<td>0.006*</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before intubation</td>
<td>80.0-112.0</td>
<td>96.77±9.09</td>
<td>107.47±8.79</td>
<td>107.50</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>97.0</td>
<td>107.50</td>
<td>105.50</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>&lt;0.001*</td>
<td>0.003*</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1min</td>
<td>110.0-141.0</td>
<td>131.20±8.32</td>
<td>120.53±9.93</td>
<td>119.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>146.0</td>
<td>133.50</td>
<td>119.0</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2min</td>
<td>122.0-160.0</td>
<td>140.97±11.02</td>
<td>121.53±8.04</td>
<td>117.53±8.80</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>142.0</td>
<td>122.50</td>
<td>118.0</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3min</td>
<td>117.0-153.0</td>
<td>134.47±10.80</td>
<td>115.67±8.58</td>
<td>112.73±8.20</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>136.50</td>
<td>117.0</td>
<td>112.50</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
<td>0.223</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5min</td>
<td>114.0-149.0</td>
<td>129.03±10.69</td>
<td>112.27±7.25</td>
<td>109.07±6.85</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>130.0</td>
<td>112.0</td>
<td>109.0</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10min</td>
<td>100.0-129.0</td>
<td>115.53±9.51</td>
<td>106.87±7.13</td>
<td>103.50±9.88</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>117.0</td>
<td>107.50</td>
<td>103.50</td>
</tr>
<tr>
<td></td>
<td>p1</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>p2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p: p-value of the ANOVA test. p1: p-value of Post Hoc test (LSD) between group 1 with other groups.

*: Statistically significant at p≤0.05.
As regards to heart rate (Table 4 & Fig. 4), no significant difference was found between the three groups throughout different time of measurement, except for a significant difference at 1 minute after intubation measured for group II & group III compared to the control group ($p < 0.005$ and $<0.001$ respectively). VAS significantly lowered in group II and group III compared to the control group (Table 5).

Table (3): Comparison of PI between the three studied groups.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>Control</th>
<th>6mg melatonin</th>
<th>9mg melatonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before premed.</td>
<td>Range</td>
<td>1.50-2.50</td>
<td>1.50-2.60</td>
<td>1.20-2.60</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.09±0.24</td>
<td>2.20±0.26</td>
<td>2.11±0.37</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.05</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>0.131</td>
<td>0.794</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td>0.209</td>
<td></td>
</tr>
<tr>
<td>Before induction</td>
<td>Range</td>
<td>1.40-2.70</td>
<td>2.0-2.90</td>
<td>1.80-2.70</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.10±0.33</td>
<td>2.35±0.25</td>
<td>2.29±0.24</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.10</td>
<td>2.30</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>0.001*</td>
<td>0.009*</td>
<td>0.428</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before intubation</td>
<td>Range</td>
<td>2.50-4.50</td>
<td>2.80-4.20</td>
<td>2.60-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>3.18±0.45</td>
<td>3.26±0.38</td>
<td>3.12±0.43</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.15</td>
<td>3.10</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>0.426</td>
<td>0.581</td>
<td>0.179</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.0-2.80</td>
<td>1.60-3.10</td>
<td>2.0-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.02±0.49</td>
<td>2.36±0.41</td>
<td>2.53±0.39</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.0</td>
<td>2.50</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1min</td>
<td>Range</td>
<td>2.0-2.60</td>
<td>2.20-3.10</td>
<td>2.30-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.29±0.19</td>
<td>2.78±0.22</td>
<td>2.59±0.35</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.30</td>
<td>2.80</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.007*</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2min</td>
<td>Range</td>
<td>2.10-3.0</td>
<td>2.20-3.20</td>
<td>2.30-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.56±0.28</td>
<td>2.86±0.22</td>
<td>2.71±0.33</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.55</td>
<td>2.85</td>
<td>2.60</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>&lt;0.001*</td>
<td>0.044*</td>
<td>0.039*</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3min</td>
<td>Range</td>
<td>2.50-3.50</td>
<td>2.30-3.50</td>
<td>2.60-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.97±0.30</td>
<td>3.18±0.26</td>
<td>2.92±0.27</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.0</td>
<td>3.20</td>
<td>2.90</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>0.004*</td>
<td>0.544</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>5min</td>
<td>Range</td>
<td>2.60-3.70</td>
<td>2.60-4.0</td>
<td>2.60-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>3.13±0.31</td>
<td>3.33±0.38</td>
<td>3.05±0.26</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.15</td>
<td>3.30</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>0.015*</td>
<td>0.330</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10min</td>
<td>Range</td>
<td>2.60-3.70</td>
<td>2.60-4.0</td>
<td>2.60-4.0</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>3.13±0.31</td>
<td>3.33±0.38</td>
<td>3.05±0.26</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.15</td>
<td>3.30</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>$p_1$</td>
<td>0.015*</td>
<td>0.330</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>$p_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$p$ : $p$-value of ANOVA test.

$p_1$ : $p$-value of Post Hoc test (LSD) between group I with each other group.

$p_2$ : $p$-value of Post Hoc test (LSD) between 6mg melatonin and 9mg melatonin.

* : Statistically significant at $p≤0.05$. 
Table (4): Comparison of HR in beats/min between the three studied groups.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>Control</th>
<th>6mg melatonin</th>
<th>9mg melatonin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>62.0-113.0</td>
<td>59.0-123.0</td>
<td>64.0-114.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>85.47±14.83</td>
<td>85.33±17.61</td>
<td>85.53±15.19</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>81.50</td>
<td>85.0</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_1</td>
<td>0.974</td>
<td>0.987</td>
<td>0.961</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>55.0-111.0</td>
<td>58.0-120.0</td>
<td>55.0-112.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>84.73±15.19</td>
<td>81.90±17.26</td>
<td>80.30±15.64</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>83.50</td>
<td>80.0</td>
<td>78.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_1</td>
<td>0.496</td>
<td>0.288</td>
<td>0.701</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>78.0-151.0</td>
<td>71.0-133.0</td>
<td>70.0-112.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>107.80±18.64</td>
<td>96.13±16.61</td>
<td>92.60±10.95</td>
<td>0.001 *</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>102.0</td>
<td>93.50</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>1min</td>
<td>p_1</td>
<td>0.005 *</td>
<td>&lt;0.001 *</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td>1min</td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>74.0-123.0</td>
<td>67.0-125.0</td>
<td>68.0-113.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>95.93±13.46</td>
<td>90.87±16.01</td>
<td>90.30±11.66</td>
<td>0.227</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>92.0</td>
<td>88.0</td>
<td>87.50</td>
<td></td>
</tr>
<tr>
<td>2min</td>
<td>p_1</td>
<td></td>
<td></td>
<td></td>
<td>0.874</td>
</tr>
<tr>
<td>2min</td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>69.0-115.0</td>
<td>65.0-114.0</td>
<td>75.0-110.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>90.27±13.0</td>
<td>85.67±13.91</td>
<td>88.60±11.66</td>
<td>0.380</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>87.0</td>
<td>82.50</td>
<td>85.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_1</td>
<td>0.159</td>
<td>0.618</td>
<td>0.381</td>
<td></td>
</tr>
<tr>
<td>3min</td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>71.0-109.0</td>
<td>65.0-103.0</td>
<td>68.0-108.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>85.60±10.88</td>
<td>80.60±12.06</td>
<td>84.33±11.50</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>82.50</td>
<td>78.50</td>
<td>81.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_1</td>
<td>0.096</td>
<td>0.670</td>
<td>0.212</td>
<td></td>
</tr>
<tr>
<td>5min</td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>56.0-100.0</td>
<td>51.0-91.0</td>
<td>53.0-98.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>72.47±10.18</td>
<td>67.67±9.83</td>
<td>72.80±10.29</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>70.0</td>
<td>68.50</td>
<td>71.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p_1</td>
<td>0.069</td>
<td>0.899</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>10min</td>
<td>p_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* : Statistically significant at \( p \leq 0.05 \).

\( p \) : p-value of ANOVA test.

\( p_1 \) : p-value of Post Hoc test (LSD) between group I with each other group.

\( p_2 \) : p-value of Post Hoc test (LSD) between the 6mg melatonin and 9mg melatonin.

Table (5): Comparison between the 3 groups as regard changes in the verbal anxiety score.

<table>
<thead>
<tr>
<th>Timely points</th>
<th>Groups</th>
<th>( p )-value between I &amp; II</th>
<th>( p )-value between I &amp; III</th>
<th>( p )-value between II &amp; III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration</td>
<td>Group I (N=30)</td>
<td>Group II (N=30)</td>
<td>Group III (N=30)</td>
<td>0.157</td>
</tr>
<tr>
<td>After one hour</td>
<td>5.06±1.94</td>
<td>5.80±1.97</td>
<td>6.30±2.00</td>
<td>5.00±2.36</td>
</tr>
<tr>
<td>After two hours</td>
<td>7.55±1.90</td>
<td>5.96±2.10</td>
<td>7.00±2.67</td>
<td>6.00±2.87</td>
</tr>
</tbody>
</table>
Discussion

The current study aimed at assessing the usefulness of Melatonin in attenuating the pressor response to direct laryngoscopy and tracheal intubation. Previous studies showed that melatonin could decrease MBP in healthy women [13] and men [14]. The mechanism of action of melatonin on circulation is complex and unclear. Melatonin may bind to specific melatonin receptors in the blood vessels, interfering with the vascular response to catecholamine [15]. Furthermore, melatonin may interfere with the peripheral and central autonomic system, causing a reduction in adrenergic outflow and catecholamines levels [16]. In addition, it may induce relaxation of the smooth muscle of the arterial walls via increasing nitric oxide availability [17]. Finally, melatonin may influence blood pressure also via its specific receptors MT 1 (melatonin type 1) or MT2 (melatonin type 2) localized in peripheral vessels or in blood pressure modulating structures of the central nervous system [18,19].

The potential protective effect of melatonin in hypertension may go far beyond its beneficial hemodynamic actions. Due to its hypotensive, sympatholytic and oxygen load reducing action melatonin limits pathologic remodeling of the left ventricle. Melatonin reduced LVH in hyperthyroid rats [20], decreased collagenous protein and hydroxyproline level in L-NAME-induced cardiac hypertrophy [21] and, in a regression experiment with SHR, it lowered collagen content and insoluble collagen concentration in the LV [22].

Zanoboni, et al., stated that pinealectomy in rats results in melatonin deficiency, peripheral vasoconstriction and hypertension [23]. In another
brief procedures premedicant as midazolam had multiple side effects proved the operating conditions during cataract pressure

...hypertension. Moreover, melatonin has endothelium-relaxing effects, it is a potent scavenger of free radicals (which negatively influence blood pressure), and it may work via epigenetic mechanisms at the level of the area postrema to regulate blood pressure [25].

In our study there was a significant decrease in the anxiety scores according to the verbal anxiety score in group II and group III which received 6mg and 9mg melatonin tablets relative to group I which received placebo. Moreover there was a significant decrease in the anxiety scores between group III which received 9mg melatonin tablets relative to group II which received 6mg melatonin tablets.

Mohamed Naguib, et al., stated that premedication with 0.05mg/kg melatonin was associated with preoperative anxiolysis and sedation without impairment of cognitive and psychomotor skills and without prolonging recovery [26].

Ismail, et al., stated that premedication with oral melatonin provided anxiolysis, enhanced perioperative analgesia, decreased the IOP, and improved the operating conditions during cataract surgery under topical anesthesia [8].

In Indian Journal of Anesthesia; the study done as evidence based data on melatonin for anxiolysis in children. Considering the great deal of interest in the potential uses of melatonin in perioperative setting; melatonin was preffered in pediatrics as premedicant as midazolam had multiple side effects including paradoxical reactions, interactions with opioid, variable bioavailability and elimination half-life and delayed discharge from PACU after brief procedures [27].

Melatonin improves tourniquet tolerance and enhances postoperative analgesia in patients receiving intravenous regional anesthesia. Melatonin is an effective premedication before IVRA since it reduced patient anxiety, decreased tourniquet-related pain, and improved perioperative analgesia [28].

In another study of American Society of Anesthesiologists (ASA) I patients (mean age 33 years, n=200) having unspecified surgery, sub-lingual melatonin (0.2mg/kg) decreased preoperative anxiety and increased sedation versus placebo [29].

Pediatric surgery, the first published study investigated the effects of melatonin in children (two to five years, n=105) undergoing minor general surgical procedures. It found that oral melatonin and midazolam at 0.25 and 0.5mg/kg were equally effective in decreasing separation anxiety and anxiety associated with the introduction of an anesthetic face mask at induction [30].

In elderly patients, melatonin premedication for anxiolysis has also been less encouraging. In patients over 65 years of age (n=138) having a variety of surgical operations, patients who received 1 mg melatonin orally had no significant decrease in anxiety pre- or postoperatively versus a placebo group [31].

The mechanism of melatonin for anxiolysis and sedation seems to relate to both melatonin receptor activation and an effect on gamma-aminobutyric acid transmission [32,33] It has been reported that melatonin administration in rats increases central nervous system levels of gamma-aminobutyric acid and flumazenil also decreases the effects of melatonin [34,35].

Perfusion index was measured in our study as an indicator for the analgesic effect of Melatonin premedication. Intubation is a stimulus able to increase endogenous catecholamines and thus leading to vasoconstriction possibly declining the perfusion index. Melatonin in (group II and group III) attenuated the release of norepinephrine limiting the changes in regional perfusion index [36].

Patients treated with melatonin preoperatively presented a greater reduction in pain and required lower morphine consumption in the postoperative period. The benefits of these interventions were statistically and clinically significant to produce postoperative anxiolysis, which led to lower postoperative pain, as well as lower morphine consumption throughout the first 24 hours after surgery [9].

Two further studies involved patients undergoing total abdominal hysterectomy under epidural anesthesia with propofol sedation (epidural catheter removed immediately postoperatively). The first of these looked at ASA I/II patients (n=33, mean age 44 years) randomized to receive oral melatonin (5mg) or placebo the night before surgery and one hour prior to the operating theatre. The treatment group reported significantly lower postoperative
pain and anxiety for the first 36 hours and also had decreased morphine consumption [31]. The second study investigated ASA I/II patients (n=59), who were randomized to receive either oral melatonin (5mg), oral clonidine (100 μg) or placebo both the night before and one hour prior to anesthesia. The melatonin and clonidine groups had lower anxiety scores, lower pain scores and lower morphine consumption in the first 24 hours postoperatively. However, subgroup analysis indicated that the decrease in pain scores and morphine consumption was only significant in those patients with higher preoperative anxiety scores [37].

The analgesic effects of melatonin are mediated by MT 1 and MT2 receptors (3 8) with a subsequent reduction in intracellular cAMP, and a consequent effect on potassium and calcium channels [39]. Other analgesic effects may relate to indirect increases in endogenous opioids [40], an effect on the MRNA expression to inhibit arachidonic acid release [41] and a direct free radical scavenging effect [42].

References
23- ZEMAN M., DULKOVA K., BADA V. and HERICHova I.: Plasma melatonin concentrations in hypertensive


Polymorphism of CAG Repeat in Androgen Receptor Gene in Egyptian Women with Polycystic Ovary Syndrome

DOAA SHAHIN, M.D.* and SHERIN M. SOBH, M.D.**
The Departments of Clinical Pathology*, Faculty of Medicine, Mansoura University and Obstetrics & Gynaecology**, Faculty of Medicine, Cairo University, Egypt

Abstract

**Background:** Trinucleotide repeats CAG (n) in androgen receptor gene is thought to be central to PCOS genetic susceptibility. However, previous studies of PCOS have shown variable association of CAG (n) polymorphism with PCOS.

**Objective:** To assess the association of the AR gene CAG repeat length polymorphism with PCOS among Egyptian patients, and its influence on clinical and biochemical androgen traits.

**Patients and Method:** A cohort of 80 women with oligo-anovulatory cycles and ultrasound features of PCOS and 67 healthy women of reproductive age were investigated for CAG repeat lengths using genescan. Assay of serum total testosterone, free testosterone and DHEAS were carried out. These parameters were correlated with the CAG repeat.

**Results:** AR (CAG) n range were 8-25 with a median of 19 in PCOS patients and 10-29 with a median of 22 in control group. The difference in median repeat number was significant comparing PCOS with control (p<0.001). A higher frequency of short AR (CAG) n alleles (≤15) tended to be more frequent in PCOS women than controls (p=0.005). We found significant difference between the both groups as regard BMI, Hirsutism, Total testosterone, Free testosterone and DHEAS.

**Conclusion:** Our findings support the hypothesis that there is association of shorter CAG repeats with Egyptian PCOS patients suggesting inherited alteration in androgen sensitivity may contribute to PCOS.

**Key Words:** CAG repeats – Androgen receptor – Polycystic ovary syndrome – Genescan.

Introduction

THE androgen receptor (AR) is a member of the family of ligand-activated transcription factors that regulate many biological processes and is encoded by a gene located at Xq11-q12q. The androgen receptor (AR) contains a polyglutamine tract of variable size in the N-terminal transactivation domain that can modulate the ability of the receptor to enhance transcriptional events in vitro [1]. This tract is encoded by a highly polymorphic CAG repeat microsatellite in exon 1 of the AR gene [2].

The AR gene contains a highly polymorphic (CAG) n repeat in exon 1 encoding aglutamine tract in the N-terminal transactivation domain of the protein, which becomes active only after AR binds to its ligand [3].

The polyglutamine tract length is inversely correlated to the transcriptional competence of the receptor, with longer tracts being associated with lower levels of AR-mediated transcription in both normal and disease states [4].

CAG repeat number normally ranges between 8 and 35 and demonstrates a stable inheritance [5].

Theoretically, an inverse relationship exists between repeat number and AR activity whereby tracts of shorter size confer greater activity than tracts of larger size. In vitro studies have indicated that ARs with shorter polyglutamine tracts have greater ability to activate reporter genes with androgen response elements [6].

Consistent with these functional studies, various metaanalyses indicated that the a smaller number of CAG repeats has been associated with hirsutism, premature pubarche, and ovarian hyperandrogenism in women [7], as well as androgen-dependent skin disorders in both men and women [8].

Polycystic Ovary Syndrome (PCOS), the leading cause of anovulatory infertility among premenopausal women, is now essentially known as androgen excess disorder. It is characterized by three key features viz., hyperandrogenism, chronic

Correspondence to: Dr. Doaa Shahin
E-mail: Doaashahin2007@yahoo.com
In view of this, a number of studies have tried to investigate the role of CAG repeats of the AR gene in PCOS yielding contrasting results. Nevertheless, some of these studies reported a trend for shorter CAG alleles to be more frequent among PCOS cases than the controls which is consistent with the in vitro evidence of the greater receptor activity of the shorter CAG alleles. The pattern of association of CAG repeat polymorphism with the PCOS are not studied in any detail among the Egyptian Women, so we investigated the association between CAG repeat numbers [i.e. (CAG) n] and an increased risk of PCOS and its influence on clinical and biochemical androgen traits.

Subjects and Methods

The study group consisted of 80 women with PCOS aged 22.5 ± 7.2 (mean ± SD) years. The subjects of this study were selected from Egyptian Women which were recruited from infertility and antenatal clinics, Elkasr Elainy Hospital, Cairo, Egypt. Due to ethnic differences in allele frequency for the human AR (hAR) (CAG) n polymorphism, women from other countries were excluded from the study.

All of these women were subject to the same infertility assessment protocol, which included reproductive history, pelvic ultrasound for ovarian morphology, blood tests for hormonal profiles, and height and weight measurements. Women with abnormal androgen levels were further assessed for adrenal hyperplasia and Cushing’s syndrome that form exclusion criteria for the diagnosis of PCOS. PCOS was based on the criteria of hyperandrogenism and anovulation.

The control group consisted of 67 healthy Egyptian women with normal menstrual cycles (28-30 days) and no signs of hyperandrogenism.

Five mls of venous blood was collected from each one and divided into 2 samples. The first one is 2ml venous blood was left to clot in plain polypropylene tube at 25 °C for 30 minutes, then the separated serum was used for Hormone analysis (total testosterone, free testosterone and DHEAS) by electrochemiluiminescence assays (automatic elecsys 2010, Germany).

The other blood sample is 3ml blood on EDTA was used for DNA extraction and CAG-repeats analysis by automated ABI 3 10 Genetic Analyzer.

CAG repeats:

DNA was isolated from blood samples using the QIAamp RNA blood mini kits catalog No. 52304 (Qiagen, Gmbh, Hilden). Testing condition was done according to the manufacturer’s recommended protocol.

The CAG repeat was genotyped using a PCR-based assay. Genomic DNA was amplified by PCR using fluorescently labeled primers that flank the CAG repeat. Amplification was performed in a reaction volume of 20 µL containing 0.1 µg genomic DNA, 8 pmol fluorescently labeled forward primer (5’-TCC AGA ATC TGT TCC AGA GCG TGC3’), 8 pmol unlabeled reverse primer (5’-GCT GTG AAG G- TT GCT GTT CCT CAT-3’), 0.1 mmol/l dNPTs, and 1U Taq polymerase. Amplification was performed as follows: Initial denaturation/ enzyme activation at 94 °C for 7 minutes; followed by 30 cycles for 45 seconds at 94 °C, 45 seconds at 55 °C, and 45 seconds at 72 °C; followed by a final extension of 72 °C for 10 minutes. Amplification was performed in an automated thermal cycler 9700 (PE, Applied Biosystems, USA).

Two microliters of PCR products were mixed with 24 µL formamide and 1 µL of the internal size standard TAMRA 500. The mixture was denatured at 95 °C for 3 min and placed on ice until analysis. Electrophoretic analysis was performed using POP4 gel in ABI 3 10 Genetic Analyzer (ABI Prism 310; Applied Biosystems, Perkin Elmer, USA). The amplified products were analyzed by Gene-scan software (Applied Biosystems). Accurate sizing of alleles was estimated by comparison with the internal standard TAMRA 500.

Statistical analysis:

The data collected were statistically analyzed using statistical package for social sciences (SPSS/version 16) software. (Inc., Chicago, USA). Unpaired t-tests were used to compare clinical characteristics between women with and without PCOS.

The primary genotypic unit used in the association analyses was the biallelic mean (the mean of the CAG repeat number from the two alleles in each subject). Biallelic mean was considered in two ways. First, it was analyzed as a continuous quantitative variable. Second, it was treated as a binary qualitative variable, with the two states being either less than or greater than or equal to the median repeat number observed in the control group. Significance was taken as \( p < 0.05 \). Correlations among variables were done by spearman’s correlation coefficient. Qualitative data are pre-
sented as number and percent. Quantitative data are presented as Mean±Standard deviation or median (minimum-maximum) where appropriate.

**Results**

This study was conducted on 80 women with PCOS and 67 healthy Egyptian women.

The clinical characteristics of the women with PCOS were as follows:

Of 80 patients 43 (38.4%) had irregular periods, 69 (61.6%) had Oligomenorrhea, 32 (40%) had hirsutism, 15 (18.75%) had acne, 69 (61.6%), and only 2 (2%) had alopecia areata. 80% of cases were defined polycystic ovaries as the presence of at least eight peripheral cysts less than 10mm in diameter, with increased ovarian stroma on ultrasound, occurring bilaterally.

Hyperandrogenism was found in 71.25% of cases. 58.75% of our studied group were obese (BMI >27kg/m²), and 68.75% were infertile.

Table (1) presents Comparison of hormonal and AR-CAG repeat length between 80 PCOS cases and 67 control women. We found significant difference between the both groups as regard BMI, Hirsutism, Total testosterone, Free testosterone and DHEAS.

The range of CAG repeats was 8-25, with a median of 19 in the women with PCOS, while in control women CAG repeat numbers ranged from 10 to 29, with a median of 22. The difference in median repeat number was significant comparing PCOS with control (p<0.001).

The allele distribution pattern was different in the PCOS group compared to control group. 45/80 (56.25%) of PCO womens carrying the very short (≤15) AR CAG repeats, while in control group 18/67 (30%) only carrying the very short (≤15) AR CAG repeats (p=0.005).

Furthermore, there were less PCOS women 25/80 (31.25%) carrying the longest length alleles (>20) than in the control group 40/67 (59.7%). In the entire cohort, biallelic mean of repeats was associated with PCOS.

GeneScan results are presented as the intensity of the fluorescence peak (y axis) vs amplicon size (x axis). Blue peaks representing PCR product fragments. The GeneScan TAMRA 500 size standard are the smaller red peaks.

**Discussion**

Hyperandrogenism is a key feature of the PCOS syndrome and might contribute to IR, which is often observed in PCOS women [13-15]. At the molecular level, testosterone effects are mediated through activation of the AR. The ability of the receptor to enhance transcription of testosterone-regulated genes was shown in vitro to depend on a highly polymorphic CAG repeat microsatellite in exon 1 [16]. Therefore, it appears reasonable to consider the CAG length polymorphism, when investigating the PCOS.

Thus, we hypothesized that the shorter alleles would be more frequent and preferentially more active among the PCOS women than the controls. To the best of our knowledge, There is no studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Hirsutism (%)</th>
<th>Total testosterone (ng/dl)</th>
<th>Free testosterone (pg/ml)</th>
<th>DHEAS (ng/ml)</th>
<th>CAG repeat length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.5±7.5</td>
<td>22.5±3.5</td>
<td>0 (0%)</td>
<td>28±7.9</td>
<td>0.36±0.24</td>
<td>854.6±316</td>
<td>22 (10-29)</td>
</tr>
<tr>
<td>PCO</td>
<td>28.9±1.9</td>
<td>31.5±5.3</td>
<td>32 (40%)</td>
<td>76±24.9</td>
<td>0.82±0.45</td>
<td>1789.9±857.8</td>
<td>19 (8-25)</td>
</tr>
<tr>
<td>χ² (p)</td>
<td>8.5 (0.044)</td>
<td>0.36 (0.014)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>5.5 (0.005)</td>
<td>(0.003)</td>
<td>(0.001)</td>
</tr>
</tbody>
</table>

p>0.001, compared with control group.
of its kind on Egyptian women to examine the association of AR polymorphism with PCOS.

Our study revealed the range of CAG repeats was 8-25, with a median of 19 in the women with PCOS, while in control women CAG repeat numbers ranged from 10 to 29, with a median of 22. We found a significant association between shorter repeat length and the presence of PCOS.

As smaller biallelic mean was associated with increased frequency of PCOS. Our results are consistent with some recent studies where Xita et al., [11] and Shah et al., [10] found shorter alleles to be more frequent among the PCOS cases than controls.

However Dasgupta et al., [17] study revealed a range of 8-31 CAG repeats in the AR gene of South Indian PCOS women and the controls. And neither the distribution of biallelic mean values nor the mean repeat sizes were found significantly different between the PCOS cases and controls. Similarly, another study found no overall association of CAG repeats with PCOS but observed higher mean-CAG repeats in a subgroup with higher testosterone levels [18].

Alternatively, in the study by Hickey et al., [19] paradoxically a longer CAG repeat biallelic mean was associated with an increased risk of PCOS in Australian Caucasian women, a finding that appears to be contrary to functional data. These contradictory results can be attributed to the different ethnic background of studied population, in which there is a significant variation in the number of AR gene CAG repeats in different populations and this may account for the inconsistent findings of association studies in different populations.

In our cohort study, There was correlation between hirsutism, total testosterone, free testosterone, DHEAS and the CAG length, which is in accordance with a recently published study [20]. Some prior studies found an association between CAG repeats and testosterone levels [21-23]. Other investigators found that the CAG repeat length of the AR influenced the relationship between free testosterone and insulin resistance [measured by homeostatic model assessment (HOMA-IR)], such that at lower CAG lengths, free testosterone and HOMA-IR were positively correlated [24]. Another study found that shorter CAG repeats were associated with increased androgen levels only in the presence of longer alleles of the SHBG (TAAAA) variant, suggesting that simultaneous lesions in both androgen availability and sensitivity could result in hyperandrogenism [11]. Presumably alterations in androgen sensitivity could lead to altered androgen secretion, similar to altered insulin secretion when insulin resistance is present.

However other study revealed no significant association between testosterone levels and CAG repeat length [17]. The allele distribution pattern was different in PCOS group compared to control group in Egyptian women. 45/80 (56.25%) of PCO womens carrying the very shortest (≤15) AR CAG repeats. Furthermore, there were less PCOS women 25/80 (31.25%) carrying the longest length alleles (>20) than in the control group 40/67 (59.7%). Our result in concordance with Jaaskelainen et al., [25] study in which all subjects with CAG repeat length 15 or less had PCOS, suggesting some influence of the shorter CAG repeat in the pathogenesis of PCOS.

In this study, significant association between CAG repeat length and BMI. This also was reported by some recent studies as Dasgupta et al., [17] who found significant heterogeneity in the CAG biallelic mean between the obese and lean PCOS cases. The lean PCOS women, who have a significantly higher frequency of the middle range CAG repeats, would possibly be conferred with a moderate receptor activity as compared to the obese PCOS cases. This is can explain the differential receptor activity among them, which may also probably explain the manifestation of hyperandrogenic features in such cases.

The difference of our results results can be explained by the difference in sample size, heterogeneous group of patients, and genetic background of patients.

As our study is the first of its kind from Egypt, Although many studies about this contradictory subject were carried on different ethnic populations. Egyptian are known to be of mixed ethnic origin (Middle Eastern, African and European), so Egyptian studies are expected to add to the data available for different ethnic backgrounds [26]. And therefore further studies are required among other ethnic groups, encompassing the geographic heterogeneity of Egypt, including Delta regions, Upper Egypt and Nubia before reaching any conclusions on the precise role of androgen receptor CAG repeat polymorphism in the manifestation of this immensely heterogeneous PCOS phenotype in the populations of this region.

References


Comparative Study between Right Liver Lobe Diameter/Albumin Ratio and Platelet Count/Spleen Diameter Ratio as a Non-Invasive Predictor of Oesophageal Varices in Patients with Liver Cirrhosis

HAMDY M. MOSTAFA, M.D.; KHALED A. EID, M.D.; MONA M. ABDEL MEGUID, M.D. and SAFIA A. MOHAMED, M.D.

The Departments of Hepatology & Gastroenterology, Faculty of Medicine, Al-Azhar University, Assiut and Clinical Pathology, Qena Faculty of Medicine, South Valley University

Abstract

Objective: Oesophageal varices development is among the major complications of liver cirrhosis. Repeated endoscopic examinations are unpleasant for patients, and have cost impact, while only half of cirrhotic patients have varices. For these reasons, non-invasive predictors for the presence and size of varices has been studied.

Aim of the Work: To compare between platelet count/spleen diameter (PC/SD) ratio and right liver lobe diameter/albumin (RLLD/alb) ratio as non-invasive predictors of oesophageal varices and their grading in patients with liver cirrhosis.

Patients and Methods: Forty patients with liver cirrhosis were included in this study, with oesophageal varices (OV) and 10 without OV as control group. All patients were subjected to the following: Complete blood count including platelet count & liver function test including serum albumin, upper endoscopy and abdominal ultrasound including liver right lobe longest diameter and spleen bipolar diameter. Child-Pugh’s score was calculated.

Results: The PC/SD ratio is significantly decreased and the RLLD/Alb ratio is significantly increased in the all patients with OV and their subgroups than the control group (p=0.001 for each). A significant negative correlations were found between both PC/SD and RLLD/Alb ratios and the size of OV and Child-Pugh’s score (p=0.001 for each correlation between PC/SD ratio and size of OV and Child-Pugh’s score and p=0.0.16 and 0.011 for correlations between RLLD/Alb ratio and size of OV and Child-Pugh’s score respectively). The sensitivity of PC/SD ratio in the prediction of the size of OV was 96%, the specificity was 91% and the accuracy was 87.2% at a cut off value of 528.6. For RLLD/Alb ratio, the sensitivity was 93%, the specificity was 95% and the accuracy was 96.5% at a cut off value of 4.7.

Conclusion: There is a good correlation between PC/SD and RLLD/Alb ratios and oesophageal varices presence and grades. Further studies are needed on a large number of patients for confirmation of these findings.

Key Words: Oesophageal varices – RLLD/Alb ratio – Spleen diameter – PC/SD ratio.

Introduction

OESOPHAGEAL varices development is among the major complications of liver cirrhosis, with an essential prevalence of approximately 50%. The mortality from each episode of variceal bleeding is 17%-57% [1]. Bleeding episodes can be predicted by the presence of red signs “red cherry spots” on the varices and by the variceal size [2].

The incidence of bleeding can be reduced with non selective beta-blockers [3]. It is also suggested that prophylactic endoscopic variceal ligation can decrease the incidence of first variceal bleeding and the mortality in patients with liver cirrhosis who have large varices [4]. Therefore, annual endoscopic variceal screening is highly recommended for patients with small oesophageal varices while the procedure should be conducted once every two years for patients suffering from liver cirrhosis without diagnosed varices. Nevertheless, repeated endoscopic examinations are unpleasant for patients, and have cost impact on health care insurances, while only half of cirrhotic patients have varices. For these reasons many non-invasive predictors for the presence and size of varices has been studied [5].

The pathophysiologic mechanisms are combined based on the integration of two non-invasive parameters, i.e. platelet count and spleen size into one ratio. Calculation of this ratio is very easy for routine clinical practice [6].

Taking into account the results of previous studies in the field, Alempijevic and colleagues [7] also combined laboratory and ultrasongraphic...
parameters and counted an original ratio. For the first time they reported the value of the right liver lobe diameter/serum albumin concentration in assessment of portal hypertension. They used serum albumin concentration as parameter of liver function in combination with right liver lobe size.

In this study, we compare between platelet count/spleen diameter (PC/SD) ratio and right liver lobe diameter/albumin (RLLD/Alb) ratio as non-invasive parameters in the prediction of oesophageal varices and their grading in patients with liver cirrhosis.

**Patients and Methods**

A case control study conducted on 50 patients with liver cirrhosis admitted to the Gastroenterology and Hepatolgy Department, Al-Azhar University Hospital between April 2011 and April 2012. Forty of them had OV and 10 without OV used as control group. The diagnosis of cirrhosis was based on clinical features including laboratory tests, and imaging diagnosis. Consent was taken from each participant after explanation of the research idea.

We excluded from the study patients with any disease that could influence the spleen and/or liver size and platelet count and/or serum albumin as: Patients on regular use of human albumin infusion, taking beta-blockers and porto-systemic shunts. Patients with hepatic focal lesion, portal vein thrombosis, blood disease or co-existing illness or infection that could influence the liver and/or spleen size were also excluded.

The following was conducted to all patients:

1- **Full medical history with special stress on:**
   - History of liver disease.
   - History of viral hepatitis or other risk factors e.g. blood transfusion, operations ... etc.
   - Upper and lower gastrointestinal bleeding
   - Symptoms of liver cell failure.

2- **Clinical examination with special stress on:**
   - Liver and spleen size.
   - Ascites.
   - Signs of liver cell failure as jaundice, palmer erythema, lower limb edema or encephalopathy ... etc.

3- **Laboratory investigations including:**
   - **Liver function tests:** Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct serum bilirubin, total protein and serum albumin using standard laboratory tests.
   - **Renal function tests:** Serum sodium, serum potassium, serum creatinine and blood urea nitrogen (BUN).
   - **Coagulation profile:** Prothrombin time (PT), and international normalized ratio (INR), by standard laboratory tests.
   - **Complete blood picture including platelet count.**

All patients were classified according to Child-Pugh’s criteria to Child-Pugh’s score A, B&C [8].

4- **Abdominal Ultrasonography:**

   With Hitashi, EUB-5500 Measurements were performed after overnight fasting and the patient in supine position with emphasis on:
   - The right liver lobe diameter (cm).
   - Splenic bi-polar diameter (long axis) (cm).
   - Ascites.
   - Presence of periportal thickening.
   - Portal vein Diameter (mm) and patency.

5- **Upper endoscopy:**

   Using Pentax EG 2940 Scopeto to evaluate the presence of oesophageal varices and its grade. Oesophageal varices classified into 4 grades as follows:
   - Grade (I): Varices at the level of mucosa.
   - Grade (II): Varices smaller than 5mm & filling less than 1/3 of the oesophageal lumen.
   - Grade (III): Varices larger than 5mm & filling more than 1/3 of the oesophageal lumen.
   - Grade (IV): Varices occupying more than 2/3 of oesophageal lumen [9].

6- **Statistical analysis:**

   All collected data were expressed as Mean ± SD and analyzed by using SPSS version 13 using the following tests: Student t-test, Chi-square test, Wilcoxon Rank Sum test, Receiver operating curve (ROC) to detect area under curve (AUC), cut off value (COV) for best sensitivity, specificity and accuracy. $p$-value <0.05 was considered significant and at <0.001 was considered highly significant, while at >0.05 was considered not significant.

**Results**

The age of the studied group ranged from 33-69 years with Mean±SD (52.3±7.76); 26 of them are males & 14 are females and in the control group it ranged from 35-66 years with Mean±SD (44.9±8.7); 4 of them are males & 6 are females (Table 1).
Serum albumin was significantly decreased in the study group than the control group [2.66±0.79 gm/dl vs 3.53±0.42gm/dl respectively (p=0.01)] and platelet count was significantly decreased in the study group than the control group [105.05±30.4 x10^9/L vs 183.4±47.94 x10^9/L respectively (p=0.001)] (Table 2).

According to the results of upper GIT endoscopic findings, cirrhotic patients were classified into 5 subgroups (Table 3):
- Control Group: 10 cirrhotic patients without OV (Fig. 1).
- Subgroup I: 8 cirrhotic patients with grade I OV (Fig. 2).
- Subgroup II: 8 cirrhotic patients with grade II OV (Fig. 3).
- Subgroup III: 12 cirrhotic patients with grade III OV (Fig. 4).
- Subgroup IV: 12 cirrhotic patients with grade IV OV (Fig. 5).

Child-Pugh classification in the control group and the studied subgroups (Table 4 & Fig. 6):

In the control group 90% were classified as Child A, 10% Child B and no one was classified as Child C. In patients with OV, 9 (22.5%) were Child A, 14 (35%) were Child B and 17 (42.5%) were Child C. In subgroup I, 4 (50%) were Child A, 3 (37.5%) were Child B & 1 (12.5%) was Child C. In subgroup II, 3 (%37.5) were Child A, 3 (37.5%) were Child B & 2 (25%) were Child C. In subgroup III, 1 (8.3 %) was Child A, 5 (41.66%) were Child B & 6 (50%) were Child C. In subgroup IV, 1 (8.3%) was Child A, 3 (25%) were Child B & 8 (66.66%) were Child C.

A highly significant positive correlation was found between Child score and oesophageal varices (r=0.462 & p=0.001) (Table 5 & Fig. 7).

The splenic diameter and portal vein diameter were significantly increased between the studied subgroups (Table 6 & Figs. 8,9,10).

There was a significant decrease in PC/SD ratio between the studied subgroups (subgroup I through IV) from 435.446±430.965 in the control group to 355.568±222.343 in subgroup IV (p=0.001) while the RLLD/Alb ratio is significantly increased from 3.67±0.56 in the control group to 5.74±2.02 in subgroup IV (p=0.001) (Table 7 & Figs. 11,12).

A highly significant negative correlation was found between PC/SD ratio and oesophageal varices & Child score (r=-0.645 & -0.394 respectively and p=0.001 for each) (Table 8 & Figs. 13,14) and a significant positive correlation between RLLD/Alb ratio and oesophageal varices & Child score (r=0.305 & =0.432 and p=0.01 6 & =0.01 1 respectively) (Table 8 & Figs. 15,16).

The sensitivity, specificity & accuracy of PC/SD ratio in the prediction of OV was 96 % & 91% & 87.2% respectively with the best cut off value at 528.6 (AUC=0.987), whereas that of RLLD/Alb ratio was 93% & 95% & 96.5% respectively with the best cut off value at 4.683 (AUC=0.953) (Figs. 17, 18 respectively). (Fig. 19) shows a comparison between the sensitivity and specificity of RLLD/Alb and PC/SD ratios in the prediction of OV.

Table (1): Age & Sex distribution of the studied groups.

<table>
<thead>
<tr>
<th>Age Range (yrs)</th>
<th>Study group (n=40)</th>
<th>Control group (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>33±69</td>
<td>35±66</td>
<td>0.011</td>
</tr>
<tr>
<td>Sex: M/F %</td>
<td>26/14</td>
<td>4/6</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table (2): Laboratory data in the control and study groups.

<table>
<thead>
<tr>
<th>Test</th>
<th>Study group (n=40)</th>
<th>Control group (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>64.83±34.08</td>
<td>47.32±27.72</td>
<td>0.02</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>58.36±28.18</td>
<td>44.32±20.39</td>
<td>0.012</td>
</tr>
<tr>
<td>T.Bil (mg/dl)</td>
<td>2.18±1.40</td>
<td>1.23±0.54</td>
<td>0.01</td>
</tr>
<tr>
<td>D.Bil (mg/dl)</td>
<td>0.90±0.69</td>
<td>0.34±0.23</td>
<td>0.001</td>
</tr>
<tr>
<td>T. Protein</td>
<td>6.84±0.95</td>
<td>6.73±0.32</td>
<td>0.124</td>
</tr>
<tr>
<td>S.Alb (g/dl)</td>
<td>2.66±0.79</td>
<td>3.53±0.42</td>
<td>0.01</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>15.91±3.00</td>
<td>12.97±1.54</td>
<td>0.001</td>
</tr>
<tr>
<td>INR</td>
<td>1.62±0.35</td>
<td>1.17±0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.84±0.30</td>
<td>0.81±0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>WBCs (x10^3/ml)</td>
<td>6.89±1.43</td>
<td>5.40±1.78</td>
<td>0.131</td>
</tr>
<tr>
<td>RBCs (x10^6/ml)</td>
<td>3.8±0.71</td>
<td>4.40±0.72</td>
<td>0.01</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.83±1.97</td>
<td>11.78±1.73</td>
<td>0.02</td>
</tr>
<tr>
<td>Platelet (x10^3/ml)</td>
<td>105.05±30.4</td>
<td>183.4±47.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table (3): Upper GIT endoscopic findings in the studied groups.

<table>
<thead>
<tr>
<th>Endoscopy results</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
</tr>
<tr>
<td>Subgroup I</td>
<td>8</td>
</tr>
<tr>
<td>Subgroup II</td>
<td>8</td>
</tr>
<tr>
<td>Subgroup III</td>
<td>12</td>
</tr>
<tr>
<td>Subgroup IV</td>
<td>12</td>
</tr>
</tbody>
</table>
Comparative Study between Right Liver Lobe Diameter/Albumin Ratio

Table (4): Child-Pugh classification in the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Child A</th>
<th>Child B</th>
<th>Child C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=10)</td>
<td>9 (90%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total patients (n=40)</td>
<td>9 (22.5%)</td>
<td>14 (35%)</td>
<td>17 (42.5%)</td>
</tr>
</tbody>
</table>

Study subgroups (n= 40):

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Child A</th>
<th>Child B</th>
<th>Child C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup I (n=8)</td>
<td>4 (50%)</td>
<td>3 (37.5%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Subgroup II (n=8)</td>
<td>3 (37.5%)</td>
<td>3 (37.5%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Subgroup III (n=12)</td>
<td>1 (8.3%)</td>
<td>5 (41.66%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Subgroup IV (n=12)</td>
<td>1 (8.33%)</td>
<td>3 (25%)</td>
<td>8 (66.66%)</td>
</tr>
</tbody>
</table>

Table (5): Correlation between oesophageal varices and Child score.

<table>
<thead>
<tr>
<th>Child score</th>
<th>Pearson correlation (r)</th>
<th>(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophageal varices</td>
<td>0.462</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Fig. (1): Image of normal esophagus without oesophageal lesion.

Fig. (2): Image of grade I oesophageal varices.

Fig. (3): Image of grade II oesophageal varices.

Fig. (4): Image of grade III oesophageal varices.

Fig. (5): Image of grade IV oesophageal varices.

Fig. (6): Child-Pugh classification in the studied groups.

Fig. (7): Correlation between oesophageal varices and Child score.
Table (6): Ultra-sonographic findings in the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Study subgroups</th>
<th>Control Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I (n=8)</td>
<td>Group II (n=8)</td>
<td>Group III (n=12)</td>
</tr>
<tr>
<td>Splenic diameter (cm)</td>
<td>14.13±1.43</td>
<td>14.98±1.83</td>
<td>16.34±0.98</td>
</tr>
<tr>
<td>Rt liver lobe diameter (cm)</td>
<td>13.23±2.48</td>
<td>13.64±2.19</td>
<td>12.54±1.79</td>
</tr>
<tr>
<td>PV diameter (cm)</td>
<td>12.18±1.88</td>
<td>13.85±1.26</td>
<td>15.09±1.98</td>
</tr>
</tbody>
</table>

Table (7): PC/SD ratio & RLLD/Alb ratio in the studied groups and subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Subgroups (n=40)</th>
<th>Control Group (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total patients</td>
<td>Group I (n=8)</td>
<td>Group II (n=8)</td>
</tr>
<tr>
<td></td>
<td>(n=40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC/SD ratio</td>
<td>682.042±274.697</td>
<td>983.014±239.817</td>
<td>777.897±296.798</td>
</tr>
<tr>
<td>RLLD/Alb ratio</td>
<td>4.91±1.42</td>
<td>4.24±0.89</td>
<td>4.75±1.35</td>
</tr>
</tbody>
</table>

Fig. (8): Splenic diameter (cm) in studied groups.

Fig. (9): Right lobe diameter in studied groups.

Fig. (10): Portal Vein diameter in studied groups.

Fig. (11): PC/SD ratio in the studied groups.

Fig. (12): RLLD/Alb ratio in the studied groups.
Table (8): Correlations between RLLD/Alb and PC/SD ratios with the presence of OV and Child score.

<table>
<thead>
<tr>
<th></th>
<th>RLLD/Alb ratio</th>
<th>PC/SD ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>OV</td>
<td>0.305</td>
<td>0.016</td>
</tr>
<tr>
<td>Child score</td>
<td>0.432</td>
<td>0.011</td>
</tr>
</tbody>
</table>

![Fig. (13): Correlation between PC/SD ratio and OV.](image)

![Fig. (14): Correlation between PC/SD ratio and Child Score.](image)

![Fig. (15): Correlation between RLLD/Alb ratio and OV.](image)

![Fig. (16): Correlation between RLLD/Alb ratio and Child score.](image)

![Fig. (17): ROC curve for the best sensitivity and specificity of PC/SD ratio in prediction of OV.](image)

![Fig. (18): ROC curve for the best sensitivity and specificity of RLLD/Alb ratio in prediction of OV.](image)

<table>
<thead>
<tr>
<th>AUC</th>
<th>COV</th>
<th>Sens.</th>
<th>Spec.</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.987</td>
<td>528.6</td>
<td>69%</td>
<td>91%</td>
<td>87.2%</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>AUC</th>
<th>COV</th>
<th>Sens.</th>
<th>Spec.</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.953</td>
<td>4.683</td>
<td>93%</td>
<td>95%</td>
<td>96.5%</td>
</tr>
</tbody>
</table>

Discussion

Egypt has the highest countrywide prevalence of hepatitis C virus infection in the world [10]. The majority of cases develop chronic hepatitis that is usually asymptomatic for years. Twenty percent of those with HCV caused chronic hepatitis progress to cirrhosis and a proportion of them die as a result of liver cirrhosis complications [11]. Oesophageal variceal bleeding is a potentially deadly complication in patients with liver cirrhosis and portal hypertension. In patients with cirrhosis, the incidence of oesophageal varices ranges from 35% to 80%. The risk of initial bleeding from varices is 25% to 35% in 2 years, with most first-bleeding episodes occurring within a year of detection of varices [12]. The mortality from each episode of variceal bleeding is 17%-57% [13].

Bleeding episodes can be predicted by the presence of red signs “red cherry spots” on the varices, and by the variceal size [4]. Therefore, annual endoscopic screening is highly recommended for patients with small oesophageal varices while the procedure should be conducted once every two years for patients suffering from liver cirrhosis without diagnosed varices [14].

The aim of the current study was to compare non-invasive parameters based on ultrasound measurements and laboratory tests (platelet count to spleen diameter (PC/SD) and right liver lobe diameter to serum albumin (RLLD/Alb) ratios that have a potential in assessment of presence and degree of oesophageal varices in patients with liver cirrhosis.

Serum albumin level was found to be significantly lower in patients with OV than that of the control group (p=0.01) (Table 2). These results were similar to the results of some researchers [15,16] which can be explained by the fact that the rate of albumin synthesis is reduced up to 50% in chronic liver disease. Also serum albumin is reduced in cirrhosis due to an elevated distribution volume in haemodilution, particularly in association with ascites [7].

We found in this study that patients with OV showed a significant decrease in platelet count than the control group (p=0.001) (Table 2). This goes with what has been reported in other studies [15,17-19], platelet count of <100,000 can be used as a predictor of OV and of <90,000 is associated with increased risk of having OV by nearly 2.5 folds [20]. Gana and colleagues [18], found that the most accurate noninvasive test used for choosing children for endoscopy to identify oesophageal varices is the platelet count. However, thrombocytopenia was found to be a common and highly specific manifestation of hypersplenism, but with low sensitivity for presence of portal hypertension [21].

Thrombocytopenia associated with chronic liver disease and OV is probably a reflection of the degree of portal hypertension and possibly other factors. Splenic sequestration or antibody-mediated destruction of platelet has been believed to be the cause of thrombocytopenia in patients with cirrhosis. However, other studies have implicated reduced hepatic production of liver-derived thrombopoietic growth factor (thrombopoietin) or rapid degradation and suppressive effects of viruses on bone marrow may also add to thrombocytopenia [20]. It is also indicated that platelet count and thrombopoietin level are returning to referent values following liver transplantation [22]. Other potential explanations for this phenomenon are presence of antithrombocytic antibodies and thrombocyte associated immunoglobulin, which can be found in the sera of patients with liver diseases [23].

The Child-Pugh scoring system is frequently used to assess prognosis and survival of liver cirrhosis [24]. In this study, with advancing Child-Pugh class, the percentage of patients with varices is increased as 22.5% of patients with OV were classified as Child class A (quiescent cirrhosis), 35% were class B (mild liver failure) and 42.5% were class C (advanced liver failure) whereas in the control group (without OV) 90% were class A, 10% was child B and no patient was class C (Table 4 & Fig. 6). These results came in line with the results of Said et al. and Tafarel et al., [15,25].

Also, with increasing the size of OV demonstrated by upper endoscopy, the number of patients increased with the advancement in Child score. A
highly significant positive correlation was also found between Child score and the size of OV ($p=0.001$) (Table 4 & Fig. 6), similar to Kim and coworkers [36] who found that OV was correlated significantly with Child-Pugh classifications B & C ($p=0.001$). However, Mahassadi et al., [27] found that cirrhotic patients with mild liver failure (those in Child-Pugh class B) were at less risk of having large OV, and those with advanced liver failure (those in Child-Pugh class C) had no risk of having large OV, compared to those with quiescent cirrhosis (those in Child-Pugh class A). One plausible explanation is the probable role of toxic effect of herbal remedies frequently prescribed to cirrhotic patients by traditional healers in Africa that might result in liver decompensation even in those with quiescent cirrhosis [28].

Zaman et al., [20] found that platelet count (PC) and Child-Pugh score were factors associated with the presence of OV. Also low PC and palpable spleen or radiologic splenomegaly were accurate to predict the presence of large OV as demonstrated by Peck-Radosavljevic and Sharma [29,30]. These studies however included patients with various etiologies of cirrhosis mainly related to hepatitis C virus or alcohol ingestion.

The ultra-sonographic parameters showed a high significant increase in splenic diameter and PV diameter ($p=0.001$ & $=0.001$ respectively) between control group and studied subgroups (Table 6 & Figs. 8, 9, 10). Splenomegaly is understandably common in patients with cirrhosis [31]. Esmat et al., 2012 [32] found a high statistically significant correlation between the presence and grade of oesophageal varices with the splenic diameter ($p<0.001$). Watanabe and colleagues [33] calculated the splenic index (length X width X height of the spleen) on computed tomography and showed that patients having an index greater than 963 have oesophageal varices, and that a high index may predict oesophageal bleeding in patients with liver cirrhosis. When portal resistance increases in cirrhosis, stagnant portal blood flow causes increased resistance of splenic venous outflow, which leads to congestive splenomegaly. In addition, the increase of splanchnic inflow also causes splenomegaly, which worsens portal hypertension. The increase in portal pressure not only facilitates the formation of oesophageal varices but also aggravates splenomegaly [6,34].

Amarapurkar et al., 1994 [35] reported that splenomegaly alone was a significant predictor for the development of large oesophageal varices. The use of Splenic diameter (SD) to predict the presence of oesophageal varices has two advantages. First, splenic diameter can concomitantly be measured during routine biannual US screening for hepatocellular carcinoma in patients with cirrhosis; hence, it does not add additional costs for detection of the severity of oesophageal varices. Second, measurement of splenic index and mean portal vein diameter (PVD) can be easily performed at the outpatient clinic [36]. However, Mahassadi et al., 2012 [27] found a lower diagnostic accuracy of SD (in Ivorian cirrhotic patients) suggesting that splenomegaly in the African context might not be useful as predictor of OV. In fact chronic parasitism and anemia, more prevalent in Africa due to various etiologies, might result in splenomegaly misleading the diagnostic accuracy of the SD [37]. To some extent, this may explain the finding that the portal vein diameter was not significant to predict OV as previously demonstrated by [20,38]. This is probably related to an underlying portal hypertension which could increase the size of portal vein [27].

In the present study, there was significant decrease in platelet count/spleen diameter (PC/SD) ratio in patients with OV over the control group ($p=0.001$) (Table 7). Also, a highly significant negative correlation was found between PC/SD ratio and the size of OV ($r=0.645, p<0.001$) and Child score ($r=0.394, p<0.001$) (Table 8 & Figs. 13,14). These results were similar to other studies [6,7,15] which suggested the PC/SD ratio as informative while Sharma and Aggarwal, 2007 [30] have proposed PC/SD as a predictor of oesophageal varices. However, Giannini et al., 2003 [17] proved that this parameter was independent of the Child-Pugh classification thus allowing a more confident use even in patients with compensated disease.

The PC/SD ratio was chosen by Giannini and colleagues [17] in the prediction of OV in patients with cirrhosis as it allows identifying the degree of thrombocytopenia which most likely depends on hypersplenism. The use of this ratio is of interest and is not redundant, and this hypothesis is supported by a number of both clinical and statistical reasons. Firstly, from a clinical point of view, platelet count may decrease for several reasons in patients with chronic liver disease [39]. Thus the use of platelet count alone as a non-invasive predictor of OV can be misleading and cannot be solely attributed to portal hypertension. So, the use of the PC/SD ratio bypasses this possible drawback since it “normalizes” platelet count to splenic sequestration. Secondly, from a statistical point of view, the PC/SD ratio was the only parameter independently associated with the presence of OV [17]. Financially, the ratio is easy to calculate and can be used at the bedside. Biannual calculation
of the ratio will not generate additional costs in
the management of cirrhotic patients, because
platelet count is assessed routinely and abdominal
ultrasonography is usually performed at least semi-
annually to monitor hepatocellular carcinoma [6].
In fact, spleen bipolar measurements consistently
show high reproducibility and low intra- and inter-
observer variability [23,40].

In the current study, the sensitivity of PC/SD
ratio was 96%, specificity was 91% and the accu-
racv of the test was 87.2% under the receiver
operating characteristic curve (area under the curve
(AUC) = 0.987) with the best cut off point value
at 528.6. Four patients (out of 100) with OV would
have been missed by application of PC/SD ratio.
In comparison to our results, Giannini et al., 2003
[17] in a study of 145 patients with cirrhosis found
that the negative predictive value of PC/SD ratio
for presence of varices was 100% with 100% sensitivity and 71% specificity (lower than ours)
at a cut off value of 909 and concluded that the
PC/SD ratio is the only parameter which is inde-
pendently associated with the presence of OV. In
2006, Giannini and coworkers [6] found that PC/SD
ratio yielded high diagnostic accuracy to predict
the presence of OV. This was confirmed by Agha
et al. [41], using the same cut off of 909. Esmat &
Rashid, [42] found that the PC/SD ratio gave the
highest accuracy (94%) at a cut-off value of
1326.58. Patient having the ratio greater than cut-
of value should not receive nonselective beta-
blockers prophylactic therapy because they are
less likely to develop oesophageal varices and they
should less frequently undergo endoscopy [43].

However, Said et al., [15] found that the PC/SD
was a predictor for presence of varices with best
cut off value of 415 where the sensitivity was
(80%) and the specificity was (60%) which are
much lower than our results. Sen and Griffiths,
2008 [44] found, however, that the PC/SD ratio is
a simple clinical predictor based on the best cut-
of values obtained individually for platelet count
and spleen size was the most sensitive predictor
of the presence of OV.

Using a PC/SD ratio with a cut-off value of
909, yielded low negative and positive predictive
values of only 73% and 74% respectively [45]. Also
in their study [46], the sensitivity of PC/SD ratio
to predict OV was 77.5%, the specificity was
45.5%, the positive predictive value was 79.5%,
the negative predictive value was 42.6% and the
accuracy was 68.9%. Besides this, in their multi-
ivariate analysis, PC/SD ratio did not even correlate
with the existence of OV. This could imply that
the association between both variables in the
univariate analysis could have been influenced by
the association between a low platelet count and
the presence of OV, since this was the only variable
related to OV after the multivariate analysis. So,
the authors do not consider PC/SD ratio to be an
adequate index to predict the existence of OV in
cirrhotic patients and they must be submitted to a
screening endoscopy in order to verify for the
presence of OV at the moment of their diagnosis
[45,46].

Together with other researchers [47], we found
that the RLLD/Alb ratio had a significant positive
correlation with the size of OV and Child score
(Table 8 & Figs. 15,16). The sensitivity & speci-
city of RLLD/Alb ratio was 93% & 95% respecti-
vatively and the accuracy of the test was 96.5% with
the best cut off point value at 4.683 (AUC=0.953).
Seven patients (out of 100) with OV would have
been missed by application of RLLD/Alb ratio.
Alempijevic et al., 2007 [7] found that the sensitivity
of RLLD/Alb ratio was 83.1% and the specifici-
y was 73.9% the best cut-off value of 4.425. The
RLLD/Alb ratio may, however, prove to be more
consistently reliable in cirrhosis [44].

When we compare between PC/SD ratio and
RLLD/Alb ratio in predicting the presence of OV
we found that the PC/SD ratio had more sensitivity
but lower specificity and accuracy than did
RLLD/Alb ratio (Figs. 17,18,19), although the two
ratios had good correlations with the grade of the
OV(Table 8 & Figs. 13,15). So, PC/SD ratio had
a higher sensitivity, even at the cost of a lower
specificity which is important to ensure that patients
with varices are not missed [44].

In conclusion, platelet count/spleen size and
right liver lobe diameter/serum albumin concentra-
tion ratios are non-invasive parameters that can
provide accurate information pertinent to determi-
nation of the presence and grade of oesophageal
varices in patients with liver cirrhosis. Despite a
good correlation between these ratios and oesoph-
geal varices grade, it is unlikely that these ratios
could be used to exclude patients from initial
endoscopic screening. Nevertheless, these ratios
may serve for selection of patients who need more
frequent endoscopies. These ratios will help identify
patients at higher risk for development of oesoph-
geal varices. It will provide insight into the rela-
tionship between clinical, biochemical, hematol-
ogical and imaging abnormalities and development
of clinically significant oesophageal varices. Further
validation of the results will be achieved through
long-term follow-up of the patients and a larger
number of studied subjects.
Comparative Study between Right Liver Lobe Diameter/Albumin Ratio

References


Neoadjuvant Docetaxel (Taxotere) Plus Cisplatin and 5-Flurouracil Followed by Concomitant Chemo radiotherapy in Patients with Locally Advanced Squamous Cell Carcinoma of the Head and Neck

The Departments of Clinical Oncology & Nuclear Medicine, Faculty of Medicine, Mansoura University

Abstract

Background: Concomitant chemoradiotherapy (CRT) has become the standard of care for patients with inoperable head and neck squamous cell carcinoma (HNSCC). More recently, induction chemotherapy (IC) has been adopted as an approach in the management of these patients. The primary objective of this study is to evaluate PFS (progression-free survival) after sequential therapy, (taxotere, cisplatin, and 5-FU induction therapy followed by concomitant CRT) in patients with locally advanced HNSCC. Secondary objectives include: overall response rate (ORR) to treatment, duration of response, overall survival (OS) and safety profile.

Patients and Methods: 30 patients with stage III-IV M0 HNSCC, Eastern Cooperative Oncology Group performance status (ECOG PS) of zero to one were prospectively treated with 3 courses of TPF induction chemotherapy followed by concomitant CRT. IC consisted of Docetaxel (Taxotere) 75mg/m\(^2\) day 1 followed by cisplatin 75mg/m\(^2\) day 1 and 5-Flurouracil 750mg/m\(^2\) day 1-5 continuous infusion, given every 3 weeks. Concomitant CRT started 4 weeks after the last cycle of chemotherapy with the goal of delivering a total dose of 66Gy concomitant with cisplatin 80mg/m\(^2\) for 2 cycles during RT.

Results: At a median follow-up period of 18 months (ranging from 8 to 30m), the median PFS was 15 months, the PFS was 46.7% and the OS was 53.3% while the median OS was not reached. The one year PFS and OS were 70% and 90% respectively. The overall response rate (ORR) was 90%. Median duration of CR was 20 months and median duration of ORR was 15 months. The most common grade III toxicity during IC was neutropinic fever (13.3 %).

Conclusions: Induction TPF is active, feasible, well tolerated, with acceptable toxicity and does not compromise the delivery of subsequent CRT in patients with ECOG PS of 0-1.

Key Words: Induction chemotherapy – Concomitant chemoradiation – Head and neck cancer.

Introduction

TUMORS arising from the head and neck are the seventh most common neoplasms worldwide. In 2010, an estimate of 634,760 new cases were diagnosed and 356,705 deaths occurred secondary to these tumors globally [12]. Most (about 90%) of these cases are squamous cell carcinomas (SCC) that arise from the upper aerodigestive tract [3].

The most important risk factors are tobacco and alcohol consumption and the combined exposure to alcohol and tobacco has a synergistic effect on carcinogenesis [4]. Other risk factors for oral cancer are snuff or chewing tobacco and chronic use of betel nut (paan). Marijuana use is also considered to be a potential risk factor [8]. Human papilloma virus (HPV) is a recently appreciated cause of head and neck squamous-cell carcinoma (HNSCC). It encompasses many different subtypes, with HPV- 16 and HPV- 18 being the most common oncogenic variants in HNSCC [6].

About two-thirds of patients with HNSCC present with advanced stage disease, commonly involving regional lymph nodes. Distant metastases at initial presentation are uncommon, arising in only 10% of patients [7].

The low overall cure rate in combination with a poor functional outcome in a significant percentage of patients with locally advanced HNSCC has led to alternative strategies of management. Historically, this has consisted of surgery and postoperative radiotherapy (RT) or RT alone for unresectable disease. These efforts yielded low locoregional control rates and 5-year survival rates from 10% to 40% [8].

The addition of chemotherapy to locoregional treatment has revolutionized the treatment of patients with locally advanced HNSCC. In the early 1990s, the focus shifted to administering chemotherapy concomitantly with radiation, to take advantage of the radiation enhancing properties of cytotoxics active in HNSCC. Evidence from a large
meta-analysis of individual patient data from randomized trials demonstrated that adding chemotherapy led to a real survival advantage of around 4% at 5 years over that seen with locoregional treatment alone [9,10].

More recently, efforts have focused on testing induction chemotherapy followed by concurrent chemoradiation (CRT) to improve organ preservation rates, reduce locoregional and distant failure and improve overall survival. Cisplatin-based induction chemotherapy doublets have been generally used as induction chemotherapy, and cisplatin plus 5-fluorouracil (5-FU), PF, has become a common treatment standard. The addition of a taxane to induction chemotherapy, in the form of a cisplatin-taxane doublet, or, more frequently, a taxane, cisplatin, and 5-FU triplet, has improved the activity of induction chemotherapy. Several trials have shown very promising results and confirmed the superiority of the triplet docetaxel, cisplatin, and 5-FU (TPF) regimen over PF, followed by RT or CRT, in terms of response rate (RR), overall survival (OS) and progression free survival (PFS) [11-13]. These data are supported by a meta-analysis of randomized clinical trials, in which a direct comparison showed an overall and progression-free survival advantage for docetaxel and cisplatin-based induction chemotherapy over PF induction chemotherapy [14]. However, all of these randomized trials compared two different induction regimens and were not designed to compare induction therapy or sequential treatment to chemoradiation alone. Many large randomized phase III trials comparing TPF induction chemotherapy followed by chemoradiation to chemoradiation alone are currently planned or underway. GSTTC, (the Gruppo di Studio sui Tumori della Testa e del Collo) conducted a randomized phase II trial of induction TPF followed by CRT or CRT alone. CR rates (primary end point) were higher with TPF (50% versus 21.2%, p=0.004). Median progression-free survival and overall survival were also higher with TPF (30.4 vs 19.7 and 39.6 vs 33.3 months respectively). Hematologic and non-hematologic toxic effects during CT/RT were similar in the two arms [15].

The primary objective of this study is to evaluate PFS after sequential therapy, (taxotere, cisplatin, and 5-FU induction therapy followed by concomitant CRT) in patients with locally advanced HNSCC. Secondary objectives include; overall response rate to treatment, duration of response, overall survival and safety profile.

**Patients and Methods**

This study was conducted at Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Mansoura University over the period from August 2010 to April 2013.

Patients aged 18-65 years were eligible if they had histological or cytological proven stage III-IV M0; inoperable SCC of the head and neck (oral cavity, oropharynx, nasopharynx, hypopharynx, larynx or nasal/paranasal); one or more measurable lesion; Eastern Cooperative Oncology Group performance status (PS) of zero to one [16]; adequate hematologic, hepatic, and renal function; and no prior chemotherapy, radiotherapy, or surgery for HNSCC. Inoperability criteria were technical un-resectability (tumor fixation/invasion to either base of the skull, cervical vertebrae, nasopharynx, or fixed lymph nodes), low surgical curability (T3-T4, N2-N3 excluding T 1 N2) as assessed by an experienced surgeon, and organ preservation.

Exclusion criteria include; pregnant or lactating women; previous or other malignancies at other sites; any prior treatment with radiotherapy or chemotherapy; symptomatic peripheral neuropathy or altered hearing ≥grade 2; patients with contraindications to use of prednisone and patients with other serious illness or medical conditions such as unstable cardiac disease, history of significant neurological or psychiatric disorders, active un-controlled infection, and acute peptic ulcer. Patients were required to provide oral informed consent before inclusion in the study.

**Treatment plan:**

After complete diagnosis and staging work-up, patients started IC (TPF) Taxotere 75mg/m² (1 hour IV infusion, D1) followed by cisplatin 75mg/m² (30-minutes IV infusion with adequate hydration, D1) and 5-FU 750mg/m²/day (continuous IV infusion, D1-5, starting after cisplatin) repeated every 3 weeks-up to a total of 3 cycles followed by concomitant RT 66Gy (using two-dimensional conventional techniques in two phases, 200Gy/F) and cisplatin 80 mg/m² D1& D28 (2 cycles during the radiotherapy course) with a minimum interval of 4 weeks after the last cycle of induction chemotherapy. During IC, the dose of the next cycle was reduced by 25% in patients with grade III-IV toxicities. G-CST (100 gg/day) was given for grade III-IV neutropenia or febrile neutropenia. Patients was considered evaluable for response if he or she received 2 cycles of induction chemotherapy and all his or her baseline lesions have been assessed. Patients with progression...
before cycle 2 will be evaluable as early progression. Tumor assessment to IC was done clinically and radiologically just before CRT.

All patients received the following premedications: 5-HT3 (5-hydroxytryptamine) antagonists and H2 (histamine 2) blockers 30 minutes before chemotherapy; dexamethasone 8mg/12 hours, IV for 3 days, one day before and 2 days after docetaxel infusion; adequate hydration with normal saline solution three liters on day 1; calcium gluconate, magnesium sulphate and potassium chloride ampoules before cisplatin infusion (IV); and mannitol 20% 200cc IV push after cisplatin infusion. Antibiotic prophylaxis (oral ciprofloxacin 500mg twice daily) was given after each cycle (days 5-15).

Patients then followed clinically every 2 months in the 1st year and every 3 months in the 2nd year (including endoscopy). CT scan or MRI was performed every 3-6 months (unless suspicion of progression) up to the time of documented progression, death or lost follow-up.

End points (objectives):

The primary objective of this study is to evaluate PFS after sequential therapy (TPF followed by concomitant CRT) in patients with locally advanced HNSCC. Secondary objectives include, overall response rate, duration of response, overall survival and safety profile. PFS was calculated from the date of inclusion to the date of progression or date of death whichever occurs first. OS was calculated from the date of inclusion to the date of death. Response rate was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) [17]. Clinical examination and toxicities will be graded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE, v3.0) [18].

Statistical analysis:

At the end of follow-up, the patients’ clinico-pathological and outcome data were collected, and subjected to statistical analysis using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data of scores were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher’s exact test) was used to examine the relation between qualitative variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Univariate cox regression was done to calculate the hazard ratio for the different prognostic factors. Binary logistic regression was used to determine significant predictors for the outcome. A p-value <0.05 was considered significant.

Results

Patient characteristics:

Thirty patients with histologically confirmed diagnosis of locally advanced HNSCC presented to Clinical Oncology and Nuclear Medicine Department at Mansoura University Hospital over the period from August 20 10 to January 2012 were included in this study. Baseline clinico-pathological characteristics of the patients are shown in (Table 1). The median age for all patients was 52 years (ranged from 20-65) with a mean value (±SD) of 49.63 (± 14.66) years and male to female ratio of 5:1.

Table (1): Baseline clinico-pathologic characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No=30 (%)</th>
<th>(frequency) (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td>Mean±SD</td>
<td>52.0 (20-65)</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>49.63±14.66</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>83.3</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>PS:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26</td>
<td>86.7</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Smoking:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ ve</td>
<td>19</td>
<td>63.3</td>
</tr>
<tr>
<td>− ve</td>
<td>11</td>
<td>36.7</td>
</tr>
<tr>
<td>Site:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>Larynx</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Nasal/paranasal</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Grade:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G I</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>G II</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>G III</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>6</td>
<td>20.0</td>
</tr>
<tr>
<td>Stage G:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>IV A</td>
<td>11</td>
<td>36.7</td>
</tr>
<tr>
<td>IV B</td>
<td>3</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Response to treatment and survival:

After IC, 5 patients (16.7%) had complete response (CR), 20 patients (66.6%) had partial response (PR) and 5 patients (16.7%) had stable disease (SD). Overall response rate (PR+CR) to IC was 83.3%.

Following IC/CRT, 11 more patients went into CR making a total of 16 patients (53.3%) in CR.

Inas I. Abdelhalim, et al.
Eleven patients (36.7%) had PR and 3 patients (10%) had SD. Pathological CR was confirmed in 3 patients. The overall response rate at the end of the protocol was 90%. Median duration of CR was 20 months (ranged from 10-30 months) and median duration of ORR was 15 months (ranged from 8-30 months).

Response to treatment (CR, PR, and SD) after IC/CRT for tumors in different sites in head and neck is summarized in (Table 2). All cases of oropharyngeal SCC had PR, progressed locally, died in the first 9-14 months of follow-up and had the worst prognoses than other sites.

<table>
<thead>
<tr>
<th>Primary site of tumor</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharynx</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Larynx</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Nasal/paranasal</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Total/% 17/56.7 10/33.3 3/10 30/100

At a median follow-up period of 18 months (ranging from 8 to 30 months), the median PFS was 15 months, mean PFS was 20.71 months (95% CI of 17.37-24.05) and the overall PFS was 46.7% (Fig. 1).

At the end of the study there were 16 patients alive and 14 patients died. The OS was 53.3%, mean OS was 22.17 (95% CI of 19.12-25.22) while the median OS was not reached (Fig. 2). The one year PFS and OS were 70% and 90% respectively.

Toxicity:

During induction TPF, 27 patients (90%) completed all planned there cycles of IC while 3 patients (10%) received only 2 cycles (these 3 patients developed NF and refused to receive the 3rd cycle). Grade III toxicities were seen in 26.7% (8 patients) and include neutropinic fever 13.3% (4 patients), mucositis 10% (3 patients) and diarrhea 6.7% (2 patients). Age of the patients was an important factor in determining the toxicity to IC. In this study 13 patients (44.3%) were &gt; 60 years and had grade III toxicities more than patients &lt;60 years with Odds ratio of 6.43, 95% CI 1.03 to 40.26 and a significant p-value of 0.035.

During CRT, all patients completed the scheduled total dose of radiation therapy (66 Gy/33 F, 5F/w). Grade III toxicities include mucositis which were seen in 3 patients (10%).

As a late toxicity, grade III laryngeal oedema was seen 1 patient (3.3%).

Univariate analysis of different factors affecting outcomes:

The following factors: Age, P.S (0 vs 1), grade, stage, IC dose reduction, IC interruption, interruption of RT period and response to treatment were analyzed and statistically evaluated in relation to RR and PFS.

RR:

When analyzing those factors using Chi-Square test and OR (Odds Ratio); factors associated with a statistically significant impact on RR include tumor stage and interruption of RT period (Table 3).

Patients with stage III disease showed higher CR rate than patients with stage IV disease with
an odds ratio of 0.005, 95% CI of 0.000-0.090 and a significant \( p \)-value of <0.001.

Also patients with no RT delay showed higher CR rate than patients with RT delay with an odds ratio of 0.018, 95% CI of 0.002-0.194 and a significant \( p \)-value of <0.001.

**PFS:**

When analyzing those factors using Kaplan-Meier Log-Rank test and Cox regression analysis, factors associated with a statistically significant impact on PFS include also tumor stage and interruption of RT period in addition to CR response to treatment. Patients with CR to treatment (IC/CRT) had a better PFS than patients with PR or SD with odds ratio of 10.833, 95% CI of 1.961-59.834 and a significant \( p \)-value of 0.004 (Table 4).

Other factors including: age, ECOG PS (0 vs 1), grade, IC dose reduction and IC delay had no significant impact on RR and PFS.

**Logistic regression for predictors of disease progression:**

Binary logistic regression model was used to determine the predictors of disease progression. The dependent variable was the progression (progressed or non-progressed) while the independent variables were: age, ECOG PS (0 vs 1), grade, stage, IC dose reduction, IC interruption, interruption of RT period and RR to treatment.

### Table (3): Univariate analysis of different factors affecting RR (CR vs no CR).

<table>
<thead>
<tr>
<th>Factor</th>
<th>RR</th>
<th>Odds R</th>
<th>95% CI</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: &lt;60y vs &gt;60y</td>
<td>CR</td>
<td>11 (64.7%)</td>
<td>6 (35.3%)</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>5 (38.5%)</td>
<td>8 (61.5%)</td>
<td></td>
</tr>
<tr>
<td>ECOG PS: 0 vs 1</td>
<td>CR</td>
<td>15 (57.7%)</td>
<td>11 (42.3%)</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td></td>
</tr>
<tr>
<td>Grade: low G (I, II) vs high G (III, undifferentiated)</td>
<td>CR</td>
<td>6 (35.3%)</td>
<td>11 (64.7%)</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>10 (76.9%)</td>
<td>3 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>Stage: III vs IV</td>
<td>CR</td>
<td>15 (93.8%)</td>
<td>1 (6.2%)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>1 (7.1%)</td>
<td>13 (92.9%)</td>
<td></td>
</tr>
<tr>
<td>IC dose reduction: 3 vs 2 cycles</td>
<td>CR</td>
<td>14 (51.9%)</td>
<td>13 (48.1%)</td>
<td>0.538</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>IC interruption (delay): No delay vs delay</td>
<td>CR</td>
<td>12 (50%)</td>
<td>12 (50%)</td>
<td>2.000</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>RT delay: No delay vs delay</td>
<td>CR</td>
<td>3 (92.9%)</td>
<td>1 (7.1%)</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>No CR</td>
<td>3 (18.8%)</td>
<td>13 (81.2%)</td>
<td></td>
</tr>
</tbody>
</table>

### Table (4): Univariate analysis of different factors affecting PFS.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds R</th>
<th>95% CI</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: &lt;60y vs &gt;60y</td>
<td>0.468</td>
<td>0.107-2.046</td>
<td>0.310</td>
</tr>
<tr>
<td>ECOG PS: 0 vs 1</td>
<td>0.733</td>
<td>0.089-6.041</td>
<td>0.773</td>
</tr>
<tr>
<td>Grade: Low (G I, II) vs High G (III, Undifferentiated)</td>
<td>2.531</td>
<td>0.557-11.512</td>
<td>0.225</td>
</tr>
<tr>
<td>Stage: III vs IV</td>
<td>0.092</td>
<td>0.017-0.510</td>
<td>0.004</td>
</tr>
<tr>
<td>IC dose reduction: 3 cycles vs 2 cycles</td>
<td>0.625</td>
<td>0.050-7.749</td>
<td>0.713</td>
</tr>
<tr>
<td>IC interruption (delay): No delay vs Delay</td>
<td>1.692</td>
<td>0.259-11.065</td>
<td>0.580</td>
</tr>
<tr>
<td>RT delay: Yes vs No</td>
<td>0.164</td>
<td>0.032-0.834</td>
<td>0.024</td>
</tr>
<tr>
<td>RR to treatment: CR vs PR, SD</td>
<td>10.833</td>
<td>1.961-59.834</td>
<td>0.004</td>
</tr>
</tbody>
</table>

### Table (5): Binary logistic regression analysis for predicting disease progression.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Score</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: &lt;60y vs &gt;60y</td>
<td>0.074</td>
<td>0.785</td>
</tr>
<tr>
<td>ECOG PS: 0 vs 1</td>
<td>0.084</td>
<td>0.773</td>
</tr>
<tr>
<td>Grade: Low G: I, II vs High G: III, Undifferentiated</td>
<td>1.475</td>
<td>0.225</td>
</tr>
<tr>
<td>Stage: III vs IV</td>
<td>19.201</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IC dose reduction: 3 cycles vs 2 cycles</td>
<td>2.549</td>
<td>0.110</td>
</tr>
<tr>
<td>IC interruption (delay): No delay vs Delay</td>
<td>0.136</td>
<td>0.713</td>
</tr>
<tr>
<td>RT delay: Yes vs No</td>
<td>14.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RR to treatment: CR vs PR, SD</td>
<td>19.201</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
The results showed that the significant predictors of progression include tumor stage, RT delay and RR to treatment (Table 5).

Discussion

Adding IC to concurrent CRT, otherwise known as sequential chemoradiotherapy, remains controversial due to lack of published randomized Phase III data demonstrating its superiority over concurrent CRT. However, encouraging results from the TAX 323 and TAX 324 studies have generated renewed interest in the use of IC in HNSCC [12, 13].

There are concerns that the toxic effects of IC may compromise or even preclude the delivery of subsequent RT [19,20]. Prolongation of RT duration through treatment interruption and failure to complete definitive RT have a significant adverse impact on locoregional tumour control and survival in HNSCC [21].

Multiple phase III randomized trials investigated the addition of taxane to PF as IC before definitive treatment also reported higher response rate with the addition of taxane. In Spanish Head and Neck Cooperative Group trial [11], the CR rate and ORR after induction PCF (Paclitaxel, cisplatin and 5-FU) were 33 and 80%. After IC/CRT, they were 88 and 98% respectively. In the Tax 323 [12] and Tax 324 [13] trials the ORR in induction-TPF/CRT arms was 72% for each trial although, Tax 323 trial used RT with altered fractionation and no concurrent chemotherapy and Tax 324 trial used weekly carboplatin during RT. In our study, the radiologic CR after TPF induction chemotherapy was 16.7%, PR was 66.6% and stable disease was 16.7%. The radiologic ORR was 83.3%. After induction-TPF/CRT, CR was 53.3%, PR was 36.7% and stable disease was 10%. The radiologic ORR at the end of treatment was 90%.

In this trial, at a median follow-up period of 18 months (ranging from 8 to 30 months), 16 patients (53.3%) were progressed or died. Of these patients, 11 progressed locally and 3 patients developed distal metastases. The remaining 2 patients died without de-commended recurrence. The median PFS was 15 months and the overall PFS was 46.7%. The one year PFS was 70% (Fig. 1).

In the sequential therapy arm (TPF followed by CRT) of Italian Collaborative Group Study [15], the 2 years PFS was 55.6% which was better than that in this study (46.7%). This may be due to smaller size of population in our study (30 vs 50 patients) or more RT delay. In our study the median duration of RT delay was 7 days ranging from 7-21 days; while in Paccagnella et al trial the median duration of of RT delay in TPF sequential arm was 7 days ranging from 3-10 days.

In addition the survival advantage in Paccagnella et al trial may be due to surgical intervention. Radical surgery was used in case of residual disease after IC/CRT and prophylactic neck dissection for patients with initial stage of N2-N3.

The one year OS rate was 90% which was nearly equal to 1-year survival result of TPF arm obtained by Italian Collaborative Group study [15] which was 86%. Survival at 18m in our study was dropped to 53.3% while, at Italian Collaborative Group Study dropped to 61% at 2 years indicating a better survival out come in Italian Collaborative Group Study possibly due to causes mentioned above.

Prophylactic G-CSF was not given during IC in this study like some other trials and grade III toxicities were seen in 26.7% (8 patients of all 30). NF was the most common and developed in all neutropinic patients; 13.3% (4 patients) followed by mucositis; 10% (3 patients) and diarrhea; 6.7% (2 patients). During CRT, grade III toxicities include mucositis 10% (3 patients) and severe laryngeal edema as a late toxicity in 3.3% (one patient). In Italian Collaborative Group trial [15], the most common grade 3-4 toxicity during IC was neutropenia (52%; 26 of 44 assessable patients), with 8% (n=4) experiencing febrile neutropenia followed by alopecia (18%), stomatitis/mucositis (6.0%), and nausea (4.3%). During CRT, Grade III toxicities include mucositis/stomatitis (27.7%), skin toxicity (18.6%), and dysphagia (20.7%).

Conclusions:

Induction chemotherapy using TPF followed by CRT is active, feasible, well tolerated, with manageable toxicities and provide a higher overall response rate in patients with locally advanced SCC of the head and neck.

References


High-Risk Pregnancy and its Outcome among Women Attending Antenatal Clinics in Abha, Saudi Arabia

AESHA FARHEEN, M.D.
The Department of Family & Community Medicine, King Khalid University, Abha, Saudi Arabia

Abstract

Background: Pregnancy can be complicated by presence of risk factors. These risk factors are detrimental to maternal and fetal health and can lead to adverse pregnancy outcomes.

Objectives: To identify distribution of at-risk cases in the risk subgroups; To compare the selected outcomes in the risk group with the non-risk group women; and To compare differences in outcome within the risk subgroups.

Material and Methods: A Cross-sectional study was carried out on women who had already delivered during 2012. Their records were studied to classify them into various risk subgroups. Selected outcomes (Caesarean section, abortion and birth weight) were compared between the risk and non-risk group as well as within the risk subgroups.

Results: Most women had a single risk factor in current (40.1%) or in previous pregnancy (31.5%). Multiple risk factors were found in (16.1%) of women. Women with risk in current pregnancy had significantly higher rate of abortion (91.7%). They also had higher rate of low birth weight (55.8%) and macrosomic babies (42.8%). Higher rate of delivery by Caesarean section (50.9%) was found in women with risk in previous pregnancies.

Conclusions: Having a risk factor in a previous pregnancy leads to a higher rate of Caesarean section. Having a risk factor in the current pregnancy increases the probability of abortion, and delivering low birth weight or high birth weight babies.

Key Words: Risk factors – Pregnancy – Abortion – Outcome – Low birth weight – Saudi Arabia.

Introduction

A HIGH-RISK pregnancy is one in which some condition puts the mother, the developing fetus, or both at higher-than-normal risk for complications during or after the pregnancy and birth [1]. A completely normal physiological state like young age (> 16 years) or age <3 5 years could be a reason of high-risk pregnancy. A complication or adverse outcome in previous pregnancies (e.g. pre-eclampsia or stillbirth) or an obstetric or medical cause in the current pregnancy (antepartum hemorrhage or gestational diabetes) makes the pregnancy a high-risk one.

Studies have shown that having a bad obstetric history [2,3], being at extremes of reproductive age [4-6] and grand multiparity [7] adversely affect the outcome. Obesity during pregnancy is now a common condition [8]. Studies have confirmed the risk of adverse outcome in obese mothers [9,10]. Hypertension and diabetes during pregnancy can result in several adverse outcomes including, fetal growth restriction, premature birth, placental abruption, and stillbirth [11,12]. Severe medical illness including cardiac or renal disease leads to adverse outcomes [13].

There is a dearth of information regarding the high-risk pregnancies in Saudi Arabia. Evidence suggests that the prevalence rates for chronic illnesses in the general population in Saudi Arabia are on the rise [14]. Consequently, this may affect women within the reproductive age group, and will definitely affect the high-risk pregnancy rates and adverse birth outcomes.

This study was conducted to enrich the existing information on this subject. The aim of the study was to find out the pattern of high-risk among pregnant women and to assess the effect of risk on outcome of pregnancy.

Material and Methods

This study was carried out at the Antenatal care (ANC) clinics of Al-Manhal, Al-Mansak and Al-Wasat primary health care centers in Abha City, Southwestern Saudi Arabia.
The Kingdom of Saudi Arabia follows the WHO guidelines for basic antenatal care. Each woman at the booking visit is issued an antenatal card and all details of current and past pregnancies are recorded in it. The card outlines the criteria for assessment of high risk pregnancy. A data-driven form was developed to collect the data from the ANC cards [18].

The risk group included women who had delivered during 2012 (n=200) and had any risk factor while the control group comprised women who had delivered in the same time period and had absence of any high risk (n=200). Eventually, the sample size comprised 162 cases and 197 controls due to missing information.

In the antenatal care card, assessment of risk is based on obstetric complications and medical illnesses in previous and current pregnancies. There are 17 criteria of which the presence of any single risk factor classifies the woman as a high-risk case.

For the purpose of this study, women with risk factor(s) were classified into 5 subgroups based on number of risk factors (single or multiple) and timing of risk (previous or current pregnancy). The subgroups were; single obstetric risk factor in previous pregnancy, multiple obstetric risk factors in previous pregnancy, single obstetric risk factor in current pregnancy, multiple obstetric risk factors or medical illness in current pregnancy, single/multiple obstetric risk factors/medical illness in both previous and current pregnancy.

Outcomes of pregnancy were: Birth outcome (livebirth, abortion, perinatal death), methods of delivery and birth weight. Data were analyzed using SPSS 17.0.

**Results**

Table (1) describes the distribution of risk subgroups among the study sample. It reveals that more women had illness or obstetric complications in the current pregnancy 65 (40.12%) than in previous pregnancy 51 (31.48%). Multiple risk factors were found in 20 women, 10 (6.2%) each in previous and current pregnancy. Twenty six women (16.1%) had risk in previous as well as in current pregnancy.

Table (2) describes the distribution of outcome by type of risk. Considering the delivery method, it was observed that the Caesarean section was high (62.5%) in the risk group compared to non-risk group. Abortion, as a birth outcome, was higher in the risk group (54.5%). Proportions of low birth weight (53.1%) and macrosomia (77.8%) were higher among the risk group.

Table (3) shows the comparison of outcomes within the risk groups. To compare the outcomes within the risk groups, 5 risk subgroups were reclassified into three groups as risk factor(s) in previous pregnancy, risk factor(s) in current pregnancy, and risk factor(s) in both previous and current pregnancy. Comparison of outcome within the study group showed that risk of Caesarean section was higher (50.9%) in the group of women with history of risk in previous pregnancy. Abortion was significantly higher in women with current pregnancy risk ($p<0.001$). Comparison of birth weight showed that more low birth weight babies (55.8%) as well as large babies (42.8%) were born to women with risk in current pregnancy.

<table>
<thead>
<tr>
<th>Risk subgroups</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single obstetric risk factor in previous pregnancy</td>
<td>51 (31.48)</td>
</tr>
<tr>
<td>Multiple obstetric risk factors in previous pregnancy</td>
<td>10 (6.17)</td>
</tr>
<tr>
<td>Single obstetric risk factor or medical illness in current pregnancy</td>
<td>65 (40.13)</td>
</tr>
<tr>
<td>Multiple obstetric risk factors or medical illness in current pregnancy</td>
<td>10 (6.17)</td>
</tr>
<tr>
<td>Single or multiple obstetric risk factors/illness in both previous and current pregnancy</td>
<td>26 (16.05)</td>
</tr>
<tr>
<td>Total</td>
<td>162 (100.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Non Risk n (%)</th>
<th>Risk n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods of Delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal delivery</td>
<td>154 (61.8)</td>
<td>95 (38.2)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>33 (37.5)</td>
<td>55 (62.5)</td>
</tr>
<tr>
<td>Birth outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live birth</td>
<td>185 (55.6)</td>
<td>148 (44.4)</td>
</tr>
<tr>
<td>Abortion</td>
<td>10 (45.5)</td>
<td>12 (54.5)</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight</td>
<td>38 (46.9)</td>
<td>43 (53.1)</td>
</tr>
<tr>
<td>Normal</td>
<td>157 (58.4)</td>
<td>112 (41.6)</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>2 (22.2)</td>
<td>7 (77.8)</td>
</tr>
</tbody>
</table>

# Excluding abortion.
Table (3): Comparison of adverse outcome of pregnancy within the risk subgroups.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Previous pregnancy n (%)</th>
<th>Current pregnancy n (%)</th>
<th>Previous &amp; Current pregnancies n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>1 (8.3)</td>
<td>11 (91.7)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>28 (50.9)</td>
<td>18 (32.7)</td>
<td>9 (16.4)</td>
<td></td>
</tr>
<tr>
<td>Low birth weight</td>
<td>14 (32.6)</td>
<td>24 (55.8)</td>
<td>5 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Macrosomic</td>
<td>2 (28.6)</td>
<td>3 (42.8)</td>
<td>2 (28.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion

In this study, most mothers with any risk factor had a single risk factor, more commonly in the current pregnancy. Multiple risk factors were found equally in previous and current pregnancy. This suggests that women who have a bad obstetric history do not necessarily have complications in subsequent pregnancies. A similar result was reported in a prospective study in India, which concluded that the outcome in current pregnancy improves as a result of optimal antenatal care in women with a bad obstetric history [3]. Several studies have shown adverse outcomes with bad obstetric history [2,11]. This study revealed that more Caesarean deliveries occurred in the risk group. When compared within the risk groups, it was found that nearly half of the Cesarean sections occurred in women with history of risk in previous pregnancies. This could be due to a higher number of women requesting for Cesarean section in addition to the specialist opting for Cesarean delivery to avoid complications. From the available data, the proportion of emergency Cesarean sections could not be ascertained to support this view. Findings in various studies suggest that delivery by Cesarean section is commoner in women with bad obstetric history [3].

Abortion, as an outcome of birth, occurred in 22 pregnancies and their proportion in risk and non-risk groups was not significantly different. However, when compared in different subgroups of risk, it occurred significantly more in the women who had a complication in the current pregnancy. Similar finding was reported in a study in Riyadh [16]. This indicates that current illness in pregnancy has more effect on abortion rather than complications in previous pregnancies. This could be explained by the availability of better care in current pregnancy to those women who have bad previous history as they are under specialist care from early on. This assumption is also supported by the finding that the perinatal death rate is the same in both groups. However, more studies are needed to make any conclusion.

Both low birth rate and macrosomic infants were born more to mothers with risk in their pregnancy. However, the difference was not significant when compared to non-risk group as well as when compared within the various subgroups of risk. Other studies have reported low birth weight babies in women with current high risk as extremes of reproductive age [4,5], heart disease [13,17], gestational diabetes [12,18] and renal disease [16].

Conclusions:

Having a risk factor in pregnancy has a definite effect on the pregnancy outcome. Women with history of risk in a previous pregnancy have higher rate of delivery by Cesarean section. Women with risk in current pregnancy have a significantly higher rate of abortion. Higher proportions of low birth weight and macrosomia are found in women who have a risk factor in the current pregnancy.

Acknowledgements:

The author wishes to thank the ethical committee of the University for allowing to conduct this research. Special mention must be made of the efforts and guidance of Professor Shamsun Nahar, Department of Family and community medicine, KKU, Abha.

References


15- WHO criteria for basic antenatal care Accessed online at website http://www.who.int/ pmnch/ media/ publications/ aonsectionIII_2.pdf.
Effect of Resisted Exercise Training on Interleukin-6 in Patients with Chronic Heart Failure

ZEINAB M. HELMY, Ph.D.; SHERIN H. MOHAMMED, Ph.D. and GABER S.A. SOLIMAN, Ph.D.

The Department of Physical Therapy for Cardiovascular/Respiratory Disorder and Geriatrics, Faculty of Physical Therapy, Cairo University

Abstract

Background and Purpose: Resisted exercise training for patients with chronic heart failure (CHF) improves exercise capacity, restores endothelial function and skeletal muscle changes. The effects of three months resisted exercise training on inflammatory cytokines (interleukin-6) in patients with CHF were studied.

Patients and Methodology: Forty male chronic heart failure patients as a result of coronary artery disease with age of 50-60 years were included in the present study. Their body mass index (BMI) >30kg/m2. They were randomly divided into two groups, each group consisted of 20 patient, the study group (group A) received a program of resisted exercise training (40-50min, resisted exercises, 3 times/week), the control group (group B) did not receive any program of exercise. Both groups received their medical treatment as prescribed by physician. The biochemical changes in inflammatory markers (interleukin-6) were measured at the beginning of the study and after twelve weeks.

Results: Showed that a program of resisted exercise training had effect to slightly decrease Interleukin-6 but not statistically significant reduction by (0.43%) while the control group showed slightly increase by (0.28 %).

Conclusion: It was concluded that a program of resisted exercise training showed non statistically significant reduction in Interleukin-6 in chronic heart failure patients.

Key Words: Resisted exercise – Interleukin-6 – Chronic heart failure patients.

Introduction

HEART failure (HF) is generally defined as the inability of the heart to supply sufficient blood flow to meet the needs of the body. Heart failure can cause a number of symptoms including shortness of breath, leg swelling, and exercise intolerance. The condition is diagnosed with Echocardiography. Treatment commonly consists of lifestyle measures (such as smoking cessation, light exercise including breathing protocols, decreased salt intake and other dietary changes) and medications, and sometimes devices or even surgery [5].

Chronic heart failure (CHF) is characterized by an intolerance to exercise, early fatigue and shortness of breath. Such symptoms impact upon one's ability to perform activities of daily living, thus significantly contributing to reduced participation and poor quality of life. Up until the late 1980s, exercise was considered unsafe for the patient with CHF. It was unclear whether any benefit could be gained from rehabilitation, and concern also existed regarding patient safety, with the belief that additional myocardial stress would cause further harm. Since this time, the evidence resoundingly suggests that exercise for these patients is not only safe but also provides substantial physiological and psychological benefits. As such, exercise is now considered an integral component of the non pharmacological management of these patients [9].

Interleukin-6 (IL-6) is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. In humans, it is encoded by the IL6 gene. IL-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. IL-6 also plays a role in fighting infection, as IL-6 has been shown in mice to be required for resistance against bacterium Streptococcus pneumoniae [8].

Inflammatory activation with increased serum cytokine levels has been described by several authors as an important factor in the progression of the syndrome of chronic heart failure (CHF). IL-6 is a “myokine”, a cytokine produced from
muscle, and is elevated in response to muscle contraction. It is significantly elevated with exercise, and precedes the appearance of other cytokines in the circulation. During exercise, it is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery [6].

Resistance training might elicit a different exercise-induced response than endurance training. These differences, exercise training has been shown to positively influence circulating levels of inflammatory cytokines in patients with chronic kidney disease and coronary heart disease [9].

Chronic heart failure is associated with increased levels of IL-6 and markers of endothelial damage. No Studies have been conducted to investigate the effect of resisted exercises on inflammatory markers (interleukin-6) in chronic heart failure patients. So there was a need to investigate the effect of resisted exercise on inflammatory markers (interleukin-6) in chronic heart failure patients in an attempt to decrease muscle wasting, cachexia and decrease early fatigability [16].

**Purpose of the study:**
To investigate the effect of resisted exercise training on inflammatory markers (interleukin 6) in chronic heart failure patients due to coronary artery disease.

**Patients and Methods**

**Patients:**
Forty male chronic heart failure patients with age ranged from 50-60 years, they were selected from Cairo University teaching Hospitals, outpatient clinics from department of cardiology from 2012 to 2013. The Forty male chronic heart failure patients were randomly divided into two groups; each group consisted of twenty patients. The first received a program of resisted exercise training (group A) and the second group was the control group (group B) that did not receive any program of exercise, both groups were under their medical treatment prescribed by the cardiologist. All patients were diagnosed as having chronic heart failure due to coronary artery disease diagnosed by coronary angiography, Ejection Fraction ranged from (30%-40%) measured by Echocardiography and NYHA classification grade 2 and 3.

Any patient had Hepatic disease, severe life limiting illness (cancer, renal failure), other endocrinial disorders, orthopedic limitation, severe valvular disorder, Moderate valvular disorder and Chronic pulmonary disease was excluded from the study.

**Instrumentation:**
A bicycle ergometer was used for warming up exercise (Tunturi original ergometer W 1 electronics), Small free weights (e.g. 0.5, 1 or 3kg), Sphygmomanometer and stethoscope for measuring blood pressure before, during and after training sessions, Weight & height scale: (Healthy scale 160kg) to evaluate the height, weight & BMI, Kits and Tubes of blood samples.

**Procedure:**

**A- Evaluation session:**
After selection of the patients An informed consent was taken from all patients who accepted to participate in the study, Before starting the study all patients were informed about the nature, benefits and procedure of the study, The sample was randomly divided into two groups equal in number, 20 for each group; group A received a program of resisted exercise training and group B did not receive any program of exercise and Plasma or serum samples were obtained by venipuncture (arterial cannula used in Larsen's study) and stored on ice. Concentration of interleukin 6 was measured by commercially available enzyme-linked immunosorbent assays (ELISAs) [13].

**B- Training:**
1 - All patients in the study (group A) attended the program of resisted exercises for 12 weeks according to the following parameters:
- **Mode of exercise** was resisted exercises in the form of circuit weight training.
- **Intensity of exercise** according to the 1 RM method (i.e. maximum weight which can be lifted in one full range of motion), a typical workout should consisted of 8 exercises to cover the major muscle groups, which includes the chest, shoulders, arms, back, abdomen, thighs, and lower legs. The resistance or weight lifted should be moderate, which is defined as 30% to 40% of 1 RM for upper body exercises and 50% to 60% of 1 RM for lower body exercises [5].
- **Duration:** The first five to ten minutes of each session was dedicated to warming up exercise on a bicycle ergometer and the same for the cooling down phase. There was a twenty to thirty minute of resisted exercise.
- **Frequency:** Three times per week for 12 weeks.
- **Resistance training recommendations for patients with CHF** were described by Warm up and cool
down duration from 5-10 minutes, circuit weight training graduated from 2-3 circuits, each circuit 8 muscles, there was 1 minute rest between circuits, time was 2-3 sec. for lifting and 2 sec. for descending, the Duration of session was 40-50 min., the mode was resistance exercises training, begin with weight increased gradually until reach to the end weight and avoiding valsalva maneuver.

- Blood pressure was measured before, during and after session.

2- After 12 weeks program, another blood sample was taken and interleukin 6 was measured and then the pre and post samples for the two groups were compared.

Statistical analysis:

Descriptive statistics was done in the form of mean and standard deviation and Inferential statistics assessed Changes in interleukin 6 using independent \( t \)-test between the two groups and dependent \( t \)-test was used to assess changes within group, analysis was done using SPSS version 17, Relatives changes percentage was calculated according to:

\[
\text{Relatives changes percentage} = \frac{\text{Post} - \text{Pre}}{\text{Pre}} \times 100
\]

Results

Demographic and clinical characteristics of the patients:

In this study, 40 patients with chronic heart failure were assigned randomly into two groups: Group (A) (Study group) and Group (B) (Control group).

There was no significant difference between both groups in their ages, weights, heights, BMI, ejection fraction, end diastolic dimension, and end systolic dimension where their \( t \) and \( p \)-values were (0.45, 0.65), (0.49, 0.62), (0.5 5, 0.5 8), (0.08, 0.93), and (0.66, 0.51), (0.64, 0.52), (0.54, 0.59) respectively.

Table (1): Demographic and clinical characteristics of patients in both groups (A & B).

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>Group A</th>
<th>Group B</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>( t )-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>55.5 ± 2.7</td>
<td>55.1 ± 2.84</td>
<td>0.45</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>96.55 ± 10.36</td>
<td>95.05 ± 8.88</td>
<td>0.49</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.67 ± 0.05</td>
<td>1.66 ± 0.06</td>
<td>0.55</td>
</tr>
<tr>
<td>BMI (Kg/m(^2))</td>
<td>34.24 ± 2.47</td>
<td>34.17 ± 2.32</td>
<td>0.08</td>
</tr>
<tr>
<td>Ejection fraction (EF %)</td>
<td>34.75 ± 1.99</td>
<td>34.35 ± 1.81</td>
<td>0.66</td>
</tr>
<tr>
<td>End Diastolic dimension (Cm)</td>
<td>6.72 ± 0.21</td>
<td>6.76 ± 0.17</td>
<td>0.64</td>
</tr>
<tr>
<td>End Systolic dimension (Cm)</td>
<td>5.17 ± 0.19</td>
<td>5.2 ± 0.15</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* SD : Standard deviation.  S : Significance.  
\( p \) : Probability.  NS : Non-significant.

Table (2): The number and percentage of patients who take medications in both groups (A & B).

<table>
<thead>
<tr>
<th>Medications</th>
<th>Group A</th>
<th>Group B</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
<td>Number</td>
</tr>
<tr>
<td>Diuretics</td>
<td>20</td>
<td>100%</td>
<td>20</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>12</td>
<td>60%</td>
<td>10</td>
</tr>
<tr>
<td>Angiotiesin converting enzyme inhibitors (ACEI)</td>
<td>20</td>
<td>100%</td>
<td>20</td>
</tr>
<tr>
<td>Statins</td>
<td>15</td>
<td>75%</td>
<td>17</td>
</tr>
<tr>
<td>Digitalis</td>
<td>8</td>
<td>40%</td>
<td>10</td>
</tr>
</tbody>
</table>

* SD : Standard deviation.  S : Significance.  
\( p \) : Probability.  NS : Non-significant.

Comparison inside each group before and after training as regarded to Interleukin 6:

Group (A):

Table (3) and Fig. (1) demonstrated the Interleukin 6 pre and post treatment for group (A). There was no significant difference in the paired \( t \)-test between pre and post training for Interleukin 6 as the mean value of pre treatment Interleukin 6 was (6.92 ± 0.79), and for post training It was (6.89 ± 0.8) where the \( t \)-value was (1.42) and \( p \)-value was (0.17). The percentage of reduction was 0.43%.
Table (3): Mean and ±SD, t and p-values of Interleukin 6 pre and post training of group (A).

<table>
<thead>
<tr>
<th>Group A</th>
<th>Interleukin 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre treatment</td>
</tr>
<tr>
<td>Mean</td>
<td>6.92 ±0.79</td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.03</td>
</tr>
<tr>
<td>Percentage of improvement</td>
<td>Decreased by 0.43%</td>
</tr>
<tr>
<td>t-value</td>
<td>1.42</td>
</tr>
<tr>
<td>p-value</td>
<td>0.17</td>
</tr>
<tr>
<td>S</td>
<td>NS</td>
</tr>
</tbody>
</table>


Fig. (1): Mean and ±SD of Interleukin 6 pre and post training of group (A).

Group (B):
Table (4) and Fig. (2) demonstrated the Interleukin 6 pre and post training for group (B). There was no significant difference in the paired t-test between pre and post treatment for Interleukin 6 as the mean value of pre treatment Interleukin 6 was (6.94±0.82) and for post training it was (6.96±0.8) where the t-value was (0.38) and p-value was (0.7). The percentage of increase was 0.28%.

Table (4): Mean and ±SD, t and p-values of Interleukin 6 pre and post training of group (B).

<table>
<thead>
<tr>
<th>Group B</th>
<th>Interleukin 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre treatment</td>
</tr>
<tr>
<td>Mean</td>
<td>6.94 ±0.82</td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.02</td>
</tr>
<tr>
<td>Percentage of improvement</td>
<td>Increased by 0.28%</td>
</tr>
<tr>
<td>t-value</td>
<td>0.38</td>
</tr>
<tr>
<td>p-value</td>
<td>0.7</td>
</tr>
<tr>
<td>S</td>
<td>NS</td>
</tr>
</tbody>
</table>


Fig. (2): Mean and ±SD of Interleukin 6 pre and post training of group (B).

Comparison between both groups as regarded to Interleukin 6:
Table (5) and Fig. (3) revealed the independent t-test results for the Interleukin 6 pre and post training between groups A and B. There was no significant difference in pre training values where the t-value was (0.09) and p-value was (0.92), and also, there was no significant difference in the post training values where the t-value was (0.25) and p-value was (0.79).

Table (5): Independent t-test between groups A and B for Interleukin 6 pre and post training.

<table>
<thead>
<tr>
<th>Independent t-test</th>
<th>Interleukin 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre treatment</td>
</tr>
<tr>
<td></td>
<td>Group (A)</td>
</tr>
<tr>
<td>Mean</td>
<td>6.92 ±0.79</td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.02</td>
</tr>
<tr>
<td>t-value</td>
<td>0.09</td>
</tr>
<tr>
<td>p-value</td>
<td>0.92</td>
</tr>
<tr>
<td>S</td>
<td>NS</td>
</tr>
</tbody>
</table>


Fig. (3): Mean and ±SD of Interleukin-6 pre and post training of groups (A,B).
Discussion

The purpose of the present study was designed to study the effect of resisted exercise (40-50 minutes exercise 3 times/week) on inflammatory markers (interleukin-6), in chronic heart failure patients due to coronary artery disease.

They were randomly assigned into two groups; each group consists of twenty patients. The first received a program of resisted exercise training (group A) (40-50 minutes exercise 3 times/week), the second was the control group, that did not receive any program of exercise (group B).

The results showed that program of resisted exercise had effect to slightly decrease interleukin-6, but statistically non significant reduction by (0.43%: p>0.05 versus increase 0.28%; p>0.05) when compared with control group.

Several possible mechanisms can explain why moderate intensity resistance exercise training reduced chronic inflammation. First, Long-term resistance exercise may protect against chronic systemic low grade inflammation via IL6 independent pathways, which IL-6 may partly contribute to the anti-inflammatory activities (e.g., decreased production of TNF-\(\zeta\)), because TNF-\(\zeta\) stimulates the production of IL-6. In return, IL-6 inhibits the transcription of TNF-\(\zeta\) and stimulates the production of anti-inflammatory cytokines and the shedding of TNF receptors that bind TNF-\(\zeta\) with high affinity. Second, resisted exercise training can protect against chronic systemic low-grade inflammation through improve blood pressure, insulin resistance, and muscle mass [1].

Inflammatory markers that included (interleukin-6) were recorded in the two groups at two intervals; the starting of the experiment (pre) and at the end of the twelve weeks (post program).

The result of the present study was supported by Kishiko et al., who approved that resistance exercise-induced muscle hypertrophy was associated with slightly reduction of interleukin-6. Twenty-one elderly women (Mean age ±SD, 85.0±4.5 years) participated in 12 weeks of moderate intensity resistance exercise training. Muscle thickness and circulating levels of interleukin-6 (IL-6) were measured before and after the exercise training. The training-induced slightly reductions in IL-6 were not significantly and associated with increased muscle thickness. Resistance training may assist in maintaining or improving muscle volume and reducing low-grade inflammation.

In agreement with present study results of Suleen et al., who approved that resistance exercise had been shown to slightly lower levels of interleukin-6. The effect of 12 weeks of moderate-intensity resisted exercise was examined on IL-6 compared to no exercise in overweight and obese individuals. IL-6 levels were not significantly decreased at week 12 compared to baseline in the resistance group. Therefore, resistance exercise training may be physiologically relevant in decreasing the risk of developing chronic diseases.

A randomized, controlled trial was done by Josef which documented the effects of resisted exercise training on inflammatory markers (interleukin-6) in patients with chronic heart failure, inflammatory mediators such as tumor necrosis factor-alpha, interleukin-6, and nitric oxide can produce effects that mimic features of heart failure, including (but not limited to) progressive left-ventricular dysfunction, pulmonary edema, left-ventricular remodeling, and cardiomyopathy. This study performed on 50 patients with age ranged from 40 to 60 years old these patients asked to perform 12 weeks of moderate intensity resistance exercise training. The study delimited to normal weight patients and underwent coronary artery bypass graft (CABG). Circulating levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-\(\zeta\), IL-6 were measured before and after finishing the exercise program, the result was reduction in (TNF)-\(\zeta\) by 18%. CRP by 23% but IL-6 was not statistically significantly decreased. However, there are several randomized trials which unanimously document that chronic-as opposed to acute bouts of-exercise does not only lead to a reduction of cytokines (interleukin-6 but non significantly) and oxidative stress, but that patients dramatically benefit by the increase in maximal oxygen consumption, exercise capacity, quality of life, reduction in hospitalization, morbidity, and mortality.

Similarly, Hilde et al., defined the effects of chronic resisted exercise on inflammatory markers (interleukin-6) in patients compared with healthy controls, to determine whether exercise elicits an abnormal inflammatory response in those patients for 3 months of mild to moderate intensity exercise. Circulating levels of serum amyloid A (SAA), tumor necrosis factor (TNF)-\(\zeta\) and IL-6 were measured before and after the exercise training. 60 persons with mean age 45 years were included in this study. These patients were diagnosed as chronic heart failure. The result was decreased in (TNF)-\(\zeta\) by 14%, decreased serum amyloid A
(SAA) by 17% and no significantly changed in IL-6. Chronic inflammatory diseases strike millions of people all over the world, and exercise is often prescribed for these patients to improve overall fitness and quality of life. Evidence was found that chronic resistance exercise training programs can lower systemic inflammation in patients with chronic heart failure compared to healthy matched controls.

In agreement with present study results, Neil et al., conducted a study to assess the effect of resisted exercise training on interleukin-6 and tumour necrosis factor alpha in Heart Failure. Results showed that Data for 106 CHF patients with moderate LV systolic dysfunction (EF 30±6.9%), and 78% of them (83 patient) had ischemic cardiomyopathy. After exercise training, peak VO2 increased 1.4±3.4ml/kg/min, serum TNF-alpha was significantly decreased 1.9 ± 8.6pg/ml and IL-6 was not significantly changed for the whole group. Baseline and post-training peak VO2 changes were not correlated with change in cytokine levels. Exercise training reduces levels TNF-alpha but not IL-6 in CHF. However, across a heterogenic patient group, change in peak VO2 was not correlated with alterations in cytokine levels. While greater exercise volume (hours) was superior in improving peak VO2.

Similarly, Miguel et al., examined the effect of resisted exercises training in heart failure on TNF-α, IL-6 and IL-10. A program of resisted exercise training with moderate intensity applied to thirty patients for three months. Circulating levels of tumor necrosis factor (TNF)-α, IL-6 and IL-10 were measured before and after the exercise training. The exercise training-induced cardiovascular benefits of physical exercises performed at intensities ranging from mild to moderate. (TNF)-α was significantly decreased, IL-10 significantly increased and IL-6 decreased at small percentage that is not statistically significant. Altogether, these data indicate a possible anti-inflammatory effect induced by physical training in HF. The “anti-inflammatory effect” induced by resisted exercises training seems to be primarily mediated by IL-10.

The results showed by Stephan et al., which came coincided with results of the current study, assessed the effects of resisted exercise training in the skeletal muscle of patients with chronic heart failure; Twenty male patients with stable CHF (left ventricular ejection fraction 25 ±2%; age 54±2 years) were randomized to a training group (n=10) or a control group (n=10). At baseline and after six months, serum samples and vastus lateralis muscle biopsies were obtained. Serum tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, and IL-1 beta levels were measured. Serum levels of IL-6, and IL-1 beta remained unaffected by training, TNF-alpha decreased significantly by 32%.

On the other hand, Balducci et al., investigated the effect of different exercise modalities on high sensitivity-C reactive protein (hs-CRP) and other inflammatory markers (IL-1, IL-4, IL-6, IL-10, CRP) in patients with type2 diabetes and the metabolic syndrome. Eighty-two patients were randomized into four groups: (A) sedentary control; (B) receiving counseling to perform low-intensity physical activity; (C) performing prescribed and supervised high-intensity aerobic exercises; (D) Resistance exercise for 12 months. Inflammatory biomarkers was performed at baseline and every three months. Levels of hs-CRP decreased in all three exercising groups, but the reduction was significant only in Groups C and D, and particularly in group D. Changes in VO2 max and the exercise modalities were strong predictors of hs-CRP reduction independent of body weight. Interleukin-6 was significantly decreased also TNF-alpha was significantly decreased in group (D) and whereas adiponectin increased in Groups C and D. Interleukin-1, whereas anti-inflammatory interleukin-4 and 10 increased only in Group D. Exercise training is associated with a significant reduction of hs-CRP and other inflammatory biomarkers, independent of weight loss.

On the contrary, Niebauer et al., determined the effect of resisted exercise training in chronic heart failure patients on inflammatory cytokines and markers of endothelial damage. Measured tumor necrosis factor α (TNF-α), its soluble TNF-receptors 1 and 2, interleukin 6 (IL-6), in 18 patients with CHF and 9 age-matched controls in a randomized cross-over study of 8 weeks of exercise training (3 days/week, resisted training 30min/day) versus 8 weeks of rest. At baseline, patients had significantly elevated levels of TNF-α and TNF-R2; after a program of resisted exercises there was significant reduction in TNF-α, TNF-R1 and 2 and IL-6 levels in the training group also exercise training was effective and led to an increase in peak VO₂ in CHF.

In contrast, Conraads et al., who assessed the relation between resistance training and TNF-alpha and IL-6 levels in patients with chronic heart failure and coronary artery disease Physical reconditioning of patients with chronic heart failure (CHF) improves exercise capacity and restores endothelial function and skeletal muscle changes. The effects of four months resistance exercise training on
cytokines and cytokine receptors in patients with CHF were studied. Blood sampling for measurement of plasma concentrations (ELISA) of interleukin-6, tumor necrosis factor-alpha, soluble TNF receptor-1 (sTNFR1) and 2 (sTNFR2) as well as cardiopulmonary exercise testing were performed at baseline and after 4 months. Training induced no significant decrease in sTNFR1, sTNFR2 concentrations and IL-6 levels were significantly altered. Cytokine concentrations remained unchanged in an untrained age-and sex-matched control group.

Conclusion:
A program consisted of resisted exercise training showed slightly reduction in interleukin-6, but not statistically significant more than patients who did not receive any program of exercise, at the end of program, in chronic heart failure patients, in a short term (up to twelve weeks) which could preserve muscle strength and prevent cachexia.

References
Imaging Findings of Interstitial Lung Disease Using High Resolution Multi-Detector CT

AHMED A. ISMAIL, M.D.* and EMAN SHABL, M.D.**
The Departments of Diagnostic Radiology* and Chest**, Faculty of Medicine, Zagazig University

Abstract

Purpose: Aim of this study was to assess the different imaging findings of some interstitial lung diseases using high resolution multi-detectors CT.

Patients and Methods: This study was carried out between May 2010 and June 2012. Thirty cases of chest patients suspected to have interstitial lung disease (ILD) by clinical, laboratory, and X-ray examinations. The patients age range 18-68 years old. High resolution CT was done for all cases using 64 Multi-detector scanners (MDCT).

Results: In this study the highest cause percentage among the intestinal lung diseases were the idiopathic interstitial pneumonia (30%), followed by sarcoidosis (16.7%), Alveolar proteinosis (10%), idiopathic fibrosis (10%), hypersensitivity pneumonitis (10%), silicosis (6.7%), and Langerhan’s cell histiocytosis (6.7%). Drug induced interstitial LD (6.7%), and the lowest frequent disease was lymphangitis carcinomatosis (3.3%). MDCT findings included the pattern of parenchymal abnormalities (The linear, reticular, nodular opacities, ground glass, honey combing, cystic lesions and mosaic appearance). The distributions and location of the findings were reported. The Linear opacities and ground glass opacities (GGO) were the most frequent findings in 15 & 13 of the patient (50% & 43.3%) respectively.

Conclusion: Multi-detector CT is of great importance in better detection and assessment of the distribution and characterization of different findings of the interstitial lung diseases (ILDs).

Key Words: Interstitial lung disease – Multidetector CT.

Introduction

INTERSTITIAL lung diseases (ILDs), also called diffuse infiltrative lung disease, are a heterogeneous group of disorders and vary widely in etiology, clinic-radiologic presentation, histopathologic features, and clinical course [1].

Diffuse ILDs are possible to subcategorize to number of groups, the large group of idiopathic interstitial pneumonias (IIPs), the second group of granulomatous disease (e.g Sarcoidosis), moreover, group of intriguing diffuse lung diseases that include Langerhans’ cell histiocytosis (LCH) & lymphangioleiomyomatosis (LAM), and group of ILDs secondary to known cause (e.g collagen vascular diseases) [2].

DILD implies an acute or chronic increase in the radiographic density of the lungs due to an abnormal accumulation of fluid, cells, or other tissue elements within the smaller air spaces or interstitium, or both [3]. Generally, the most common chest X-ray patterns are nodular, reticular, reticulonodular, linear, consolidation, and ground-glass (GG). The first radiological diagnostic approach to DILD is classically based on chest X-ray evaluation. Among the most difficult tasks in radiology, the standard chest radiograph remains unsurpassed in relation to its cost, radiation dose, availability, and ease of performance [4]. However, the chest radiograph has several limitations especially due to the superimposition of structures and the relatively low-contrast resolution. The chest radiograph is normal in 10 to 15% of symptomatic patients with proven DILD; also it can mimic interstitial disease in patients with different lung lesions (e.g., paraseptal emphysema and small-airway disease), and it is often quite nonspecific, although abnormal [5,6].

High resolution multi-detector CT scanner have several advantages for imaging of ILD and include increased speed and the ability to use thinner collimation, thus increasing spatial resolution and reducing partial volume averaging [7]. Additional advantages in imaging ILD include retrospective reconstructions of thinner and thicker sections and creation of maximum intensity projection (MIP).
and minimum intensity projection (minIP) images from the same raw data using post-processing techniques [8].

Many different radiological features obtained by MDCT scans like diffuse, bilateral interstitial or reticular, nodular infiltrates, small but variable sized nodules, patchy ground glass opacity honeycombing & bronchiactasis [9,10].

**Patients and Methods**

This study was carried out between May 2010 and June 2012. 30 cases of chest patients suspected to have interstitial lung diseases by clinical, laboratory, and X-ray examinations. The study was approved by local ethics committee. The patient's age ranged between 18-68 years old.

All patients were subjected to:

- Careful history taking and general clinical examinations.
- Pulmonary function tests.
- Laboratory tests as (CBC, sputum analysis).
- Digital chest X-ray.

- MDCT protocol: All patients were studied using bright speed 64 detector GE CT scanners. One-mm collimation was used with 120-140 KV, 100-360mAs, helical full rotation 0.5-second and pitch of 1.375. Patients were scanned caudo-cranially in supine position full inspiration during one breath hold (scan time about 6 seconds).

Once the patient was scanned the images were immediately transferred to a work station and reconstructed, multiplanar reformation (MPR), maximum intensity projections (MIP).

**Statistical analysis:**

The data were recorded, expressed as mean, standard deviation, and range. The frequency of signs in multi-detector HRCT expressed as number and percentage.

**Results**

The present study included 30 patients with interstitial lung disease. The patients' age ranged between 18-68 years old, the mean age 53 ± 13.64. The peak age for interstitial lung disease was 50-60 years old 30% of all cases (Table 1). There was female predominance 60% among the interstitial lung disease cases, while the male cases represent 40%.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>3 (10)</td>
</tr>
<tr>
<td>20-30</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>30-40</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>40-50</td>
<td>6 (20)</td>
</tr>
<tr>
<td>50-60</td>
<td>9 (30)</td>
</tr>
<tr>
<td>60-70</td>
<td>3 (10)</td>
</tr>
</tbody>
</table>

**Table (1): Frequency of age of the interstitial lung disease cases.**

<table>
<thead>
<tr>
<th>Lung changes</th>
<th>MDCT findings</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased lung capacity</td>
<td>Linear abnormalities</td>
<td>15 (50%)</td>
<td>15 (50%)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Ground glass opacity</td>
<td>13 (43.3%)</td>
<td>17 (56.7%)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Reticular abnormalities</td>
<td>7 (23.3%)</td>
<td>23 (76.7%)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Nodular</td>
<td>5 (16.7%)</td>
<td>25 (83.3%)</td>
<td>30</td>
</tr>
<tr>
<td>Decreased lung capacity</td>
<td>Honeycombing</td>
<td>7 (23.3%)</td>
<td>23 (76.7%)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Cysts or cyst like appearance</td>
<td>5 (16.7%)</td>
<td>25 (83.3%)</td>
<td>30</td>
</tr>
<tr>
<td>Areas of decreased attenuation without walls</td>
<td>Mosaic appearance</td>
<td>5 (16.7%)</td>
<td>25 (83.3%)</td>
<td>30</td>
</tr>
</tbody>
</table>

Table (2): MDCT findings & frequency of signs among 30 patients with interstitial lung diseases.

Linear pattern may correspond to (a) thickening of the interstitial fiber network of the lung owing to fluid, (b) fibrous tissue, or (c) interstitial infiltration by cells or other material. Linear pattern best imaged with MIP, whatever their cause. It was found in 15 cases of our studying group (50%) (Fig. 1). Ground glass pattern found in 13 cases (43.3%) (Fig. 2). The reticular pattern was found in 7 cases (23.3%), which appeared as abnormal irregular parenchymal opacities (Fig. 3). The nodular pattern (Fig. 4) found in 5 cases (Table 2).

The honey combing was found in 7 cases (23.3%) and cystic changes in Langerhan's cell histocytosis (LAH) (Fig. 5) in 5 cases (16.7%). Mosaic appearance was found in 5 cases (16.7%). Other finding like traction Bronchiectasis and pleural effusion were found only in 4 cases (13.3%) for each, while fissural thickening was found only in one case (3.3%).
The highest percentage among the ILDs cases in the present study was the Idiopathic Interstitial pneumonia (IIP) in 9 cases (30%), followed by sarcoidosis, Alveolar proteinosis, Idiopathic Interstitial fibrosis, hypersensitivity pneumonitis, silicosis, Langerhan's cell histocytosis (LAH), Drug induced interstitial LD and the lowest frequency among our patients was lymphangitis carcinomatosis (3.3%) (Table 3).

The MDCT finding for different disease reported in (Table 3).

Location & distribution of each disease after post processing reconstruction was reported in (Table 4).

Enlarged mediastinal L.Ns was found in all sarcoidosis cases and pleural calcifications found in the two silicosis cases (6.7%).

<table>
<thead>
<tr>
<th>The disease</th>
<th>N. (%)</th>
<th>MDCT location</th>
<th>MDCT distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Upper-zone</td>
<td>Mid-zone</td>
</tr>
<tr>
<td>Idiopathic Interstitial pneumonia</td>
<td>9 (30)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>5 (16.7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Alveolar proteinosis</td>
<td>3 (10)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Idiopathic fibrosis</td>
<td>3 (10)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>3 (10)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Silicosis</td>
<td>2 (6.7)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Langerhan's cell histocytosis</td>
<td>2 (6.7)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Drug induced interstitial LD</td>
<td>2 (6.7)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lymphangitis carcinomatosis</td>
<td>1 (3.3)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Fig. (1): A 38-year-old female patient, past history of operated breast cancer (Lymphangitis carcinomatosis). (A,B) coronal MIP & axial view show a linear pattern in the right upper & lower lobes caused by thickening of the interlobular septa (arrow), together with the development of a pleural effusion.

Fig. (2): A 36-year-old female with acute onset of dyspnea (A,B) axial view and MIP reformatted image show diffuse ground-glass opacity widely spread across both lungs in patient with acute idiopathic interstitial pneumonia.

Fig. (3): A 40-year-old male patient with idiopathic interstitial fibrosis complains of dry cough, gradual onset of dyspnea. (A,B) MDCT lung window axial view & MIP showing bilateral coarse reticulations, dominating in both lung bases with sub-pleural early honey combing dominating at both lower lung lobes.
Fig. (4): A 51-year-old female patient complains of chronic cough and dyspnea. (A,B) MIP & axial images showing ill defined small nodules scattered predominantly in both upper lobes and right middle lobe. These nodules have satellite appearance (white arrows). Some nodules are miliary-like.

Fig. (5): A 29-year-old male patient complains of dry prolonged cough with cystic lung changes in LCH. (A) MIP demonstrating variable size, thin walled cysts and small nodules (arrow), with an upper to mid-zone Predominance with sparing of the costophrenic angles. (B) Axial view demonstrating irregular bizarrely shaped, thin-walled cysts (arrow); with ground glass opacities.

**Discussion**

MDCT scanner now is most useful for chest disease especially ILD diagnostic practice as it makes high resolution MPR and MIP images. The most common cause for interstitial lung disease in our study was the idiopathic interstitial pneumonia (IIP), which found in 9 cases (36.7%). These cases presented by changes like ground glass opacities (GGO) with honeycombing especially at the lower lobes, and traction bronchiactasis agreed with Tomiyama et al., [10].

In the present study, sarcoidosis found in 5 cases (16.7%), these cases presented by nodular pattern, honeycombing changes more at the upper lobes with hilar lymph node enlargement. Our findings in agreement with Eva and Caudia, [11]. Regarding Hypersensitivity pneumonitis and interstitial lung disease, both were found only in 10% for each. The imaging finding with the MDCT was bilateral diffuse ground glass attenuations and per bronchial mottling. Similarly these findings reported by other authors [12].

Silicosis, LCH, drug induced interstitial lung disease were found in 2 cases only (6.7%) for each disease. The most common findings in silicosis was the mosaic attenuation areas, reticular changes, pleural calcifications and sub-pleural honeycombing while drug induced interstitial lung disease the bizarre shaped nodular or reticular infiltrates was
the most common imaging finding. Our study finding in agreement with Semin et al., and Santigo et al., [13,14].

The linear parenchymal pattern was a common finding found in 50% of the patients. It represented by abnormal septal lines, which are easily assessed with MIP, may be related to a variety of disorders such as Hypersensitivity pneumonitis, fibrosis, or lymphangitis carcinomatosis. Moreover, Catherine et al., stated that MIP is the reformatting technique of choice for evaluating the size of pulmonary vessels [15].

In this study the ground glass opacity (GGO) was found in 13 cases (43.3%). Ground glass opacity appearance non specific finding which may be represented in many pathological changes including microscopic fine fibrosis, interstitial cellular infiltration, increased parenchymal water or tissue compression [16,17]. However, it is usually associated with other features such as nodules, areas of consolidation and interstitial abnormalities that may contribute to the specificity of diagnosis (e.g. “crazy-paving” suggesting alveolar proteinosis) [18].

On the other hand the lower accuracy described by Studler et al., [7] in detecting GGO by conventional MSCT compared to HRCT could be due to the use of 3mm reconstructions. In our study, the use of 1.25mm and of high quality reconstruction algorithms (MPR,MIP), provided by the volumetric 64-row MSCT, improved the quality of the images, crucial for a correct diagnosis.

Among our patients, nodular pattern was present in 5 cases (6.7%), either airspace nodules and interstitial nodules, both referring to multiple rounded opacities varying in diameter from 1mm to 1 cm. Nodular pattern is often difficult to evaluate solely on the basis of its attenuation and definition. More useful is the assessment of its regional distribution, both in the cranio-caudal and axial dimensions and within the secondary pulmonary lobule. MIP with progressively increasing slab thickness is very helpful because it improves the detection of small nodules and the estimation of their profusion, thereby helping in the recognition of their characteristic distribution relative to the landmarks of the secondary pulmonary lobule [15].

A pattern of multiple cysts distributed throughout the lungs is suggestive of LAM [18]. LCH is predominating in both upper lobes. Honeycombing in our study was found in 7 cases (23.7%). It refers to the characteristic appearance of extensive end-stage pulmonary fibrosis, resulting from lung destruction and obliteration of the acinar architecture. At MDCT CT, the cystic spaces in honeycombing commonly share walls, are predominantly subpleural, and occur in several layers. Honeycombing is most commonly caused by IPF, collagen vascular disease, end stage hypersensitivity pneumonitis, or asbestosis [15].

Mosaic attenuation pattern were found in 5 cases (16.7%) of our studying patients. In these cases, areas of higher attenuation represent the interstitial process disease and the areas of lower attenuation represent the normal lung [19].

Kasthoori et al., [20] found that MPR and post-processing added more information about the extent and character of the abnormalities, hence increasing confidence in the Diagnosis of ILDs. Others like Chooi and Morcos, [21] found the MPR in assessing diffuse infiltrative parenchymal lung disease did not add important new diagnostic information compared with axial imaging alone.

One of the main pitfalls of MDCT is the use of high radiation dose. According to Schoepf et al., [22], there is no significant increase in radiation dose using high resolution MDCT imaging compared with single slice CT (5.55mSv versus 5.50mSv). On the otherhand, radiation exposure by CT for young patients and pregnant women had to be considered, so lung MR may be considered an alternative to other modalities with at least similar diagnostic value [23].

Conclusion:

MSCT is of great importance in better detection and assessment of the distribution and characterization of different findings of different interstitial lung diseases (ILDs).

References


5- PADLEY S.P.G., HANSELL D.M., FLOWER C.D.R., et al.: Comparative accuracy of high resolution computed tomography and chest radiography in the diagnosis of...
Ahmed A. Ismail & Eman Shabl


13- SEMIN CHONG, KYUNG SOO and LEE JUNARY: Radiographics, 26, 59-77, 2006


17- PAOLA ROGLIANI and MARCO MUR: Respiratory Medicine, Volume 102, p. 1753-1761, 2008.


Preinduction Ultrasonographic Measurements as a Predictor of Successful Induction of Labour in Prolonged Pregnancy in Primigravidas

AHMED S.A. ASHOUR, M.D.; RANA M.A. ABDELLA, M.D.; HASSAN O. GHAREEB, M.D. and FOUAD A. ABO-HAMILA, M.D.

The Department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University

Abstract

Objective: To determine if ultrasonographic measurements as the cervical length, the fetal occiput position, the estimated fetal weight and whether the head is well flexed or not are good predictors for successful labour induction in prolonged pregnancy in primigravidas.

Design: Prospective cohort study.

Setting: Kasr El-Aini Hospital.

Patients and Methods: This study included 100 primigravidas ≥41 weeks with singleton vertex presentation, not in labour, with Bishop score ≥5 where previous uterine scar; previous operations on the cervix (e.g. cervical amputation); obstetric or medical complication with pregnancy (e.g. diabetes) were excluded. All patients were subjected to history taking, abdominal and vaginal examinations including Bishop score. The position of the occiput, estimated fetal weight and flexion of the head by abdominal ultrasonography and cervical length measurement by Transvaginal ultrasound were recorded. Labour Induction was done according to standard Kasr El Aini guidelines for induction. The primary outcome was successful attempt for vaginal delivery. Secondary outcomes were induction to delivery interval (IDI) and Apgar score at 1 and 5 minutes.

Results: In our study, there was a highly significant difference between cervical length in successful group (Mean=14.34mm) and failed group (Mean=28.25mm). There was significant difference in estimated fetal weight by ultrasound in the successful group (Mean=3235.33gm) and failed group (Mean=3700gm). The number of cases with occipito-anterior position was higher in successful group in comparison with the failed group and the difference was statistically significant (p-value:0.0001). Flexed head position showed the highest percentage of successful labour induction in comparison with deflexed and extended positions, and the difference was statistically significant (p-value:0.002).

Conclusion: In women undergoing induction of labour, prediction of outcome can be provided by determining sonographically the preinduction cervical length, occipital position and degree of flexion of the head which were superior to Bishop score.

Key Words: Induction of labour – Prolonged pregnancy – Ultrasonographic measurements.

Introduction

PROLONGED pregnancy is a real problem in modern obstetrics. It causes an anxiety among pregnant women [1]. The definition of post-term pregnancy is the pregnancy that has extended to or beyond 42 weeks (294 days) of gestation [2]. Prolonged gestation complicates 5% to 10% of all pregnancies and confers increased risk to both the fetus and mother [3]. Postterm pregnancy is associated with higher rates of stillbirth, macrosomia (birth weight >4000gm), birth injury and meconium aspiration syndrome [3].

Induction of labour at 41 weeks is associated with less intrapartum fetal compromise, meconium-stained liquor (MSL) and macrosomia (>4000gm) [4]. Induction of labour is performed in about 20% of all pregnancies and successful induction is reported to be related to cervical characteristics, or “ripeness” [5].

To date, Bishop score remains the standard method to predict the duration and outcome of induced labour. However, the preinduction “favorability” of the cervix as assessed by the Bishop score is very subjective and several studies have demonstrated a poor predictive value for the outcome of induction especially in women with a low Bishop score [6].

In women undergoing induction of labour, pre-induction sonographic assessment of cervical length
and occipital position is superior to the Bishop score in the prediction of outcome of labour [7].

The aim of the study was to determine if some ultrasonographic measurements as the cervical length, the fetal occiput position, the estimated fetal weight and degree of flexion of the head (whether the head is well flexed or not) are good predictors for successful induction of labour in cases of prolonged pregnancy in primigravidas.

**Patients and Methods**

This was a prospective cohort study carried out from March 2012 to October 2012 at the casualty department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University, was approved by the institutional review board. One hundred consecutive primigravidas during the study period, with well-dated postterm pregnancies ≥41 weeks were admitted and recruited after giving a written consent for termination of pregnancy via induction of labour.

The inclusion criteria included a singleton pregnancy with a gestational age correctly dated by either a first trimester measurement of crown-rump length or a second trimester (before 20 weeks) ultrasound examination with no gross fetal anomalies found with a cephalic, vertex presentation (brow and face presentation excluded). All patients were not in labour and with a Bishop score ≥5 and a reactive fetus. Exclusion criteria included a contraindication to vaginal delivery; previous uterine scar; previous operations on the cervix (e.g. cerclage, cervical amputation or conization); obstetric or medical complication with pregnancy (e.g. diabetes or hypertension).

All patients were subjected to full history taking, abdominal examination (for assessment of fundal level, fetal back position) and vaginal examination (to exclude cephalopelvic disproportion, confirm presentation and position). Digital examination and scoring of the cervix was done using the modified Bishop score.

All ultrasound measurements were performed by using MEDISON SONACE X-4-EXP, ultrasound machine equipped with a 3.5-MHz convex transabdominal probe and 7.5-MHz vaginal probe. Ultrasound examinations were done by a single operator to avoid interobserver variability. Fetal biometry with calculation of the fetal weight using the Hadlock formula was done, with special concern to the position of the occiput and degree of head flexion. Transvaginal ultrasound was done for assessment of cervical length where the following steps were taken; patient was asked to empty the bladder. The probe was inserted into the vagina only a few centimeters and then rocked in the antero-posterior direction to visualize the cervix, checking for the anterior and posterior lips and the line of the internal cervical canal in the midsagittal plane. The probe was then slowly withdrawn back a little with the lightest touch to avoid compression artifacts. Measurement was made from internal to external os and repeated three times taking the shortest best measurement. Any funneling (or membrane protrusion more than 5mm down the canal) was also recorded.

Based on sonographic landmarks—such as the fetal orbits for occipito-posterior position, cerebellum and occiput for occipito-anterior position and midline cerebellar echo for occipito-transverse position—the fetal occipital position could be depicted.

Flexion of the fetal head could be depicted directly while tracking the fetal spine in the sagittal plane towards the fetal head in occipito-anterior position. Various degrees of deflexion of the head were noted as a result of movement of the depicted biparietal diameter from an imaginary line parallel to the pelvic inlet, to any angle up to 90 degrees; the latter representing an acutely hyper-extended fetal head.

Induction of labour was done according to standard Kasr El Aini guidelines for induction of labour. We started induction by prostaglandin E 1 (misoprostol) using an initial dose of 50 microgram (2 vaginal tablets of vagiprost® 25 microgram each tablet, manufactured by ADWIA CO. S.A.E Egypt). Six hours later, reassessment of the cervix was done unless the clinical condition necessitated earlier assessment. A second dose and a third dose of 25 micrograms of misoprostol was given in cases of unfavorable cervix with failed ripening; each 6 hours apart. If no cervical ripening after the third dose of misoprostol, the patient was delivered by Caesarean section.

In cases that achieved cervical ripening with misoprostol, oxytocin infusion was started using 5 units in 500ml of normal saline or Ringer’s solution, 6 hours following the last dose of misoprostol-starting with a rate of 10-15 drops/minute.

Infusion rate was increased (by doubling drops/minute) at intervals of 30 minutes, until there were 3 good contractions in 10 minutes, each lasting 45-60 seconds.

During the period of induction, the fetal heart rate was monitored continuously, by means of
Ahmed S.A. Ashour, et al. 917
electronic fetal heart rate monitoring (Cardiotocography). Also, maternal monitoring was done including blood pressure measurements every 2 hours and frequent clinical evaluation (according to the condition). When a non reassuring fetal heart rate (FHR) was detected, closer monitoring of fetal heart rate was performed with simultaneous adequate conservative measures in the form of stoppage of oxytocin infusion, change in maternal position to the left lateral and oxygen administration. CS was intended to be done if persistent non-reassuring FHR was present after performing the previous maneuvers.

All patients received antepartum analgesia during the period of induction in the form of pethidine 50mg/4 hourly IM. Deliveries were performed in the operating theater and a pediatrician and anesthetist were attending.

Women's characteristics of age, height, BMI, gestational age, and initial Bishop Score were recorded. The primary outcome was successful attempt for vaginal delivery. The secondary outcomes were induction to delivery interval (IDI) and neonatal outcome in terms of Apgar score 1 and 5 minutes and neonatal birth weight. Using the definition of Watson et al., [8], an induction attempt was considered successful if the patient reached the active phase of labour as demonstrated by progressive dilatation and effacement of the cervix and followed by vaginal delivery. All women's data were recorded in a special input form.

Statistical analysis:

Data were statistically described in terms of Mean±Standard deviation (±SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t-test for independent samples. For comparing categorical data, Chi square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. p-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

One hundred primigravidas consented to participate in the study. The mean age of the studied group was 22.21 years. The range of gestational age was 40.7-42.60 weeks with the mean age being 41.4 weeks. The BMI ranged between 22.72-28.37 with a mean BMI of 24.78. The mean Bishop score was 3.15. The cohort of enrolled women was divided into two groups according to the result of induction by prostaglandin E 1 (Misoprostol) into Group A (successful induction of vaginal delivery; n=92) and Group B (failed induction; n=8). Table (1) illustrates the maternal clinical data and fetal outcome in both groups; there was evidence of statistically significant difference between both groups in terms of mean gestational age and BMI which were significantly higher in group B that failed to achieve vaginal delivery. On the other hand, there was no evidence of statistically significant difference between both groups as regards age and initial Bishop score. Apgar scores at 1 and 5 minutes were significantly higher in group A while neonatal birth weight was lower in this group. There was a highly significant difference between cervical length in the successful group (Mean=14.34mm) and failed group (Mean=28.25mm).

Table (1): Maternal clinical characteristics and fetal outcome among both groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Successful induction n=92</th>
<th>Failed induction n=8</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>Mean = 22.28</td>
<td>Mean = 21.38</td>
<td></td>
<td>.601</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>Mean = 41.42</td>
<td>Mean = 41.76</td>
<td></td>
<td>.5677</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean = 74.12</td>
<td>Mean = 76.50</td>
<td></td>
<td>.425</td>
</tr>
<tr>
<td>BMI</td>
<td>Mean = 24.67</td>
<td>Mean = 25.94</td>
<td></td>
<td>.042</td>
</tr>
<tr>
<td>Bishop score</td>
<td>Mean = 3.14</td>
<td>Mean = 3.25</td>
<td></td>
<td>.114</td>
</tr>
<tr>
<td>Cervical length by TVS (mm)</td>
<td>Mean = 14.34</td>
<td>Mean = 25.94</td>
<td></td>
<td>.376</td>
</tr>
<tr>
<td>Apgar score 1 minute</td>
<td>Mean = 6.10</td>
<td>Mean = 28.25</td>
<td></td>
<td>.018</td>
</tr>
<tr>
<td>Apgar score 5 minutes</td>
<td>Mean = 8.97</td>
<td>Mean = 8.00</td>
<td></td>
<td>.003</td>
</tr>
<tr>
<td>Neonatal birth weight (g)</td>
<td>Mean = 3016.85</td>
<td>Mean = 3580.00</td>
<td></td>
<td>.001</td>
</tr>
</tbody>
</table>
The occurrence of occipito-anterior position was significantly higher in Group A in comparison to the failed induction group. Also, flexed head position showed the highest percentage of successful labour induction in comparison with deflexed and extended positions with a statistically significant difference (Table 2).

Table (2): Relation between preinduction sonographic variables (fetal occiput position and degree of flexion) and outcome of induction.

<table>
<thead>
<tr>
<th>Ultrasound variable</th>
<th>Group</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (Successful, n=92)</td>
<td>B (Failed, n=8)</td>
<td></td>
</tr>
<tr>
<td>Position of the fetal head:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOA</td>
<td>44</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>LOP</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>LOT</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>ROA</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>ROP</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ROT</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Degree of flexion:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deflexed</td>
<td>13</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Flexed</td>
<td>77</td>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>Extended</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Fig. (1): Transvaginal cervical length measurement from the internal to external cervical os.

A strong positive correlation was found between the cervical length and the induction to delivery interval ($r=0.477$). Other variables such as ultrasound estimated fetal weight and neonatal birth weight showed a weak correlation. Bishop's score, on the other hand, showed negative correlation (inverse correlation) with induction to delivery interval ($-0.045$).

Table (3): Correlation between Bishop score, sonographically measured cervical length, and fetal weight and induction to delivery interval.

<table>
<thead>
<tr>
<th>Induction to delivery</th>
<th>Correlation coefficient= r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bishop score</td>
<td>-0.045</td>
<td>.337</td>
</tr>
<tr>
<td>Cervical length</td>
<td>0.477 (**)</td>
<td>.0001</td>
</tr>
<tr>
<td>U/S Estimated fetal weight</td>
<td>0.066</td>
<td>.265</td>
</tr>
<tr>
<td>Neonatal birth weight</td>
<td>0.157</td>
<td>.068</td>
</tr>
</tbody>
</table>

$r$ = Correlation coefficient  $p$ = Probability
** Correlation is significant at the 0.01 level (1-tailed).

Discussion

To date, Bishop score remains the standard method to predict the duration and outcome of induced labour. However, the preinduction “favorability” of the cervix as assessed by the Bishop score is very subjective and several studies have demonstrated a poor predictive value for the outcome of induction especially in women with a low Bishop score [6].

Many have evaluated and confirmed the validity of the Bishop score. Among the factors considered in assigning the score, the strongest association with successful labour seems to be with cervical dilation. The Bishop score has been criticized for not attributing more significance to cervical dilation. However, despite this criticism, none of the modifications to the original scoring system have been shown to improve predictability [9].

In the present study, 92 cases delivered vaginally after successful induction of labour. Eight cases (8%) delivered by cesarean section due to failure of progress of labour. There was a poor relationship between a high Bishop score and successful induction where many cases ($r=-0.045$) with poor Bishop score responded to induction of labour while some cases with high Bishop score did not respond to induction of labour. This is in contrast to what was revealed by Yanik et al., in 2007 who demonstrated that Bishop score significantly predicts the success of induction and the mode of delivery while transvaginal ultrasonography (TVUS) cervical length did not [10].

Measurement of cervical length was successfully achieved in all cases. The technique at term is more difficult, especially when the head is engaged and the alignment of the cervix is distorted.

Cervical length measurement was found to be the most significant parameter for the prediction of successful labour induction and showed a positive correlation to the induction to delivery interval ($r=0.477$) in contrast to the Bishop score which showed a non-significant negative correlation according to the results of the present study.

This agrees with the study done by Laencina et al., who also found cervical length measurement as a better predictor than the Bishop score for successful induction [11]. Also, Daskalakis et al., in 2006 found that cervical length proved to be an independent predictor of a successful labour induction in nulliparas [12].
Bastani et al., reported that cervical length measured by transvaginal ultrasonography has the potential to replace the traditional Bishop score, provided that such a facility is available when needed [13]. Also Tan et al., demonstrated that transvaginal sonography is significantly less painful than digital examination for Bishop score assessment [14].

In women undergoing induction of labour, the predictive value of cervical length is clearly superior to that of the Bishop score [15]. The sensitivity of sonographic assessment in the prediction of Cesarean section (CS) and likelihood of vaginal delivery within 24 hours of induction was higher than that of the Bishop score by about 20% [16].

Pandis et al., demonstrated that sonographically measured cervical length was better than the pre-induction Bishop score in predicting the likelihood of vaginal delivery within 24h of induction [17]. Torbjorn et al., found that univariable regression analyses of successful induction were significant for ultrasound measured cervical length <25mm [18].

Strobel et al., examined 97 singleton pregnancies at 41 to 42+2 weeks and reported that cervical length provided a significant prediction of spontaneous onset of labour and delivery within the subsequent 1-2 days but not within 4 days in either nulliparous or parous women [19].

Other studies for example Tan et al., however, found ultrasound measurements of cervical length and the Bishop score to be of similar value in predicting a Cesarean delivery [14]. Also, Roman et al., 2004 in their study of 106 postterm cases found that cervical length was not better indicator than the Bishop score in determining the delivery mode [20].

On the other hand, Rozenberg et al., (2005) in their study of 166 women induced with prostaglandins found the Bishop score to be better than cervical length for predicting successful outcome of induced labour [21]. Also Groeneveld et al., found that transvaginal ultrasonographic measurement of cervical length was not a significant independent predictor of vaginal delivery within 96 hours, where they chose a longer interval (96 hours) between start of induction and vaginal delivery and multiparous patients were included in their study. In their study, a maximum of 36-48 hours was given from start of induction to delivery [5].

There are number of maternal characteristics associated with successful induction such as height, weight, BMI and gestational age.

Our study showed that a lower BMI was associated with more successful induction. This agrees with the study done by Park et al., who stated that the mean BMI was significantly lower in women who had successfully induced labour and only BMI provided a significant contribution in predicting successful labour induction [22]. Also Uyar et al., concluded that BMI and transvaginal cervical length were better predictors compared to the Bishop score in determining the success of labour induction [23].

Peregrine et al., studied induction of labour in 267 women at 36 or more weeks of gestation. Logistic regression analysis was used to determine which factors best predicted the risk of cesarean delivery. They found that parity ($p<0.001$), BMI ($p<0.001$), height ($p=.05$), and ultrasonic transvaginal cervical length ($p<0.001$) are the most accurate parameters in predicting the risk of cesarean delivery after induction of labour [24].

Successful induction was also affected by certain fetal characteristics such as ultrasound estimated fetal weight, position of the occiput and degree of flexion of the head as displayed in our study. The rate of successful induction was 97.8% in the left occipito-anterior position and 100% in the right occipito-anterior position while only 50% in left occipito-posterior positions. Fetuses with well-flexed heads and smaller estimated fetal weight were associated with higher rate of successful induction and this difference was statistically significant ($p$-values 0.002 and 0.018 respectively).

A deflexed head is more commonly associated with the development or persistence of occipito-posterior positions which are associated with more failed labour induction [25].

This agrees with Peregrine et al., who demonstrated the ease of use of transabdominal ultrasonography in determining fetal head position before induction of labour and found that 96% of patients with occipito-posterior position have failed induction and delivered by CS [26]. Other studies, however, stated that determination of position of the head before induction of labour did not predict the course of labour, probably because the fetal head may rotate during labour even after premature rupture of membranes [27].

Rane et al., have also demonstrated the additional significant contribution of occipital position in predicting the outcome of induction of labour. Although occipital position is related to cervical length, being shorter in occipito-anterior and occipito-transverse than in occipito-posterior posi-
tions, the occipital position enhances the effect of cervical length in the prediction of outcome [16].

Rane et al., also found that cervical length and parity provide independent prediction of induction-to-delivery interval and the likelihood of vaginal delivery within 24 hours of induction [28]. This is also similar to the conclusions stated by De Gennaro et al., [29].

Also Rane et al., have established a series of models that classified women into high- and low-risk groups for Cesarean section and provided individual patient predictions for outcome of induction of labour [16]. For example: In a 35-year-old nulliparous woman with BMI >30 and the sonographic findings of cervical length of 30mm, and fetal OP position, the likelihood of Cesarean section is 87%. On the other hand, in a 25-years-old-parous woman with BMI <30 and cervical length of 10mm, and fetal OA position the likelihood of vaginal delivery within 24h is 95% and the likelihood of Cesarean section is 2.5% and this is highly consistent with our study.

In conclusion, preinduction sonographic measurements of cervical length, fetal occipit position and flexion of the head in postterm pregnancy are superior to Bishop score in prediction of the outcome of induction according to our findings. Thus, these can provide physicians with more precise information to plan further management of pregnancy.

It is recommended that cervical length measurement by transvaginal ultrasound should be done before induction of labor as it is highly correlated with success of labor induction.

References


18- TORBJØRN M. EGGEBØ, INGER OKLAND, CLAUDIA HEIEN, LEIF K. GIESING, PÅL ROMUNDSTAD and


Immuno histo chemical Expression of Toll-Like Receptor 3 in Verruca Vulgaris

AHMED A. SALEH, M.D.; FATMA M. EL-ESAWY, M.D. and TAGHREED A. ABDEL AZIZ, M.D.*

The Departments of Dermatology & Andrology and Pathology*, Faculty of Medicine, Benha University

Abstract

Background: Toll-like receptors are expressed on both immune and nonimmune cells and respond to specific components of microbial pathogens, their signaling results in the activation of nuclear factor B (NF-B), which triggers the production of a variety of antimicrobial and proinflammatory cytokines and chemokines.

Objective: The purpose of this study was to evaluate the role of Toll-like receptors 3 (TLR 3) in the pathogenesis of verruca vulgaris (VV) by its immnohistochemical expression in the skin lesions of VV patients.

Patients and Methods: This study was carried out on 20 patients with verruca vulgaris and 20 apparently healthy volunteers served as controls. All patients and controls were presenting at Benha Dermatology Department's Outpatients Clinic. A written consent was taken from each patient and control before participation in the study. This study was carried out during the period between March 2012 to February 2013. A 4mm punch skin biopsies were taken from wart in patient group and from normal skin in control group for histopathological staining and immnohistochemical staining of TLR 3.

Results: There was a highly significant increase ($p<0.001$) in the TLR3 expression, density and staining intensity in all cases as compared with the normal controls.

Conclusion: Epidermal TLR3 may have an important role in cutaneous innate immune responses to cutaneous human papillomavirus infections. So, Toll-like receptor agonists represent a promising approach for the treatment of verruca vulgaris.

Key Words: Verruca vulgaris – Papillomavirus – Toll-like receptors 3 – Immunohistochemical staining.

Introduction

PAPILLOMA viruses are a group of small non-enveloped DNA tumor viruses whose infection usually causes benign epithelial lesions (warts) but sometimes also cause malignancies (cervical cancer and anal cancer) [1].

Human papilloma viruses' lesions arise from the proliferation of infected basal cells. When the basal keratinocytes of the host are exposed to the virus through a disruption of the epithelial barrier, for example after minor skin abrasions or during sexual intercourse, infection occurs [2]. Keratinocytes are one of the first cutaneous cell types to encounter various microbes including the papilloma and molluscum viruses. Keratinocyte TLRs are believed to play an important role in pathogen recognition and host innate immune responses in the skin. Toll-like receptor 3, 7, and 9 were shown to recognize different kinds of viruses [3,4]. Toll-like receptors (TLRs) are a family of pattern recognition receptors that are activated by specific components of microbes and certain host molecules [5]. TLRs constitute the first line of defense against many pathogens and play a crucial role in the function of the innate immune system [6]. TLRs have been shown to play an important role in initiation and modulation of adaptive immune responses (via effects on dendritic cells), T helper subset differentiation, and immune tolerance, so they serve as a link between innate and acquired immune responses [7,8]; Toll-like receptor 3 was highly expressed in dengue viral infection [9], Toll-like receptor 3 recognize viral double-stranded RNA [10] and host messenger RNA (mRNA) as endogenous ligand [11]. Following activation of TLR by ligands of microbial origin, several reactions are possible. Immune cells can produce signaling factors called cytokines which trigger inflammation. In the case of a bacterial factor, the pathogen might be phagocytosed and digested, and its antigens presented to CD4 + T cells. In the case of a viral factor, the infected cell may shut off its protein synthesis and may undergo programmed cell death (apoptosis). Immune cells that have detected a virus may also release anti-viral factors such as interferon [12].
Patients and Methods

This case-control study was carried out on 20 patients with verruca vulgaris who had not received treatment for wart one year before the study. They were diagnosed clinically and histopathologically. Twenty age and sex matched apparently healthy volunteers served as controls. All patients and controls were presenting at Benha Dermatology Department's outpatients Clinic, during the period between October 2012 and February 2013, a written consent was taken from each patient and control before participation in the study which was approved by Ethics Committee Human Research of Benha University. The exclusion criteria were: History of keloid or hypertrophic scar, patients under anticoagulant treatment, patients with allergy to lidocaine, or patients with wart only on the joint.

Skin biopsy: Four mm punch skin biopsies were taken from wart in patient group and from normal skin in control group, were fixed in 10% neutral buffered formalin, routinely were processed and paraffin embedded. Sections 5 μm thickness from paraffin blocks were mounted on plain slides and routinely were stained with hematoxylin and eosin (H&E) and were examined by light microscopy to confirm the clinical diagnosis. Also, sections 4 μm thickness from both patients and controls were mounted on positive charged slides for immunohistochemical staining for TLR3.

Immunohistochemical staining: Paraffin-embedded tissue sections, 3-4 micron thick were mounted on positively-charged slides and heated at 60°C for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining. After antigen retrieval with microwave treatment in 10 mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6.0, endogenous peroxidase was blocked with 3% hydrogen peroxide for 15 minutes. Sections were washed 3 times with cold 0.01 M phosphate buffered saline (PBS) after blocking with 10% normal rabbit serum, slides were incubated 3 times with cold 0.01 M phosphate buffered saline (PBS) after blocking with 10% normal rabbit serum, slides were incubated for 1 hour with tol-3 antibody (Dako Cytomation Norden A/S, Glostrup, Denmark; dilution 1:50), prepared DAB-substrate-chromogenic solution was applied and incubated for 15-30 minutes until color intensity has been reached. Lastly, sections were counterstained with Mayer’s hematoxylin. Positive controls for tol-3 staining were used consisting of normal skin samples previously shown to stain with this antibody.

Statistical analysis: The results were analyzed using SPSS (version 16) statistical package for Microsoft windows. The person correlation coefficient was used for statistical analysis. p-value<0.05 was considered statistically significant.

Results

This study comprised 20 patients with verruca vulgaris, 8 females and 12 males. Their age ranged from 13 to 45 years with a mean of 24.85 ± 8.49 years. The duration of the disease ranged from 3 to 96 months with a mean of 26.4 ± 23.38 months. Two patients had single lesion 10%, 18 patients had multiple lesions 90%. The number of patients who had previous treatments with electrocautery 10 %, chemical cautery 10%, and who didn't have any previous treatments was 80 %. Twenty healthy volunteers served as control, 7 females and 13 males, their ages ranged from 16 to 42 years (Mean 26.2 ± 10.22).

Results of immunohistochemical staining of TLR3:

There was a highly significant increase in the TLR3 expression in all cases as compared with the normal controls (p<0.001). Expression of TLR3 was negative in normal human skin specimens (Fig. 1). TLR 3 was diffusely expressed throughout the horny and granular layer of epidermis in verruca vulgaris skin specimens. The expression staining intensity was moderate in two skin specimens and strong in the remaining specimens in patient group (Table 1) and (Figs. 2-7).

Table (1): Comparison between TLR 3 expression in the lesional skin of patients and control.

<table>
<thead>
<tr>
<th>TLR 3 Expression</th>
<th>Case No. %</th>
<th>Control No. %</th>
<th>Corrected χ² test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0 0.0</td>
<td>20 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately positive</td>
<td>2 10.0</td>
<td>0 0.0</td>
<td>31.28</td>
<td>0.001 HS</td>
</tr>
<tr>
<td>Strongly positive</td>
<td>18 90.0</td>
<td>0 0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20 100.0</td>
<td>20 100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(p-value<0.001).
Immunohistochemical staining of TLR 3 using Avidin-Biotin complex.

Fig. (1): A case of normal skin showing negative cytoplasmic expression of TLR 3 antibody in epidermal layers and dermis (strept-avidin-Biotin method) (IHx200).

Fig. (2): A case of verruca vulgaris immunostained by anti TLR 3 showing strong expression (brown staining) in all layers of the epidermis (strept-avidin-Biotin method) (IHx200).

Fig. (3): A case of verruca vulgaris immunostained by anti TLR 3 showing mild expression (brown staining) in the epidermis and dermis (strept-avidin-Biotin method) (IHx200).

Fig. (4): A case of verruca vulgaris immunostained by anti TLR 3 showing strong and diffuse expression (brown staining) in the epidermis and dermis (strept-avidin-Biotin) x 200.

Fig. (5): A case of verruca vulgaris immunostained by anti TLR 3 showing strong and diffuse expression in the epidermis with prominent hyperkeratosis (strept-avidin-Biotin) x 200.

Fig. (6): A case of verruca vulgaris immunostained by anti TLR 3 showing moderate expression (brown staining) in the basal layer of epidermis (strept-avidin-Biotin method) (IHx200).
Discussion

Warts are caused by double stranded human papilloma virus. Cutaneous viral infections have been proposed to induce localized inflammatory and antiviral responses that are believed to result in the resolution of viral skin lesions [14]. In order to counteract the innate host immune system, viruses should evolve mechanisms that block recognition and signaling through pattern recognition receptors, such as TLRs [15].

TLRs constitute the first line of defense against many pathogens and play a crucial role in the function of the innate immune system [6].

In the present study, TLR3 expression was negative in all control specimens. This is in agreement with Baker et al., [16], who reported that the expression of TLR3 and 4 was not detected in normal human skin. In contrast, Lebre et al., [17] demonstrated that normal keratinocytes constitutively express TLR 2, 3, and 4 mRNA, but not TLR 7. These different results might be due to the difference in the biopsy site and skin specimens, or to the staining methods.

All the patients showed statistically significant expression of TLR 3 when compared with controls. The intensity of expression was moderate in two wart specimens (10%) and strong in the 18 wart specimens (90%). Localization of TLR 3 by immune-histopathological examination revealed that it is more highly expressed throughout the horny, granular layer and basal layer of epidermis more than the dermal cells in the warts specimens. This strong epidermal expression of TLR 3 and the pure epidermal location of warts may explain that TLR 3 may have a role in recognition and response to cutaneous HPV infection. This is in agreement with Ku et al., [13], who evaluated the expression of various TLRs including TLR 3 in skin biopsies of patients with warts using PCR and immunostaining. The PCR and the immunostaining results showed markedly unregulated TLR 3 and 9 mRNA in warts and molluscum, indicating the importance of TLR 3 in cutaneous innate immune response to commonly encountered cutaneous viral infections.

The rationale for targeting TLRs as a means of treating viral infections is based on the presence of naturally occurring agonist molecules within invading viruses [18]. Poly-ICLC (Hiltonol) is a polyinosinic-polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose. It is a very stable double-stranded RNA and potent TLR 3 agonist with a strong IFN-inducing ability [19].

In this study statistical relationships between TLR 3 versus age and sex of patients, disease duration and number of warts were evaluated and were all found to be insignificant.

Conclusion:

Over expression of TLR 3 in wart skin implicate the role of TLR 3 in recognition and response to cutaneous HPV infection. Furthermore, pharmacological modulation of TLR 3 signaling or function of its downstream chemokines and/or cytokines can alter TLR 3 agonist-induced innate immune response and enhance its antiviral effects.

References


Study on the Management of Pregnancies Complicated by Late Preterm Prelabour Rupture of Membranes between 34 and 37 Weeks of Gestation in Woman's Health Centre-Assiut University: A Prospective Study

HAZEM S. MOHAMAD, M.D.
The Department of Obstetrics & Gynecology, Woman's Health, University Centre, Assiut University, Assiut, Egypt

Abstract

Background: Preterm prelabour rupture of membranes (PPROM) is the rupture of membranes during pregnancy before the end of 37 weeks gestation. It occurs in approximately 2-20% of pregnancies and is the cause of about one third of preterm deliveries. PPROM is associated with 18-20% of perinatal deaths. It can lead to significant perinatal morbidity. Treatment varies depending on gestational age, and includes consideration of delivery when rupture of membranes occurs at or after 34 weeks gestation.

Objectives: The aim of this study was to determine the scope of problem of (PPROM) in Woman’s Health Center-Assiut University and to address a question about which is better, the expectant management or the decision of labour induction.

Design and Setting: A randomised prospective controlled study was carried out in Woman’s Health Centre-Assiut University during the period from first of July 2012 to 31 of December 2012-Assiut-Egypt. Participants were randomly allocated in a 1: 1 ratio to induction of labour or expectant management using block randomisation.

Patients and Methods: One hundred, non-laboring, pregnant women with more than 24-hours of PPROM between 34 (+0) and 37 (+0) weeks of gestation were scheduled for this study. Half of them (50 women) were managed by labour induction (group A), while the other half (50 women) were treated expectantly (group B).

Every patient eligible for admission to this study was allotted serially a number from the table of random numbers, so all patients with even numbers induced labour (group A), and those with odd numbers received expectant management (group B).

The primary outcome was the presence or absence of neonatal respiratory distress syndrome as well as the mode of delivery, while the secondary outcome included neonatal sepsis, neonatal hypoglycaemia or neonatal hyperbilirubinaemia.

Results: In the expectant group (B), neonatal sepsis was found in 9 cases (18%), neonatal respiratory distress syndrome was found in 8 cases (16%), neonatal hyperbilirubinaemia was found in 5 cases (10%) and no reported cases of neonatal hypoglycaemia. In the induction of labour group (A), no reported cases of neonatal sepsis, neonatal respiratory distress syndrome was found in 29 cases (58%), neonatal hyperbilirubinaemia was found in 20 cases (40%) and neonatal hypoglycaemia was found in 18 cases (36%).

Conclusion: This current study showed that the expectant management in cases of premature prelabour rupture of membranes between 34 and the start of 37 weeks gestation remains the reasonable choice according to our local social and financial circumstances as regards the availability and cost of neonatal pediatric care units and medications needed for those preterm neonates.

Key Words: Preterm prelabour rupture of membranes – Preterm birth – Antenatal corticosteroids – Antibiotics.

Introduction

PREMATURE prelabour rupture of membranes (PPROM) refers to rupture of the foetal membranes prior to the onset of labour [1], and can occur at any gestational age even at 42 weeks, gestation. For this reason it is also referred to as prelabour ROM. PPROM can occur either at term or preterm (before 37 completed weeks gestation). Prolonged PPROM refers to that longer than 24 hours and is associated with an increased risk of ascending infection [2]. Approximately 8–10% of term pregnancies will experience spontaneous rupture of membranes prior to the onset of uterine activity [3-6]. Intra-amniotic infection and decidual haemorrhage (placental abruption) occurring remote from term, for example, may release proteases into the chorialdecidual tissues and amniotic fluid.
leading to rupture of membranes [5]. Also, invasive procedures performed during pregnancy as amniocentesis, chorionic villus sampling, fetoscopy or cervical cerclage, can damage the membranes causing them to leak [7,8].

Preterm PROM is associated with a 4-fold increase in perinatal mortality and a 3-fold increase in neonatal morbidity including respiratory distress syndrome (RDS), polymicrobial intra-amniotic infection, and intraventricular haemorrhage (IVH) [9-11]. The risk of caesarean delivery with its attendant surgical risks to the parturient is higher in preterm PROM as compared with term deliveries [12].

Preterm PROM is largely a clinical diagnosis. Diminished amniotic fluid volume by ultrasound alone cannot confirm the diagnosis but may help to suggest it in the appropriate clinical setting [2].

The initial management of a woman presenting with suspected PPROM should focus on confirming the diagnosis, validating gestational age, documenting foetal wellbeing, and deciding on the mode of delivery [13,14].

Absolute contraindications to expectant management include, intra amniotic infection (chorio-amnionitis), non-reassuring foetal testing, active labour, intrauterine foetal death or multi foetal anomalies [14,15].

A favorable gestational age (defined as >34 weeks) can also be regarded as a relative contraindications to continued expectant management in the setting of preterm PROM [2].

Several areas of controversy in the management of preterm PROM still exist. However, preterm PROM is a relative contraindication to the use of tocolytic agents [16]. There is now substantial evidence to suggest that adjunctive prophylactic (empiric) broad-spectrum antibiotics and administration of antental corticosteroids, can significantly prolong latency in the setting of preterm PROM remote from term [17,18].

This study was designed trying to settle the answer about which is better to the mother and her foetus in cases with preterm PROM, the expectant management trying to reach term or the desicion of inducing labour.

Patients and Methods

This randomized prospective-controlled study was conducted between the first of July 2012 to 31 of December 2012 at Woman’s Health Centre, Assiut University, Assiut, Egypt, and comprised one hundred pregnant women with premature PROM between 34th and the beginning of 37th week of gestation scheduled for this study. 50 Women were managed by labour induction (group A) and the other 50 were managed expectantly (group B).

All patients gave a clear written consent to participate in this study. Every patient eligible for admission to this study was allotted serially a number from the table of random numbers, so all patients with even numbers induced labour (group B) and those with odd numbers received expectant management (group A). The primary outcome was the presence or absence of neonatal respiratory distress syndrome as well as the mode of delivery, while the secondary outcome included neonatal sepsis, neonatal hypoglycaemia or neonatal hyperbilirubinaemia.

This study was approved by the Institutional Review Board of the Faculty of Medicine.

Every effort was taken to exclude contraindications to expectant management. Absolute contraindications included:

- Intra-amniotic infection (chorio-amnionitis) whose diagnosis remains primarily a clinical one with evidence of foetal tachycardia, maternal tachycardia, maternal fever (>37.8 °C) and/or uterine tenderness. Evidence of pus leaking from the cervix on sterile speculum examination can also confirm the diagnosis.
- Non-reassuring foetal testing.
- Patients actively in labour (true labour pain and cervical dilatation and/or ripening).
- Patients with multi foetal anomalies.
- Patients with previous uterine scar (CS, myomectomy or metroplasty).
- Diabetics and immunosupressed ladies.
- Intrauterine foetal death.
- Multifoetal pregnancies.

The key recommendations for conservative regimen of treatment included [19]:

- Antibiotics had to be administered to patients with PPROM because this prolongs the latent period and improves the outcomes.
- Corticosteroids had to be given to those patients to decrease the risk of intra ventricular haemorrhage, neonatal respiratory distress syndrome and necrotizing enterocolitis.
- Digital vaginal or cervical examination had not to be performed and sterile speculum usage was preferred.
- Long-term tocolysis was not indicated, although short-term regimen might be considered to facilitate maternal transport and the use of corticosteroids as well as antibiotics.
- Multiple (repeated) courses of corticosteroids were not recommended.

Termination of pregnancy was done to patients of (group A) (induction group), once the diagnosis of PPROM was established. For patients of (group B), there was no place for outpatient management, and the expectant regimen had to be undertaken in Woman's Health University Hospital because it was not possible to accurately predict which patient would develop complications as infection or placental abruption.

Modified bed rest was notified to all participants of group (B) in this study, in an attempt to enhance reaccumulation of amniotic fluid and to improve uteroplacental perfusion and thereby foetal growth.

**Neonatal sepsis was defined as follows [20]:**

- Blood culture taken at birth found positive for bacteria excluding staphylococcus epidermidis.
- Two or more symptoms of infection within 72 hours after birth (aponea, temperature instability, lethargy, feeding intolerance, respiratory distress, or haemodynamic instability).
- C-reactive protein >20nmol/L.

Data were collected and analyzed with SPSS version 11 (statistical package for social science, Chicago IL, USA). Statistical methods were applied including descriptive statistics (frequency, percentage, mean and SD) and tests of significance (by $X^2$ statistics or Fisher's exact test as appropriate). A $p$-value <0.05 was considered statistically significant.

**Results**

This study comprised 100 (one hundred) pregnant women with preterm prelabour rupture of membranes between the 34 (+0) and 37 (+0) weeks gestation. Sociodemographic data of these women are shown in Table (1).

<table>
<thead>
<tr>
<th>Table (1): Sociodemographic data.</th>
<th>Group (B) 50 cases Expectant management</th>
<th>Group (A) 50 cases Labour induction</th>
<th>Significance $p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: (years)-range (Mean±SD)</td>
<td>27.63±3.19</td>
<td>28.34±2.13</td>
<td>N.S.</td>
</tr>
<tr>
<td>Parity (Range)</td>
<td>2-4 (2.3 ± 1.0)</td>
<td>1-3 (1.8±2.0)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Body mass index (BMI) (Kg/m²) (range)</td>
<td>27.3±4.5</td>
<td>26.1±5.0</td>
<td>(17-40)</td>
</tr>
<tr>
<td>Previous history of “PPROM”</td>
<td>2 cases</td>
<td>3 cases</td>
<td>N.S.</td>
</tr>
<tr>
<td>Passive % smoking (husband is smoker)</td>
<td>6 cases (12%)</td>
<td>5 cases (10%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Crowded house % (&gt;3 persons/room)</td>
<td>26 cases (52%)</td>
<td>22 cases (44%)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Among patients had to receive expectant management 5 cases (18%) showed neonatal sepsis while no single case developed such complication among the induction group.

On other side, those offsprings who developed respiratory distress syndrome (RDS) and were incubated in the neonatal intensive care unit (NICV) for >5 days, were 29 cases (58%) among the induced group (A), while only 8 neonates (16%) among the expectant group (B) were in need for (NICV).

Neonatal hypoglycaemia (immediate postnatal blood sugar level <50mg/dl) was found in 18 cases (36%) of group (A) (induction group) while neonatal hyperbilirubinaemia occurred in 20 cases (40%) among this group. In the expectant group (B) only 1 case of neonatal hyperbilirubinaemia accrued, while no cases of neonatal hypoglycaemia was confronted in this group (Table 2).
Study on the Management of Pregnancies Complicated

Table (2): Neonatal outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Group (A) Induction group</th>
<th>Group (B) Expectant group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal sepsis (n.) (%)</td>
<td>Zero</td>
<td>9 (18%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory distress syndrome RDS (n.) (%)</td>
<td>29 (58%)</td>
<td>8 (16%)</td>
<td>Statistically significant</td>
</tr>
<tr>
<td>Intra partum foetal death (n.) (%)</td>
<td>Zero</td>
<td>Zero</td>
<td>Not available</td>
</tr>
<tr>
<td>N. hypertibirubanemia</td>
<td>20 (40%)</td>
<td>Zero</td>
<td>S.S. p&lt;0.001</td>
</tr>
<tr>
<td>Neonatal hypoglycaemia (n.) (%)</td>
<td>18 (36%)</td>
<td>Zero</td>
<td>S.S. P&lt;0.001</td>
</tr>
</tbody>
</table>

Table (3): Maternal outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Group (A) Induction group</th>
<th>Group (B) Expectant group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antepartum haemorrhage n (%)</td>
<td>1 (2%)</td>
<td>1 (4%)</td>
<td>N. S.</td>
</tr>
<tr>
<td>Cord prolapse n (%)</td>
<td>Zero</td>
<td>Zero</td>
<td>Not available</td>
</tr>
<tr>
<td>Clinical chorio amnionitis n (%)</td>
<td>Zero</td>
<td>9 cases (18%)</td>
<td>S.S.</td>
</tr>
<tr>
<td>Thromboembolic complications n (%)</td>
<td>1 (2%)</td>
<td>Zero</td>
<td>N. S.</td>
</tr>
<tr>
<td>Maternal death n (%)</td>
<td>Zero</td>
<td>Zero</td>
<td>Not available</td>
</tr>
</tbody>
</table>

Mode of delivery:
A- Vaginal delivery n (%) | 32 (64%) | 29 (58%) | N. S. |
B- Caesarean section n (%) | 18 (36%) | 21 (42%) | N. S. |

Primary outcome:
1- Mode of delivery
2- Neonatal RDS (respiratory distress syndrome)

Secondary outcome:
1- Neonatal sepsis
2- N.hypoglycaemia
3- N.hyperbiliribinaemia

Fig. (1): Flowchart of the study population.
Discussion

We conducted a relatively short prospective randomized study to compare induction of labour and expectant management in women with preterm prelabour rupture of membranes (PPROM) between 34 and 37 weeks of gestational age.

We found that, in pregnancies completed by (PPROM), labour induction reduces the incidence of neonatal sepsis compared to the expectant regimen of management. The number of neonates with respiratory distress syndrome (RDS) was comparable in both arms, and induction of labour, did not increase the risk of caesarean section. However, in this current study labour induction increases the risk of neonatal hypoglycaemia as well as hyperbilirubinaemia.

Our findings are not in line with the results of Hannah et al., [20] who compared labour induction with expectant management in (5041) women with PROM at term and showed that labour induction didn't reduce the risk of neonatal sepsis as compared to expectant management (2.5% versus 2.8%). This differences in this aspect of results may be due to some limitations in our study because of low financial sources, since our study didn't include routine cultures from all neonates to diagnose sepsis and neonatal blood samples as well as liquor cultures were taken only for clinical indications at (NICU). In consensus, neonatologists decided whether or not a newborn had suffered neonatal sepsis (suspected or proven). Despite of the lack of blood culture from neonates in this study, we believe that no case of neonatal sepsis was missed from pediatricians in our (NICV) sector.

In our current study, labour induction reduced the risk of clinical chorioamnionitis and this was coincided with the results of Naef et al., [21] and Villar et al., [22].

Our study has also described a relationship between chorioamnionitis and increased risk of neonatal sepsis, respiratory distress and even neonatal death. This was agreeable with the results of Alexander et al., [23] Lau et al., [24] and Leviton et al., [25].

The number of caesarean sections in our study was comparable in the induction as well as expectant groups and this was coinciding with the results of Hannah et al., [20]. However, Buchanan et al., [26] reported that there was an increased risk of caesarean section among the expectant management group in their study.

The current study resulted in decreased risks of neonatal hypoglycaemia and hyperbilirubinaemia in infants of women managed expectantly. This was similar to the results of Van der Ham et al., [27] and Kayem et al., [28]. This was probably, contributed to prolongation of gestational age at the time of delivery. Hyperbilirubinaemia is potentially neurotoxic especially in infants born preterm [29]. However, total bilirubin levels below 30mg/dl, when treated appropriately are not associated with adverse neuro-developmental outcome [30].

Nevertheless, limitations of this study are many. Both strict inclusion criteria and relatively low number of participating patients weaken the collected data. Another potential limitation, is that we reported few secondary, mostly neonatal, outcomes. Although, this is uncommon in maternal-fetal medicine, it is possible that a significant difference can not be found by chance [27].

In conclusion, in pregnancies complicated by PPROM between 34 and 37 weeks of gestation, the incidence of neonatal sepsis is not low. Our study showed that induction of labour did not substantially improve pregnancy outcomes compared with the expectant management.

Acknowledgments:

We thank our colleagues, residents, house officers, high nurses, and nurses as well as all staff members of the neonatal intensive care unit in paediatric hospital-Assiut University for their great help with recruitment and data collection.

References


Effect of Endurance Exercise and/or Diet Restriction on Mitochondrial Bioenergetics Function in Skeletal Muscle of Diabetic Male Albino Rats

NASHWA ELTABLAWY, M.D. and EMAN F. KHALEEL, M.D.

The Department of Physiology, Faculty of Medicine, Cairo University

Abstract

Reduced mitochondrial capacity in skeletal muscle occurs in type 1 diabetic patient and in those at increased risk for this disorder, but the extent to which mitochondrial dysfunction in type 1 diabetic patients is remediable by physical activity and/or diet restriction intervention is uncertain. The aims of our study were to assess the effect of diet restriction and or endurance training on: 1- Expression of Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) the major transcriptional coactivator regulating the expression of the oxidative phosphorylation (OXPHOS) gene, estrogen related receptor alpha (ERR α) and mitofusin2, 2- Expression of genes of mitochondrial enzymes involved in mitochondrial oxidative metabolism, 3- Lipid peroxidation as measured by (malondialdehyde) MDA and 4- Glucose metabolism.

Material and Methods: In this study six groups, 8 rats for each, were included: A sedentary fed ad libitum group, a trained fed ad libitum group, a diabetic sedentary fed ad libitum group, a diabetic trained group, a diabetic diet restricted group, and a diabetic trained-diet restricted group. For each group the following parameters were measured: 1- Serum glucose, serum insulin, 2- Glycogen, MDA level in gastrocnemius muscle and 3- Gene expression by real time PCR for PGC-1 α, Mitofusin2, GLUT4, carnitine palmitoyltransferase-1 (CPT-1) and citrate synthase.

Results: Our results showed that streptozotocin (STZ) induced diabetic rats had significant increase in glucose level and significant decrease in insulin level. Endurance training or diet restriction caused significant decrease in glucose level, while combination of diet restriction and endurance exercise synergistically caused significant decrease in comparison to diet restriction alone but still significantly higher than the normal level in the control rats. There was significant increase in MDA content of gastrocnemius muscles of STZ-induced diabetic rats which significantly decreased by diet restriction, more decreased by endurance exercise, combination of diet restriction and endurance exercise synergistically caused more decrease to the normal level as normal control rats. Glycogen content of gastrocnemius muscle of STZ-induced diabetic rats was decreased significantly which it was increased significantly by endurance exercise when compared to diabetic rats. Also our results showed significant decrease in mRNA o PGC-1 α, ERR α, Mitofusin2, GLUT4, CPT-1, citrate synthase in gastrocnemius muscles STZ-induced diabetic rats in comparison to normal control rats. Diet restriction caused insignificant increase in PGC-1 α, ERR α, mitofusin2, GLUT4, CPT-1. Endurance exercise in STZ-induced diabetic rats caused significant increased in gene expression of PGC-1 α, ERR α, mitofusin2, GLUT4, CPT-1 and insignificant increased in citrate synthase. Combining effect of Diet restriction and endurance training caused insignificant increased in PGC-1 α, Mitofusin2, GLUT4, CPT-1, citrate synthase in comparison to exercise alone.

Conclusion: Diet restriction and/or endurance training in diabetic rats induces mitochondrial bioenergetics in skeletal muscle, and enhances mitochondrial function and improve glucose metabolism by skeletal muscle possibly due to increase PGC-1 α and ERR α. Therefore represent a key strategy in the prevention and treatment of type 1 diabetes and in those at increased risk for this disorder.

Key Words: Type 1 diabetic – Exercise – Diet restriction – PGC-1 α – ERR α – Mitofusin2.

Introduction

IT is well known that increased contractile activity, such as endurance exercise training, promotes phenotypic adaptations in skeletal muscle toward a more oxidative phenotype. Specifically endurance exercise training leads to fiber type transformation, mitochondrial biogenesis, angiogenesis, and other adaptive changes in skeletal muscles along with improved insulin sensitivity and metabolic flexibility in both rodents and humans [1-5].

One of the most adaptations to endurance exercise training is an increase in skeletal muscle mitochondrial size and density [6]. A training-induced increase in mitochondrial content is associated with reduced risk for several chronic diseases, likely due, in part, to an enhanced ability to oxidize carbohydrate and lipid [7]. In contrast, decreased mitochondrial volume and a reduced capacity for substrate oxidation have been linked
to obesity [8], insulin resistance [9], aging and Type 2 diabetes [10]. Increasing skeletal muscle mitochondrial content through exercise training may, therefore represent a key strategy in the prevention and treatment of chronic diseases [11-13]. Elucidating the molecular mechanisms that promote exercise-induced mitochondrial bioenergetics is thus an important area of scientific investigation with significant therapeutic application.

Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) transcriptional coactivator identified through its interaction with PPARG in brown fat cells, was found to be greatly unregulated in brown fat and skeletal muscle in response to cold exposure [14]. Also it was found that PGC-1α mRNA and protein expressions are very responsive to endurance exercise [15,16]. It was reported that PGC-1α is activated in old animals under caloric restriction (CR) [17]. The expression of PGC-1α is decreased in skeletal muscle of type 2 diabetic patients [18] and with physical inactivity [19]. PGC-1α is now known to be involved in the regulation of thermogenesis, energy metabolism, and other biological processes of various organ systems [20-22]. PGC-1α interacts with and coactivates a growing list of transcription factors including estrogen-related receptor-α (ERR) [23], thyroid receptor [14], nuclear respiratory factor-1 (NRF-1) [24], NRF-2 [25], and myocyte enhancer factor-2 (MEF2) [26]. As a consequence, PGC-1α is involved in the coordinated regulation of nuclear-and mitochondrial-encoded genes required for contractile and metabolic adaptations in skeletal muscle [27,28]. PGC-1α upregulation (20-27%) in insulin-resistant muscles (obese Zucker rats) improved insulin-stimulated glucose transport, reduced intramuscular lipids (triaclylglycerol, diacylglycerol, ceramide), increased mitochondrial fatty acid oxidation (subsarcolemmal mitochondria only, not intermyofibrillar mitochondria), and increased expression of GLUT4 [29]. A strong positive correlation ($ r=0.74; p<0.05$) between changes in PGC-1α and cytochrome c oxidase (COX) activity was used as an index of mitochondrial adaptations [30] there is now ample evidence that a massive PGC-1α overexpression (600-2,000%) have unexpected pathophysiological consequences [31].

Leick et al., [32] reported that PGC-1α is not mandatory for exercise- and training-induced adaptive gene responses as reduced basal level mitochondrial respiratory function in a mouse model of global gene disruption of the PGC-1 gene (PGC-1 KO) was corrected by endurance exercise training and increases in hexokinase II, aminolevulinic synthase 1, and cytochrome oxidase (COX) protein expression in response to endurance exercise.

The estrogen-related receptor (ERRγ) is one of the first orphan nuclear receptors identified [33]. Both ERRγ and PGC-1α are up regulated in human skeletal muscle following endurance exercise [34,35]. ERRγ, in combination with its transcriptional co-activator PGC-1α, is known to regulate genes involved in mitochondrial energy-producing pathways in cardiac and skeletal muscle [34,36]. ERRγ activates genes involved in multiple key energy production pathways, including cellular fatty acid uptake, fatty acid oxidation [37] and mitochondrial electron transport/oxidative phosphorylation [38].

Mitofusin2 (Mfn2) is a mitochondrial membrane protein that participates in mitochondrial fusion and regulates mitochondrial metabolism in mammalian cells [39,40]. Soriano et al., [41] demonstrate a stimulatory effect of PGC-1α on Mfn2 mRNA and protein expression in muscle cells. PGC-1α also stimulated the activity of the Mfn2 promoter, ERRγ also activated the transcriptional activity of the Mfn2 promoter, and the effects were synergic with those of PGC-1α. Mfn2 loss of function reduced the stimulatory effect of PGC-1α on mitochondrial membrane potential. The previous results indicate the existence of a regulatory pathway involving PGC-1α, ERRγ, and Mfn2. Alterations in this regulatory pathway may participate in the pathophysiology of insulin-resistant conditions and type 2 diabetes (40,42).

Our study was performed in order to investigate the possible role of exercise and or food restriction on mitochondrial function and glucose metabolism in skeletal muscle of STZ-induced diabetic rats.

**Material and Methods**

The present study was carried out in the Physiology Department, Faculty of Medicine, Cairo University from March 2013 – July 2013. 48 male albino rats with body weights 150-200 grams were included in this study. The animals were and placed in the animal house of the faculty. They were housed in wire mesh cages at room temperature with 12:12 dark-light cycles.

**Animals were randomly divided into 6 groups of 8 rats each:** Group 1: Sedentary fed ad libitum group, Group 2: Trained fed ad libitum group, Group 3: Diabetic fed ad libitum group, Group 4: Diabetic diet restricted group, Group 5: Diabetic trained and fed ad libitum group and Group 6: Diabetic trained and diet restricted group.
Experimental diabetes: Was induced in fasting rats by single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (60mg/Kg body weight; Sigma Aldrich Co., Germany) dissolved in citrate buffer [43].

Diet restriction:
All groups were allowed to fed ad libitum for one week then food was restricted to the particular studied groups (Groups 4,6) by about 50% (low carbohydrate diet) [44].

Training:
The exercise protocol consisted of swimming exercise (1 hr/day, 5 days/week) [45] for 12 weeks in a swimming tank filled with water at a temperature of 37ºC. Daily swimming divided into 2 sessions each formed of 30 minutes separated by 1 hour rest. At the completion of each period of swimming exercise the rats were removed from the water, carefully dried and returned to their cages. The exercised rats underwent a swimming programme consisting o gradually increasing periods of swimming in the first 4 days the duration of exercise was gradual increased from an initial period of 15min to the maximum permissible period of 30min.

At the end of the experimental protocol, blood samples from all overnight-fasting rats were collected, by introducing fine capillary tube at the inner canthus of the eye into the venous plexus, to assess serum levels of glucose and insulin. Then, animals were sacrificed and gastrocnemius muscle were rapidly excised for further detection of GLUT4, glycogen, MDA, ERR (x), CPT-1, PGC-1 (x), citrate synthase and Mitofusin2.

Measurements of fasting blood glucose level:
Serum glucose was measured using oxidase-peroxidase method [46].

Measurement of serum insulin:
Serum insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer’s instructions [47].

Assessment of glycogen level:
Glycogen was measured in muscle sample by Colorimetric method using Glycogen Assay Kit (abnova USA) according to manufacture instruction.

Measurement of Malondialdehyde (MDA):
To measure the MDA concentration, 1 00mg of muscle tissue in 1mL PBS, pH 7.0 was homogenized with micropestle in microtube. 20% TCA was added to muscle homogenate to precipitate the protein, and centrifuged. Supernatants were collected and thiobarbituric acid (TBA) solution was added to the supernatants. After boiling for 10 minutes in water bath, the absorbance was measured. Concentration of MDA in supernatants of muscle homogenate was calculated using the standard curve [48].

Detection of gene expression by real time PCR:
Mitofusin2, citrate synthase, carnitine palmitoyltransferase- 1 (CPT- 1), ERR alpha, GLUT4 and PGC-1 alpha gene expression was measured by real time PCR briefly, Real-time quantitative PCR.

Total RNA was isolated from muscle tissue homogenate using RN easy Purification Reagent (Qiagen, Valencia, CA) according to manufacturers instruction. The RNA sample was dissolved in RNase-free water and quantified spectrophotometrically. The integrity of the RNA was studied by gel electrophoresis on a 1% agarose gel, containing ethidium bromide. First-strand cDNA synthesis was performed with the Super Script Choice System (Life Technologies, Breda, the Netherlands) by mixing 2yg total RNA with 0.5 yg of oligo (dT) primer in a total volume of 12 gL. After the mixture was heated at 70ºC for 10min, a solution containing 50mmol/L Tris˙HCl (pH 8.3), 75mmol/L KCl, 3mmol/L MgCl2, 10mmol/L DTT, 0.5mmol/L dNTPs, 0.5 g RNase inhibitor, and 200U Super-script Reverse Transcriptase was added, resulting in a total volume of 20.5 gL. This mixture was incubated at 42ºC for 1h; total volume was adjusted to 100 gL with RNase-free water and stored at 80ºC until further use.

For real-time quantitative PCR, 5 gLoF first-strand cDNA was used in a total volume of 25 gL, containing 12.5 gL 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200ng of each primer.

Primers, which shown in Table (1), PCR reactions, consisting of 95 ºC for 10min (1 cycle), 94 ºC for 15s, and 60 ºC for 1min (40 cycles), were performed on an ABI Prism 7900HT Fast Real Time PCR system (Applied Biosystems).

Data were analyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1.7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative Ct method. All values were normalized to the beta actin genes [49].
Exercise in normal rats did not significantly affect glucose and insulin levels compared to normal sedentary rats ($p>0.05$), while it reduced glucose levels significantly in diabetic trained rats (Group 5) compared to diabetic sedentary fed ad. libitum (Group 3). While exercise had no significant effect on insulin level as there was no significant difference between (Group 3) and (Group 4).

The diet restriction and diet restriction & exercise (Groups 4, 6) decreased the glucose level and the insulin level in the diabetic rats compared to diabetic sedentary fed ad. libitum (Group 3) (Figs. 1, 2).

**Table (1): Primer sequences used for RT-PCR.**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitofusin</td>
<td>F: 5’-ACGGTGGAGTCTAATGAC-3’&lt;br&gt;R: 5’GAGAAATGTTTTGGCCTG-3’</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>F: 5’-GGCCTGGAACCTCTGCTC-3’&lt;br&gt;R: 5’-GGCTCCTCCTCCATCC-3’</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Forward 5’- CCGAGCTCATGGAGGAGCTA-3’&lt;br&gt;Reverse 5’- ATCTGTGTGGAGGCTTCGTG-3’</td>
</tr>
<tr>
<td>ERR alpha</td>
<td>Forward 5’- GCG CAT CCA GAC CAA CAA TAAC-3’&lt;br&gt;Reverse 5’- GCC GAA GCT GCA TGG ACA CT-3’</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Forward 5’- AAAGAAGCCGACACTAAACC-3’&lt;br&gt;Reverse 5’- CTTCAATTCAGGAGGATCC-3’</td>
</tr>
<tr>
<td>PGC-1 alpha</td>
<td>Forward 5’- TTGGCCCAAGTCCTCCTGAC-3’&lt;br&gt;Reverse 5’- TGAGGACCGCTACGAAGTT-3’</td>
</tr>
<tr>
<td>Beta actin</td>
<td>Forward 5’ TCT GCC ACC CTTTT ACA ATG3&lt;br&gt;Reverse 5’- AGC ACA GCC TGG ATA GCA ACG3</td>
</tr>
</tbody>
</table>

**Statistical method:**

The data was coded and entered using the statistical package SPSS version 15. The data was summarized using descriptive statistics: Mean, standard deviation, median, minimal and maximum values for quantitative variables. Statistical differences between groups were tested using ANOVA (analysis of variance) with post Hoc-Bonferroni test for quantitative normally distributed variables while Nonparametric Mann Whitney test and kruskal-Wallis test were used for quantitative variables which aren’t normally distributed. $p$-values less than or equal to 0.05 were considered statistically significant.

**Table (2): Mean±Standard deviation of serum fasting glucose, insulin, PGC-1 $\alpha$, ERR$\alpha$, Mitofusin2, GLUT4, glycogen, MDA, CPT-1, and citrate synthase among the six studied groups.**

<table>
<thead>
<tr>
<th></th>
<th>Sedentary fed ad libitum Group (1)</th>
<th>Trained fed ad libitum Group (2)</th>
<th>Diabetic fed ad libitum Group (3)</th>
<th>Diabetic diet restricted Group (4)</th>
<th>Diabetic trained and fed ad libitum Group (5)</th>
<th>Diabetic diet restricted and food restrict Group (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>83.65±9.74</td>
<td>80.59±9.58</td>
<td>227.8±19.7#*</td>
<td>180.9±17.04#*</td>
<td>167.5±16.21#<em>$^</em>$</td>
<td>145.73±18.02#<em>$^</em>$</td>
</tr>
<tr>
<td>Serum insulin (ng/ml)</td>
<td>63.41±9.29</td>
<td>57.12±7.16</td>
<td>40.51±5.85#*</td>
<td>3.76±7.43#<em>$^</em>$</td>
<td>39.34±5.85#<em>$^</em>$</td>
<td>34.49±7.01#<em>$^</em>$</td>
</tr>
<tr>
<td>PGC-1 $\alpha$</td>
<td>1.24±0.27</td>
<td>1.20±0.23</td>
<td>0.38±0.15#*</td>
<td>0.46±0.18#*</td>
<td>0.59±0.20#<em>$^</em>$</td>
<td>0.67±0.14#<em>$^</em>$</td>
</tr>
<tr>
<td>ERR $\alpha$</td>
<td>0.88±0.14</td>
<td>1.19±0.37</td>
<td>0.56±0.17#</td>
<td>0.58±0.14#</td>
<td>0.99±0.11#<em>$^</em>$</td>
<td>0.97±0.24#<em>$^</em>$</td>
</tr>
<tr>
<td>Mitofusin2</td>
<td>2.63±1.26</td>
<td>2.75±0.84</td>
<td>1.69±0.46#</td>
<td>1.84±0.34#</td>
<td>2.54±0.54#<em>$^</em>$</td>
<td>2.83±0.86#<em>$^</em>$</td>
</tr>
<tr>
<td>GLUT4</td>
<td>0.64±0.13</td>
<td>0.55±0.09</td>
<td>0.14±0.05#*</td>
<td>0.17±0.05#*</td>
<td>0.44±0.11#<em>$^</em>$</td>
<td>0.52±0.13#<em>$^</em>$</td>
</tr>
<tr>
<td>Glycogen mg/mg</td>
<td>9.80±1.09</td>
<td>10.11±0.88*</td>
<td>3.69±0.82#*</td>
<td>3.54±1.13#<em>$^</em>$</td>
<td>6.09±1.15#<em>$^</em>$</td>
<td>7.41±0.79#<em>$^</em>$</td>
</tr>
<tr>
<td>MDA nmol/mg</td>
<td>1.94±0.38</td>
<td>2.02±0.62</td>
<td>10.03±1.36#<em>$^</em>$</td>
<td>7.99±1.57#<em>$^</em>$</td>
<td>4.16±1.18#<em>$^</em>$</td>
<td>3.11±1.03#<em>$^</em>$</td>
</tr>
<tr>
<td>CPT-1</td>
<td>2.11±0.35</td>
<td>2.15±0.41</td>
<td>0.98±0.33#*</td>
<td>1.38±0.30#*</td>
<td>1.80±0.27$^*$</td>
<td>1.98±0.27$^*$</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>0.22±0.04</td>
<td>0.24±0.11</td>
<td>0.07±0.03</td>
<td>0.07±0.02#*</td>
<td>0.09±0.04#*</td>
<td>0.11±0.03#<em>$^</em>$</td>
</tr>
</tbody>
</table>

*: Significant ($p<0.05$) when compared to sedentary fed ad libitum rats.  
#: Significant ($p<0.05$) when compared to trained fed ad libitum rats.  
$^*$: Significant ($p<0.05$) when compared to diabetic fed ad libitum rats.  
$^\&$: Significant ($p<0.05$) when compared to diabetic diet restricted rats.  
$^\&\$: Significant ($p<0.05$) when compared to diabetic trained fed ad libitum rats.

**Results**

**Fasting serum glucose and insulin:**

Significant increase ($p<0.05$) in glucose level in Group 3 (Diabetic fed ad lib) compared to Group 1 (Sedentary fed ad lib), Group 2 (Trained fed ad lib), Group 4 (Diabetic diet restricted), Group 5 (Diabetic trained) and Group 6 (Diabetic trained and food restricted) was seen.

There was a significant decrease ($p<0.05$) in insulin value in diabetic rats (Group 3) when compared to normal rats (Group 1, Group 2).
PGC-1α, ERRα and mitofusin2:

There was significant decrease ($p<0.05$) in mRNA of PGC-1, ERR alpha and mitofusin2 in diabetic rats (Group 3) when compared to normal rats (Groups 1,2). Their values are not affected by diet restriction (Group 4) as there were no significant change when compared by diabetic rats ($p>0.05$). Exercise in diabetic rats (Group 5) caused significant increase in comparing PGC-1α, ERR alpha and mitofusin2 with their values in diabetic rats (Group 3). Also exercise in diabetic rats (Group 5) returned ERR alpha and mitofusin2 to normal values as there was no significant change when compared to normal control rats (Groups 1,2). While PGC-1α was not returned to normal value by exercise as there was significant decrease when compared to normal control rats (Groups 1,2). Diet restriction and exercise (Group 6) showed significant increase ($p<0.05$) in PGC-1mRNA when compared to diet restriction alone (Group 4) (Figs. 3-5).
GLUT4:

GLUT4 level was not changed in normal rats by exercise as there was no significant difference between Group 1 (sedentary fed ad libitum) and Group 2 (trained fed ad libitum), while diabetes caused significant decreased ($p < 0.05$) in Group 3 (diabetic fed ad libitum) when compared to the control (Group 1). Diet restriction had no effect on GLUT4 as there was no significant difference ($p > 0.05$) between Group 4 (diabetic diet restricted) and Group 3 (diabetic rats). But exercise and exercise and diet restriction in diabetic rats (Groups 5, 6) caused significant increase ($p < 0.05$) in GLUT4 when compared to diabetic (Group 3) (Fig. 6).

Exercise and diet restriction restore GLUT4 level within normal as there was no significant difference in GLUT4 level between (Group 6) and normal rats (Groups 1, 2).

Glycogen:

There was no significant difference in glycogen in skeletal muscle of normal rats ($p > 0.05$) between trained group (Group 2) and Group 1 (sedentary fed ad libitum). There were significant decrease ($p < 0.05$) in all diabetic Groups 3, 4, 5 and 6 when compared to (Groups 1, 2).

Exercise caused significant increase ($p < 0.05$) in glycogen (Group 5) when compared to its value in diabetic rats (Group 3) and diet restriction (Group 4).

Diet restriction (Group 4) had no significant change ($p > 0.05$) in glycogen level when compared to values of diabetic rats (Group 3). Also diet restriction with exercise (Group 6) showed no significant change in glycogen when compared to its value caused by exercise alone (Group 5) (Fig. 7).

MDA:

Diabetes in group 3 caused significant increase ($p < 0.05$) in MDA when compared to normal rats (Groups 1, 2). Exercise or diet restriction in diabetic rats (Groups 4, 5) caused significant decrease in MDA when compared to its value in diabetic rats (Group 3) but still significantly higher than MDA value of normal control rats. Exercise and diet restriction in diabetic rats (Group 6) caused significant decrease in MDA that reach its value as normal control rats as there is no significant change between group 6 and 1, 2 ($p > 0.05$) (Fig. 8).

CPT-1:

There was significant decrease ($p < 0.05$) in CPT-1 mRNA in diabetic rats (Group 3) when compared to its value in normal rats (Groups 1, 2). Diet restriction in diabetic rats (Group 4) had no significant effect on CPT-1 when compared to diabetic rats,
while exercise in diabetic rats (Group 5) caused significant increase \((p<0.05)\) in its value when compared to diabetic rats. Also exercise return its level as normal control as there is no significant difference \((p>0.05)\) in CPT-1 values between diabetic rats with exercise and normal control rats (Fig. 9).

**Citrate synthase:**

There was significant decrease \((p<0.05)\) in citrate synthase in diabetic rats (Group 3) when compared to its value in normal rats (Groups 1, 2). Diet restriction or exercise in diabetic rats (Groups 4, 5) had no significant difference \((p>0.05)\) effect on Citrate synthase when compared to its value in diabetic rats. Combination between diet restriction and exercise in diabetic rats (Group 6) caused insignificant \((p>0.05)\) increase in citrate synthase when compared to its value in diabetic rats (Group 3) (Fig. 10).

**Discussion**

This study was done to investigate the possible role of exercise and or diet restriction in diabetic rats on mitochondrial function.

In the present study there was significant increase in glucose levels in all groups treated with STZ as compared with non-diabetic groups. Diet restriction or exercise alone results in significant reduction in the glucose level of diabetic rats, moreover combination of both have synergistic effect but do not restore normal control value in non diabetic rats.

These results are in agreement with previous study conducted by Filaire and his colleagues [44] who reported that diabetic rats showed hyperglycemia but training and food restriction significantly reduced blood glucose concentrations, despite; exercise training doesn’t restore normal blood glucose levels [50].

Several mechanisms may act locally to improve glucose uptake and disposal after exercise. Those include increased muscle blood flow, increased insulin binding to its receptor (IR), increased IR turnover and increases glucose transport by stimulating GLUT4 translocation to the muscle cell surface [51].

In the present study, STZ induced diabetes results in a significant decrease in insulin level as compared to non diabetic rats. The chronic exercise alone did not affect insulin level in both diabetic and normal rats, while food restriction alone [52] or in combination with exercise significantly decreases insulin level.

It is possible that food restriction exerts its effects on insulin secretion by alteration in different steps in the mechanism of insulin gene expression, biosynthesis or secretion due to the elevated levels of serum FFA accompanying food restriction [53]. The possible mechanism by which FFA exert this effect is by increasing levels of carnitine palmityl transferase-1 (CPT-1) activity in pancreatic islets that leads to decreased Fatty acyl-CoA which is an important coupling factor in the secretion of insulin by stimulating protein kinase C isoforms, activation of ATP-sensitive K+ channels and acetylating proteins to target them to appropriate membrane sites, so pancreatic islets respond less and glucose-induced insulin secretion is abolished [54].

The mRNA expression level of PGC-1α is lower in the skeletal muscles of diabetic patients [55]. Reductions in PGC-1α have also been ob-
served when fasting insulin concentrations are increased and with an increased body mass index in diabetes-prone humans [56]. Thus, there appears to be a potential role for PGC-1 (ξ) in the etiology of insulin resistance in human skeletal muscle. This linkage is strengthened by the fact that PGC-1 (ξ) is also downregulated in selected animal models of insulin resistance and type 2 diabetes [57,58].

The previous studies are in agreement with the present study which showed that Real-time RT-PCR analysis of gene expression of PGC-1 (ξ) in the gastrocnemius muscle of diabetic rats was significantly decreased compared to normal rats. Exercise did not affect PGC-1 (ξ) mRNA of in normal rats. Its level was significantly elevated in diabetic rats by exercise, not by diet restriction compared to diabetic rats fed at libtm but this elevation did not reach to its value in normal rats. Many studies showed that PGC-1 (ξ) mRNA and protein expressions are very responsive to endurance exercise [59-64]. Also our results showed that exercise with diet restriction significantly increase PGC-1 (ξ) mRNA than diet alone [65].

The possible mechanisms that might regulate this response is that exercise caused activation of AMP-activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase (CaMK) II and p38 mitogen-activated protein kinase (MAPK) which are upstream modulators of PGC-1 (ξ) expression in skeletal muscle [59,66,67]. And transcription factors that are coactivated by PGC-1 (ξ), such as myocyte enhancer factor 2 (MEF2) [68], leads to a second phase of adaptation characterized by an increased expression of PGC-1 (ξ) and higher protein content, which could serve to sustain the increase in mitochondrial content.

In the present study exercise in normal rats caused insignificant increase in ERR (ξ) mRNA when compared to normal sedentary rats. STZ induced diabetes caused significant decrease in ERR-2(ξ) mRNA in gastrocnemius muscle when compared to normal trained rats. Its level was elevated by exercise, not by diet restriction when compared to diabetic rats and this elevation reached to its level in normal trained rats.

The results of the present study are consistent with that of Cartoni et al., [64] who reported that exercise leads to increases in the gene expression of PGC-1 (ξ) and its coactivators of mitochondrial biogenesis, ERR (ξ) and NRF-2 as well as that of the mitochondrial fusion proteins Mfn1 and Mfn2.

ERR (ξ) serves as a critical nodal point in the regulatory circuitry downstream of PGC-1 (ξ) to direct the transcription of genes involved in mitochondrial energy-producing pathways in cardiac and skeletal muscle. ERR (ξ) null mice show defects in lipid metabolism and decreased expression of genes coding for fatty acid oxidation enzymes and oxidative phosphorylation components [38]. Herzog et al., [69] suggest that enhancing ERR (ξ) activity could have beneficial effects on glucose metabolism in diabetic subjects by two distinct mechanisms: Increasing mitochondrial oxidative capacity in peripheral tissues and liver, and suppressing hepatic glucose production.

Bach et al., [39,40] reported that Mfn2 expression is down regulated in skeletal muscle in animal or human obesity and in type 2 diabetic patients which is in accordance with the results in the present study which showed that STZ induced diabetes caused significant decrease in Mfn2 mRNA in gastrocnemius muscle of rat.

Our results showed that exercise in normal rats caused insignificant increase in Mfn2 mRNA when compared to normal sedentary rats. But exercise in diabetic rats increased and restore Mfn2 mRNA level to its level in normal rats. In contrast to our results Alvarez et al., [70] found that exercise caused no increase in Mfn2 in young type 2 diabetic subjects. While Little [71] et al., reported that exercise increases Mitofusin2 (71%) protein content in patients with type 2 diabetes.

The GLUT4 protein is recruited from intracellular sites by insulin and exercise by different signaling pathways. It translocates to the cell membrane and transverse tubules, where it mediates the transport of glucose into the muscle cells [72,73]. The increase in GLUT4 expression is mediated by the transcription factors myocyte enhancer factor 2 (MEF2A and D) and GLUT4 enhancer factor (GEF) [74]. PGC-1 (ξ) coactivates MEF2A and also increases MEF2A protein expression by activating NRF-1 [75], and possibly, other transcription factors that regulate MEF2A expression. Furthermore As a consequence of this adaptive increase in GLUT4, muscle glycogen storage following glycogen depleting exercise occurs more rapidly and to a greater extent in the trained than in the untrained state [76,77].

Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM [78,79]. In the present study we found that GLUT4 mRNA in the skeletal muscle of STZ-induced diabetic rats was decreased [78], but exercise training reversed diabetes-induced decrease of GLUT4 mRNA. Other studies on the skeletal muscles of rats [80,81] found
that exercise training increased GLUT4 protein expression in insulin deficiency and insulin resistance.

In the present study exercise had no effect on glycogen of the muscle in normal rats, while diabetic rats had decreased glycogen content of gastrocnemius muscle which increased by exercise but did not reach its value in normal rats. Exercise training-induced increases in muscle glycogen content could be regulated by multiple mechanisms, including enhanced insulin sensitivity, glycogen synthase expression, allosteric activation of glycogen synthase, and protein phosphatase 1 (PP1) activity [82].

PGC-1 alpha was shown to increase muscle glycogen stores via several mechanisms including stimulation of glucose import, suppression of glycolytic flux, and by down-regulation of the expression of glycogen phosphorylase and its activating kinase, phosphorylase kinase alpha. These findings identify PGC-1 alpha as a critical regulator of skeletal muscle fuel stores. Conversely, PGC-1 alpha-deficient animals exhibited reduced rates of muscle glycogen repletion post-exercise [28].

In the present study exercise in normal rats had no effect on CPT-1mRNA, while Hildebrandt et al. [83] found that CPT-1mRNA is significantly increased in gastrocnemius muscle of normal rats. Our results showed significant decrease in CPT-1 mRNA in STZ-induced diabetic rats and exercise but not diet restriction caused significant increase in CPT-1 mRNA to the normal level. In contrast to our results Huang et al., [84] found that exercise or diet restriction for 8 weeks having no effect on CPT-1 mRNA in skeletal muscle of in KKAY obese/diabetic mice. While exercise increased CPT-1 mRNA and protein in skeletal-muscle obese mice [85] and human [86]. It was also reported that exercise increased CPT-1mRNA in skeletal muscle of healthy subjects under the effect of striated muscle activator of Rho signalling (STARS) which is a transcriptional target of PGC-1α and ERRα [37]. Meredith et al., [87] demonstrated that PGC-1α stimulates CPT-1 gene expression in rat neonatal myocytes through interaction with myocyte enhancer factor 2 (MEF2).

Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of diabetes mellitus [88]. Reactive oxygen species (ROS) in turn, can profoundly modify, and even damage, mitochondrial function [89]. PGC-1α expression is induced by ROS, and PGC-1α has been shown to limit the accumulation of ROS and be protective against oxidative damage [90].

In our study, MDA (marker of lipid peroxidation) content of skeletal muscle was significantly increased in diabetic group. Exercise decreased the elevated MDA. Our result is consistent with the other studies results [91-93] who indicated an increase in lipid peroxidation in diabetes mellitus and decreased by exercise.

It was reported that caloric restriction resulted in significantly reduced malondialdehyde in hepatocytes of streptozotocin-induced diabetic rats [94]. Also our study showed that diet restriction decreased significantly the elevated MDA content of skeletal muscle.

Our results showed significant decreased in citrate synthase (CS) mRNA in diabetic rats which did not changed by diet restriction but increased insignificantly by exercise and diet restriction. Lehti et al., [95] found that Citrate synthase activity was lower in the skeletal muscles of diabetic mice compared with healthy mice (p<0.05). Trained healthy and trained diabetic mice both had higher citrate synthase activity than their respective untrained controls [96]. It was reported that overexpression of PGC-1 in skeletal muscle of rats increased the activity of citrate synthase [97].

We concluded that diet restriction and/or endurance training in diabetic rats induces mitochondrial bioenergetics in skeletal muscle, and enhances mitochondrial function and improve glucose uptake by skeletal muscle possibly due to increase PGC-1α and ERRα. Therefore represent a key strategy in the prevention and treatment of type 1 diabetes and in those at increased risk for this disorder.

References


70- MARÍA ISABEL HERNÁNDEZ-ALVAREZ, HOOD THABIT, NICOLE BURNS, SYED SHAH, IMAD BREMA, MENSUD HATUNIC, FRANCIS FINUCANE, MARC LIESA, CHIARA CHIELLINI, DEBORAH NA-ON, ANTONIO ZORZANO and JOHN J. NOLAN: Subjects With Early-Onset Type 2 Diabetes Show Defective Activation of the Skeletal Muscle PGC-1α/Mitofusin2 Regulatory Pathway in Response to Physical Activity, Diabetes Care, 33: 645-651, 2010.


The Role of Multislice CT in Imaging of Different Tracheal Lesions


The Department of Radiology, Thoracic Imaging Unit, Faculty of Medicine, Cairo University

Abstract

Background: The advent of multi-detector CT has revolutionized imaging of the airways and other thoracic structures. In comparison to single-detector helical CT scanners, multidetector scanners not only provide faster speed, greater coverage, and improved spatial resolution, but also have the unique ability to create images of thick and thin collimation from the same data set. One of the greatest benefits of this new technology is the improved quality of two-dimensional (2D) multi-planar and three-dimensional (3D) reconstruction images. These images break away from the confines of the traditional axial imaging plane and have the potential to facilitate the assessment of a variety of airway disorders. With regard to the assessment of airway stenoses, multi-planar volume reformation methods aid in the detection of mild stenoses, improve the accuracy of determining the length of stenoses, and aid in the identification of horizontal webs. Review of multi-planar volume-reformatted images has been shown to aid in the planning of stent placement or surgery.

Multiple tracheal lesions can be assessed as tracheal stricture, Relapsing polychondritis, Amyloidosis, Tracheomalacia. Tracheopathia osteochondroplastica as well as saber sheath trachea deformity.

Aim of the Work: The aim of our review study is to distinguish the features of different tracheal lesions using multi-detector CT.

Patients and Methods: This study included 20 patients; 15 males and 5 females, age range 8-83 (average of 46.67 years). Cases were referred from the ENT, Chest and Oncology Departments to Radiology Department in Kasr Al-Aini Hospital for MSCT Chest. The study was performed from March 2012 till March 2013. They were all subjected to Thorough clinical examination with history taking, general and chest examination, MSCT was done to all patients.

Results: In this study, we provide evidence that multislice computed tomography (MSCT) is able to diagnose different tracheal lesions either in symptomatizing patients or incidental in cases presenting with known or chronic illness or other clinical suspicion.

Key Words: Multislice computed tomography – Tracheal lesions.

Introduction

THE advent of multi-detector CT has revolutionized imaging of the airways and other thoracic structures. In comparison to single-detector helical CT scanners, multidetector scanners not only provide faster speed, greater coverage, and improved spatial resolution, but also have the unique ability to create images of thick and thin collimation from the same data set [1,2].

One of the greatest benefits of this new technology is the improved quality of two-dimensional (2D) multi-planar and three-dimensional (3D) reconstruction images. These images break away from the confines of the traditional axial imaging plane and have the potential to facilitate the assessment of a variety of airway disorders.

With regard to the assessment of airway stenoses, multi-planar volume reformation methods aid in the detection of mild stenoses, improve the accuracy of determining the length of stenoses, and aid in the identification of horizontal webs. Review of multi-planar volume-reformatted images has been shown to aid in the planning of stent placement or surgery.

Airway imaging is routinely performed at end-inspiration during a single breath-hold. State-of-the-art helical scanners allow the entire central airways to be imaged in less than 5sec. The speed of the examination is particularly important when imaging patients with airway disorders because many of these patients cannot tolerate the significantly longer breath-hold time required by single-detector CT scanners. Short scanning time is also an advantage for imaging during dynamic breathing.
The Role of Multislice CT in Imaging of Different Tracheal Lesions

or at end expiration in patients with suspected tracheomalacia a condition characterized by excessive collapse of the airway during expiration.

Tracheal stricture caused by damage from cuffed endotracheal tube, tracheostomy or trauma to the neck. Cuff pressure in these devices may exceed the capillary pressure leading to ischemic necrosis and subsequent fibrosis. Assessment of such localized tracheal abnormality can be achieved with contiguous 1.5-5.0mm collimation scans obtained through the area during a single breath hold [3,4].

Relapsing polychondritis is a systemic disease in which the tracheal cartilage is affected by recurrent episodes of inflammation. On CT images, fixed narrowing of the tracheal lumen with associated thickening of the wall is noted [3,4].

Amyloidosis is a condition in which a fibrillar protein is deposited in the trachea. Tracheal involvement takes the form of diffuse or multifocal submucosal infiltrates. On CT scan, narrowing of the lumen, wall thickening and calcification is noted [3,4].

Tracheomalacia is a clinical disorder associated with softening of the cartilage and loss of structural integrity of the trachea. Both primary and secondary etiologies are recognized. In pediatric patients, prematurity or prolonged mechanical ventilation is often implicated. In adults, many cases are posttraumatic or post-inflammatory with or without complicating infections [3,4].

Tracheopathyia osteochondroplastica is a rare idiopathic and usually asymptomatic disorder of older men; this disorder is characterized by multiple osteo-cartilaginous masses adjacent to the tracheal rings of the inner anterolateral wall of the trachea. Radiologically, focal tracheal thickening, calcification of the tracheal rings, multiple calcified tracheal nodules, and long-segment tracheal narrowing are typically seen.

Saber-sheath trachea deformity is a pathognomonic finding in patients with chronic obstructive pulmonary disease. The saber-sheath appearance is found when mechanical forces of hyperinflated lungs cause the coronal diameter of the intrathoracic trachea to narrow and the sagittal diameter to elongate so that the sagittal-to-coronal diameter ratio exceeds 2:1. The extra-thoracic trachea remains normal in configuration. CT may also reveal mild intra-thoracic tracheal wall thickening, frequently with ossification of the tracheal rings.

Aim of the work:
The aim of our review study is to distinguish the features of different tracheal lesions using multi-detector CT.

Patients and Methods
This study involved 20 patients; 15 males and 5 females, age range 8-83 (average of 46.67 years). Cases were referred from the ENT, Chest and Oncology Departments to Radiology department in Kasr Al-Aini Hospital for MSCT Chest. The study was performed from March 2012 till March 2013. They were all subjected to Thorough clinical examination with history taking, general and chest examination, MSCT was done to all patients.

Patients were subjected to:
- Thorough clinical examination with history taking, general and chest examination.
- Routine laboratory tests mostly complete blood picture, the other tests were considered according to case e.g. Sputum culture, etc.
- Conventional endoscopy to trachea and bronchi were attempted in 8 cases.
- Pulmonary function tests (PFT) done to 6 patients with obstructive manifestations.
- Transbronchial lung biopsy done in 5 patients.
- MSCT: GE Light Speed Plus MSCT 4 channels set present in the Radiology Department Kasr Al-Aini was used for all cases. 12 cases underwent routine CT chest [with (8 cases) or without IV contrast (4 cases)], virtual bronchoscopy was requested in 7 cases (see Table (1) for technique used) and HRCT was done in one case with bronchiectasis (see Table (2) for technique used).

Results
This study included 20 patients; 15 males and 5 females, age range 8-83 (average of 46.67 years). Table (3) summarizes the different MSCT findings in our 20 cases.

The other findings referred to in the table represent a case showing normal trachea and focal bronchomalacia in a case suspected clinically to have tracheomalacia. Table (4) summarizes the MSCT findings in the cases showing tracheal narrowing; Table (5) presents cases with tracheal masses while Table (6) presents cases with calcification.
Youssriah Y. Sabri, et al.

Table (1): Virtual bronchography technique done in cardiothoracic Imaging unit, Radiology department, Cairo University (Kasr Al Aini).

**IV contrast:** None needed.

**CT scanning:** CT scan examinations were performed using GE Light Speed Multislice 4 channels present in the radiology department at Kasr Al- Aini hospital. The examination is done in supine position. A scout is taken with Kv 120 and mA 120, then helical scanning is done in caudo-cranial direction to minimize respiration artifacts, using detector row 4, helical thickness 1.25, pitch 1.5:1, speed (mm/rot) 7.5. Detector configuration 4x1.25, beam collimation 5.00mm, interval 1.00, gantry tilt 0.0, FOV depends on the patient's body build, but is about 35cm. Kv 120-140, mA 120-160, total exposure time 16-20sec during breath hold in inspiration. The images acquired are then sent to a separate workstation to be processed, manipulated and reconstructed by resident in the cardiothoracic imaging unit in the radiology department.

**Reconstruction techniques:** Reconstruction of the images are done using different reconstruction softwares available at the workstation. Several reconstruction techniques are done each aiming for a certain diagnostic achievement as follows:

1. **3D Internal surface rendering:** Also termed virtual bronchoscopy (VB), where images simulate those of true bronchoscopy. The cursor of the virtual camera was guided manually by the resident to cover all regions from trachea till segmental bronchi taking pertinent images and naming each.

2. **3D Volume rendering:** Images show the outer features of the bronchial tree and lungs where any external deformity could be detected.

3. **2D Minimum intensity processing (minIP):** Images only detect the lowest hounsefield attenuation values available thus only detecting air column within the bronchial tree, this technique enhances the detection of internal deformities or caliber changes and shows distal air beyond an obstructed area.

4. **2D Multplanar images reconstruction (MPR):** Images are reconstructed in axial and coronal planes showing the air-way, the surrounding lesion(s), their extent, effects and relations. It also could clarify other possible lesions as pleural effusions, mediastinal extension and lymph adenopathy, pericardial changes...etc.

5. High resolution CT images of the lungs are done as a supplementary study to evaluate the lungs for possible lymphangitis carcinomatous, this is not a part of the virtual bronchographic study but we found it helpful in patient's staging and management without significant extra effort.

Table (2): HRCT technique used in Kasr Al-Aini.

<table>
<thead>
<tr>
<th>Scout</th>
<th>Kv120</th>
<th>mA20</th>
<th>Holding breath</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRCT protocol:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scan type</td>
<td>Helical full 0.5sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detector Row</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helical Thickness</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pitch</td>
<td>1.5:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed (mm/rot)</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detector configuration</td>
<td>4x1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beam collimation</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval mm</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric tilt</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOV</td>
<td>Depends on patients’ size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kv</td>
<td>120-140</td>
<td>120-160</td>
<td></td>
</tr>
<tr>
<td>mA</td>
<td>120-160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total exposure time</td>
<td>16-20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holding breath in full inspiration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstruction type</td>
<td>STD (standard)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal window images are also taken</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Different MSCT findings of the trachea.

<table>
<thead>
<tr>
<th>MSCT finding</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrowing</td>
<td>10</td>
</tr>
<tr>
<td>Focal filling defect</td>
<td>1</td>
</tr>
<tr>
<td>Widening</td>
<td>1</td>
</tr>
<tr>
<td>Tracheal diverticulum</td>
<td>1</td>
</tr>
<tr>
<td>Calcification</td>
<td>5</td>
</tr>
<tr>
<td>Tracheomalacia</td>
<td>3</td>
</tr>
<tr>
<td>Tracheal bronchus (case 1,2)</td>
<td>2</td>
</tr>
<tr>
<td>Trauma</td>
<td>1</td>
</tr>
<tr>
<td>Tracheoesophageal fistula</td>
<td>1</td>
</tr>
<tr>
<td>Other findings</td>
<td>1</td>
</tr>
</tbody>
</table>

Table (4): MSCT findings in the cases showing tracheal narrowing.

<table>
<thead>
<tr>
<th>Cause of narrowing</th>
<th>No. of cases</th>
<th>Focal/diffuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular</td>
<td>1</td>
<td>Thoracic trachea</td>
</tr>
<tr>
<td>Saber-sheath trachea</td>
<td>1</td>
<td>Thoracic trachea</td>
</tr>
<tr>
<td>Extrinsic compression</td>
<td>4</td>
<td>Thoracic trachea</td>
</tr>
<tr>
<td>Extrinsic compression</td>
<td>1</td>
<td>Focal</td>
</tr>
<tr>
<td>Chemical inhalation</td>
<td>1</td>
<td>Focal</td>
</tr>
<tr>
<td>Tracheobronchopathia</td>
<td>1</td>
<td>Focal</td>
</tr>
<tr>
<td>Osteochondroplastica</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (5): MSCT findings in the cases with tracheal masses.

<table>
<thead>
<tr>
<th>Site of lesion</th>
<th>No. of cases</th>
<th>Pathological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrinsic with tracheal infiltration</td>
<td>1</td>
<td>Bronchogenic carcinoma</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>1</td>
<td>Sarcoid</td>
</tr>
</tbody>
</table>

Table (6): Presents cases with tracheal calcification.

<table>
<thead>
<tr>
<th>Cause of calcification</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>3</td>
</tr>
<tr>
<td>Tracheobronchopathia osteochondroplastica</td>
<td>1</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1</td>
</tr>
</tbody>
</table>
Case (1): Axial CT chest (lung window) in a 23 year-old male patient showing right tracheal bronchus.

Case (2): Virtual bronchoscopy and reconstructed coronal image (lung window) in a 53 year-old female patient showing right tracheal bronchus.

Case (3): Axial CT chest images (mediastinal and lung windows) in a 83 year-old female patient showing right tracheal diverticulum.

Case (4): Male 48 years old with known esophageal carcinoma and suspected trachea-esophageal fistula Axial CT chest images (mediastinal window) showing tracheoesophageal fistula at the thoracic inlet.
Case (5): Male child 10 years old with history of penetrated neck trauma by sharp object that penetrated the anterior aspect of the trachea with severe surgical emphysema. Axial CT chest images (lung window) showing marked surgical emphysema with residual anterior mid-line tract along the path of the original penetrating object.

Case (6): Female patient 34 years old with history of sarcoidosis coming for follow-up CT. Axial CT chest images (mediastinal window) showing intrinsic sessile small left mass lesion protruding into the tracheal lumen.

Case (7): Male patient 65 years old with known left central Bronchogenic carcinoma coming for virtual bronchoscopy. Virtual bronchoscopy (scout, VB, reconstructed coronal images in MinIP), and coronal mediastinal window images) in a case of left central Bronchogenic carcinoma with upper lobar collapse, showing extrinsic compression of the trachea with displacement to the right but no evidence of tracheal infiltration.
Case (8): Male patient 45 years old with known right central Bronchogenic carcinoma coming for virtual bronchoscopy. Virtual bronchoscopy (scout, VB, axial mediastinal window, MinIP coronal images) and axial mediastinal window images in case of right central Bronchogenic carcinoma, showing marked extrinsic compression of the trachea with displacement to the left and evident tracheal infiltration.

Case (9): Tracheomalacia: Incidental finding during MSCT of the chest in a 76 year-old male patient. Axial CT chest images (lung window) in Inspiratory and expiratory phases showing marked collapse of trachea in expiration and abnormal configuration during inspiration.
Case (10): Male patient 10 years old with suspected tracheomalacia referred for follow virtual bronchoscopy. Axial CT chest images, VB and MinIP images in inspiration and expiration showing no evidence of tracheomalacia but MinIP expiratory images show tight proximal left bronchus with dilated distal bronchus a sign denoting focal bronchomalacia.

Case (11): Female patient 8 years old with clinical signs of pulmonary hypertension referred for CT chest. CT chest images (mediastinal window) showing prominent main pulmonary artery and anomalous origin of left pulmonary artery from right pulmonary artery causing concentric compression of the trachea with marked narrowing and deformity suggesting tracheomalacia.
Case (12): Saber-sheath trachea: Incidental finding during MSCT of the chest. Axial CT chest (lung window) in a 45 year-old male patient, smoker showing increased sagittal to coronal diameter ratio (2:1), finding diagnostic of saber-sheath trachea.

Case (13): Tracheomegaly: Incidental finding during HRCT of the chest done for bronchiectasis in a 53 year-old male patient. Axial HRCT chest images (lung windows) in a 53 year-old male patient showing tracheomegaly (trachea more than 3cm). HRCT shows the classic wall corrugation and right tracheal diverticulum. Basal cuts shows bronchiectasis. Mediastinal image also shows antero-lateral calcification due to aging.

Case (14): Tracheal calcification: Incidental finding during MSCT chest. Axial CT chest images (mediastinal window) showing marked tracheal calcification in 50 year-old male patient. The affection of the posterior membranous wall suggests amyloidosis.

Case (15): Female patient 57 years old with stridor of six month duration, chest X-ray showed tracheal calcification while conventional endoscopy showed tracheal narrowing. The diagnosis of tracheopathia osteochondroplastica was considered and virtual bronchoscopy was recommended. Virtual bronchoscopy, MinIP image and coronal and axial mediastinal window images in a 57 year-old female patient showing tracheal narrowing and nodular calcification in a case of tracheopathia osteochondroplastica.
Discussion

Multi-detector CT has become the imaging modality of choice for a wide range of tracheal lesions as it allows direct visualization of the cross-sectional tracheal airway and allows determination of the size, extent, and attenuation value of a lesion. In addition, recent advances in computer technology allowed multiplanar and 3D reconstruction of the acquired axial images, thus facilitating the detailed evaluation of a large variety of tracheal lesions. Also faster imaging times markedly reduced the radiation dose to the patients and more importantly the motion artifacts which could degrade the quality of the examination.[6]

Three-dimensional reconstruction of axial CT images permits navigation through the tracheobronchial tree via real-time simulated bronchoscopy known as virtual bronchoscopy (VB). Such technique overcame many of the drawbacks of conventional bronchoscopy as it allowed the visualization of the relationship between a tracheal lesion and the surrounding mediastinal structures as well as airway patency beyond a stenosis, through which a bronchoscope can never pass.[6]

The majority, 13 out of 20 (65%), of the tracheal lesions encountered throughout our study were not suspected clinically and were incidentally discovered during scanning for another clinical suspicion. This was consistent with the study conducted by Marom E et al.,[7] who stated that a patient with tracheal affection usually does not present with a typical clinical presentation.

In the two patients in which a tracheal bronchus was detected, it was an incidental finding on CT and in both cases it was arising from the right tracheal wall and supplying the apical segment of the right upper lobe. This is consistent with the study done by Masayuki et al.,[8], who reported that tracheal bronchi arose from the right lateral wall of the trachea supplying one or more right upper lobe segments and is detected in about 0.3 1% of the overall patient population.

In the single case of tracheal diverticulum (Case 3) we detected on CT, it was an incidental finding in an 83 year-old female patient. It was arising near the thoracic inlet, along the posterolateral right tracheal wall appearing as an air filled sac communicating with the tracheal lumen in some of the axial cuts and as an isolated paratracheal air cyst in the lower ones. This site and CT appearance typically matches a study done by Goo et al.,[9] who reported that most cases of tracheal diverticulum is located at the right posterior-lateral side of the trachea and appears to have one or multiple connections with the trachea. Two types have been reported; congenital and acquired. Congenital tracheal diverticulum is smaller, located approximately 4.5 cm below vocal cords or just above the carina. It's more common in males than females.

In our case of tracheomegaly (Case 13), the tracheal diameter was more than 3 cm with tracheal wall corrugations and basal CT cuts showed bronchiectatic changes. These are consistent with a study done by Hubbard M. et al.,[10] which states that dilatation of the trachea and tracheal wall corrugations are characteristic features of tracheomegaly, and that bronchiectasis is a common association as seen in our case.

In our three patients who were diagnosed as tracheomalacia (Case 9, 10, 11), collapse of the trachea with bowing of the posterior membranous portion anteriorly during expiration was noted in two cases and acquired tracheomalacia secondary to prolonged vascular compression was seen in one case. In a retrospective study done by Heussel and Hafner (May 2001-July 2008),[11] a patient group (n=27) of children with bronchoscopic evidence of tracheomalacia, and a control group (n=320) underwent inspiratory and expiratory CT. The patient group showed significantly greater cross-sectional area change of the trachea (57.2% ± 22.2% vs. 10.6%±11.2%, p<0.001) than the control group.

In our case of saber sheath trachea, it was an incidental finding in a 45 year-old male patient, smoker, in whom MSCT showed increased sagittal to coronal diameter ratio (2:1). This is set to be the diagnostic feature of saber sheath trachea according to the study done by Ochs R. et al.,[12], on 176 patients, out of which 124 (70%) displayed an increase in the sagittal to coronal diameter ratio of the trachea.

In the case of tracheoesophageal fistula (case 4), it was in a patient suffering from an esophageal neoplasm which was markedly compressing the trachea causing narrowing of its lumen, the diagnosis of an acquired tracheoesophageal fistula was clinically suspected. On CT, the fistulous communication between the trachea and esophagus was demonstrated. This was consistent with a study done by Yalcin et al.,[13] which involved five females and two males who were subjected to tracheal intubation. The presenting symptoms were respiratory difficulty (n=3), coughing (n=2), and dysphagia with coughing (n=2). The site of the fistulae were proximal (n=3) and middle (n=1) trachea, left main bronchus (n=1), and nearly the entire posterior wall of the trachea (n=2).
The CT findings of the trachea in the case of sarcoidosis (Case 6) coming for follow-up were limited to a mural sessile mass lesion protruding into the tracheal lumen with preserved diameter and shape of the trachea. In a study by done by Nunes H. et al., [14] on 67 patients with sarcoidosis, out of whom 48 patients (72%) showed diffuse tracheal narrowing associated with calcified nodular masses while 19 patients (28%) showed mural sessile mass lesion as in our case.

In the patient who was clinically suspected tracheopathia osteochondroplastica (Case 15) virtual bronchography, reconstructed volume rendering and MinIP images as well as the mediastinal window images constituted an integrated study that showed tracheal wall thickening with luminal narrowing and nodular calcification of the wall. These are consistent with the study done by Leske et al., [15] that showed the value of virtual bronchography in evaluating wall of trachea on CT, 3D volume rendering and virtual bronchoscopy and delineated the distinct nodular mucosal lesions as well as tracheal stenosis and its extent.

In the case diagnosed as having tracheal amyloidosis showed, CT showed tracheal calcification with evident affection of the posterior membranous wall. These findings are consistent with a study conducted by Marchiori E. et al., [16] that stated that amyloidosis involving the trachea presented irregular nodular narrowing of the tracheal lumen with mural calcification favoring the posterior tracheal wall.

In our three patients with bronchogenic carcinoma (Case 7,8), the axial CT images compression and displacement of the trachea by the mediastinal lesion in all three cases, with infiltration of the tracheal lumen in one of them. This was clearly demonstrated by the aid of volume rendering, coronal MinIP as well as virtual bronchoscopic images. These findings are consistent with the study conducted by Herth F.J. et al., [17] who stated that virtual bronchoscopy has a great role in evaluation of tracheal involvement in mediastinal lesions by allowing non-invasive intra-luminal assessment of the tracheo-bronchial tree and easy detection of endo-luminal invasion of the trachea in such lesions.

Conclusion:

Multidetector CT has greatly overcomed the several limitations associated with routine axial CT images suffice for evaluating many airway abnormalities such as limited ability to detect subtle airway stenoses; underestimation of the cranio-caudal extent of disease; difficulty displaying complex 3D relationships of the airways; and inadequate representation of the airways that are oriented obliquely to the axial plane.

References:

6- PHILLIP M. BOISSELLE and DAVID A. LYNCH: CT of the Airways, 121-151, 2008.
Combined Tailored Lateral Internal Sphincterotomy with V-Y Advancement Flap Versus Lateral Internal Sphincterotomy Alone in Treatment of Chronic Anal Fissure

TAREK HEGAZI, M.D.* and SALAH S. SOLIMAN, M.D., M.R.C.S.**
The Department of General Surgery, Faculty of Medicine, Cairo* and Fayoum** Universities

Abstract

Background: Anal fissure is one of the most common ano-rectal diseases and 10% of patients ultimately receive surgery. Lateral internal sphincterotomy is highly effective and is the surgical treatment of choice for curing chronic anal fissure with hypertonicity after failure of conservative measures. LIS has a high success rate, but can have complications as bleeding and incontinence. There can be delayed or non healing of the sphincterotomy surgical site, and persistence of symptoms or recurrence of fissure.

The Aim of the Study: Was to evaluate the results of V-Y advancement flap combined with tailored open lateral internal sphincterotomy in comparison with open lateral internal sphincterotomy alone in treatment of chronic anal fissure.

Patients and Methods: Between April 2011 and December 2012, twenty patients fulfilling the criteria of having chronic anal fissure with persistence of symptoms inspite of conservative treatment for a period of 4-6 weeks were randomly assigned into two treatment groups.

Group A was assigned to do open lateral internal sphincterotomy (LIS) alone, while in Group B, Tailored lateral Internal sphincterotomy (TLIS) combined with V-Y advancement flap was done.

Response to the treatment was assessed in terms of postoperative pain, time and rate of fissure healing and occurrence of complications. Follow-up of the patients was done every week for three months to detect short term postoperative outcomes and every 3 months for one year to detect recurrence.

Results: In Group B, no severe pain was present and less time interval and doses for analgesia was observed. By the end of second week 90% in Group B showed complete healing and acceptance of V-Y-flap whereas only 20% showed complete healing in Group A. By the end of fourth week the rest of patients in Group A showed complete healing except one patient with failure of healing due to infection. Postoperative Bleeding, and infection was found in Group A and flap dehiscence was present in one patient in Group B. Soiling was noticed in all patients of Group A, during first and second week while in Group B, soiling was present in three patients only on the first day. No incontinence was observed in Group B and only one patient in Group A showed incontinence with no recurrence in both groups.

Conclusion: Tailored lateral internal sphincterotomy with V-Y advancement flap appears to produce the greatest and rapid healing rate, with few complications and no incontinence and recurrence rate.

Key Words: Anal fissure – V-Y advancement flap – Lateral internal sphincterotomy.

Introduction

An anal fissure is a nonhealing linear tear or ulceration in the epithelial lining of the distal anal canal, distal to the dentate line (mucocutaneous Junction) [1]. Although this is an extremely common condition, if not the most common anorectal problem encountered in practice, it is surprisingly difficult to know the exact incidence, as many people avoid seeking treatment and many fissures will resolve without intervention [2].

It commonly occurs between 2nd and 4th decades of life with an equal distribution between men and women [3]. It is usually located in the posteriomidline but occurs anteriorly in fifth of patients [4]. Anal Fissures can be primary (typical) or secondary (atypical). It can be divided into two clinical subtypes, depending upon the duration of disease, the acute and chronic fissures [5]. The aetiology of typical fissure is not clear. A common initiator is trauma from large or hard stool, but many traumatic fissures heal and others do not [6].

Diagnosis is suspected on history alone, it may cause bright red bleeding with bowel movements, and anal pain persisting for one to two hours after bowel movement [7]. The most consistent finding on physical examination is spasm of the anal canal
due to hypertrophy and hypertonicity of the internal anal sphincter, which can be so severe causing ischaemia, pain and non healing of the fissure. It has been postulated that fissures associated with internal anal sphincter hypertonicity are ischaemic in nature [8].

Studies using ambulatory manometry in patients with anal fissure have shown persisting high resting anal pressure with poor spontaneous relaxation. Another study examining the influence of ischaemia, showed that the higher the sphincter pressure, the lower the anodermal blood flow. This was most pronounced posteriorly, and was followed by a return of normal blood flow after sphincterotomy [4].

All management options aim to reduce anal tone by relaxing the internal anal sphincter and subsequently providing symptomatic relief and healing of the fissure. The first approach in treatment include general measures, such as dietary fiber supplements, adequate fluid intake, topical analgesics, medical treatments, such as Glyceryl trinitrate (GTN) ointment, calcium channel blockers and botulinum toxin [9]. An anal fissure is likely to be non healing, if the fissure persists beyond 4-6 weeks of medical management and surgery is reserved for those patients [10].

Previous procedures such as manual anal dilatation, and posterior midline sphincterotomy are rarely used because of high recurrence and incontinence rates and longer time for wound healing [11]. Lateral internal sphincterotomy is the first line surgical option for all fissures, associated with hypertonicity of the internal anal sphincter. It can be performed using open or closed approach depending upon surgeon’s preference. It also has associated problems of faecal incontinence which can reach up to 10% and controversy still exists with regard to minor variations in operative technique as Tailored LIS to reduce this complication, also recurrence after LIS is 5% [13].

An anal advancement flap is effective in healing an anal fissure as primary line of treatment, also is a good choice for those who have recurrent anal fissures post LIS and is followed by minor complications. Various flaps have been described, such as rotational or V-Y flap [14]. This study was designed to evaluate the results of V-Y advancement flap combined with open Tailored lateral internal sphincterotomy in comparison with open lateral internal sphincterotomy alone in treatment of chronic anal fissure.

**Patients and Methods**

The study was conducted in Kasr El-Ainy Hospital Faculty of Medicine, Cairo University, from April 2011 to December 2012. The study included twenty patients aged from 20-46 years and all patients fulfill the criteria of having chronic anal fissure with persistence of symptoms inspite of conservative treatment for a period of 4-6 weeks and with increased resting anal pressure.

Patients excluded were those who had previous surgical procedure in the perianal region with cictricial deformation, those with acute anal fissure, large sentinel pile, inflammatory bowel disease, hemorrhoids, anal fistula, old age patients, anal abscesses, and those with coagulopathy. The patients were randomly allocated into two groups, ten patients each:

- **Group (A):** Will have open lateral internal sphincterotomy alone.
- **Group (B):** Will have combined open tailored lateral internal sphincterotomy with V-Y advancement flap.

All patients were subjected to Personal and Medical history, clinical examination (general and local) including P.R examination and Anal Manometric studies. Routine laboratory Investigations were done, and all patients were fit for surgery.

Patients were fully informed about the risks and benefits of the two procedures. Informed consent was obtained from every patient.

The day prior to surgery, all patients were kept on fluid diet. Laxative was given and an enema was done by night, if possible. The perianal region was shaved if needed. All patients received a standard regimen of intravenous antibiotics (1.5gm of ampicillin sulbactam and 500mg of Metronidazole) one hour preoperative and continued at eight hour intervals for up to 24 hours after the end of surgery.

**Operative procedure:**

All patients received spinal anesthesia, and with the patient in lithotomy position, first rectal examination was done to ensure the absence of any associated pathology.

In Group A, traditional lateral internal sphincterotomy was performed by a standard open technique, a 5-mm incision was made into the perianal skin along the intersphincteric groove. The internal
Anal sphincter was then dissected and a segment is then looped on a right angle and brought into the incision and divided under vision with diathermy to the level of dentate line. The two ends are allowed to fall back. A gap can then be palpated in internal sphincter through anal mucosa. The incision can be closed or left open to heal. Excision of the fibrotic edge of the fissure, curettage of its base and excision of sentinel pile and/or anal papilla, if present. Bleeding was secured by diathermy and a pad of gauze was firmly packed into the anus.

In Group B, Tailored lateral internal sphincterotomy was performed, with the same steps like Group A, but the extent of sphincterotomy was done to be more or less equal to the length of the fissure. Injection of 1/200000 Adrenaline+Lidocaine 10% (45ml saline+5ml lidocaine+1ml adrenaline 0.25mg) in the site of flap to control bleeding. Then the V-Y advancement flap was performed by making a V-shaped incision from the edges of the fissure extending 4cm from the anal verge and away from the midline. The V-shaped flap formed of skin and subcutaneous fat was mobilized sufficiently to allow advancement into the anal canal to cover the fissure defect.

Care was taken to preserve enough pedicle to ensure adequate blood supply in addition to the underlying vascular pedicle contained in the subcutaneous fat. Thus, it is necessary to preserve fatty subcutaneous tissue with wide mobilization to maintain flap viability.

The base of the flap was sutured to the lower anal mucosa at dentate line with interrupted 3/0 vicryl. The skin is then closed behind the V at the external portion of the perineum by silk 3/0 to push the V-flap into anal canal and widen the stenotic area.

Fig. (1): Anal fissure.

Fig. (2): Construction and wide mobilization of v-y dermal flap.

Postoperative care:

For both groups were the same. The oral feeding was resumed on the first day starting with fluids then soft diet. Urinary catheter was kept for 24 hours for fear of retention.

Analgesia was given according to severity of pain either by oral NSAID for mild pain, or injection of NSAID in moderate pain and opioid (pethidine 100mg IM) in severe pain.

The patients were given a laxative and a lubricant to avoid the bad experience of a painful first motion. The patients were discharged and followed-up clinically every week for three months, to detect shortterm postoperative outcome and every 3 months for one year to detect Recurrence rate. The comparison between the two groups includes the rate and timing of complete healing, soiling or any degree of fecal incontinence and the recurrence. Flap complications (as ecchymosis, hematoma, infection, disruption of flap, and flap necrosis), operative time, length of hospital stay, time of relieve of pain, and persistence of symptoms were also recorded. Methods of statistical study used were student $t$-test, chi-squared test and Mann-Whitney U test and a probability less than 0.05 ($p$-value <0.05) is considered statistically significant.

Results

The patients enrolled in Group A were 6 females (60%) and 4 males (40%). Their age ranged from 22-46 years with a mean age 32 years.

The patients in Group B were 6 females (60%) and 4 males (40%) their age ranged from 20-46 years with a mean age 32 years.
All patients in both groups had primary posterior chronic anal fissure; with failure of conservative treatment for 4-6 weeks, and persistence of symptoms.

Thirteen patients (65%) complained from pain, five patients (25%) complained from pain and bleeding, two patients (10%) complained from pain, bleeding, itching and perianal skin irritation.

The operative time in Group A ranged from 10-22 minutes with a mean operative time 15.6 minutes.

In Group B, the operative time ranged from 25-43 minutes with a mean operative time 33.1 minutes. The operative time was significantly longer in Group B and statistically significant difference was observed in Group A \((p\text{-value}=0.005)\).

As regard postoperative pain, it was ranged as mild, moderate and severe. In Group A, one patient showed mild pain, three patients showed moderate pain, and six patients showed severe pain. In Group B, six patients showed mild degree of pain, three patients showed moderate pain and one patient showed severe degree of pain.

Statistically significant difference in postoperative pain between both groups was observed, with less severe pain and less time interval and doses for analgesia in Group B \((p=0.005)\).

As regard hospital stay, no difference was found between both groups, as all patients were discharged after one day, except one patient in Group A who had bleeding after pack removal, that necessitated another day for observation.

As regard the rate of healing and the timing of complete healing (epithelization and coverage of the fissure in Group A and good acceptance with coaptation of the edges of flap in Group B) was observed. Any fissure showing no signs of healing or failure of acceptance of V-Y advancement flap after 4-6 weeks of the procedure is considered to show delayed healing.

In the first week no complete healing was found in Group A, but granulation tissue in abundant amount was found in five patients, the other five patients showed no signs of healing.

In the first week, good flap acceptance in all patients of Group B was noticed except one patient who had a defect due to dehiscence of one limb of V-Y flap.

On the 2nd week, complete epithelization of the fissure was observed in two patients in Group A, and incomplete healing in the rest of patients, while in Group B, nine patients showed complete healing and the one patient with wound dehiscence, showed secondary healing. On he 3rd-4th week post operative, the rest of patients in Group A showed complete healing, and only one patient showed persistent raw area with failure of healing, due to infection and bad hygiene, that was followed-up for one month with dressing and antibiotics until complete healing occurred.

Statistically significant difference was observed in Group B as regard the rate of healing and timing of complete healing with a \(p\)-value (0.0055). As regard postoperative complications, mild postoperative bleeding was observed in one patient in Group A after removal of the pack, that required repacking for another 24 hours.

In Group B, no frank bleeding was detected, only ecchymosis of the perianal area.

During the period of healing, two patients showed spontaneous blood tinged soaked underwear and five patients showed frank blood after defection in Group A.

In Group B, the patient with flap dehiscence, showed blood tinged under wear after hard stool.

Statistically significant difference as regard postoperative bleeding with no bleeding in Group B \((p=0.005)\).

No Flap hematoma, or flap necrosis was observed in patients of Group B.

Only one patient showed disruption of one limb of V-Y advancement flap due to infection. Time of relieve of pain was related to complete healing of the fissure and coverage of the raw area.
No patient in both groups showed persistence of symptoms of anal fissure after complete healing, but the disappearance of symptoms was rapid in Group B.

As regard fecal incontinence, it varied from soiling of under wears by serous or feculent discharge to frank fecal incontinence.

Soiling was noticed in all patients of Group A on the first and second week and one patient complaining of incontinence to flatus and stool.

In Group B only three patients had soiling on the first day with no soiling afterwards in all patients.

Statistically significant difference as regard soiling and incontinence was observed in favour of Group B (p=0.005). Follow-up and observation of the patient in Group A with fecal incontinence showed gradual improvement with no permanent incontinence. Follow-up for one year revealed no recurrence of fissure in both groups.

**Discussion**

Anal fissure commonly affects young adults between second and fourth decades with equal distribution between men and women and a life time incidence of 11.1% [3].

In our study, the sex distribution ratio was almost equal in both groups with a mean age of 32 years.

The combination of anal pain and bleeding is sufficiently worrisome that patients often seek medical attention. In our study, the majority of patients complained of pain (65%), 25% from pain and bleeding and 10% from pain, bleeding, and itching. Acute fissures may heal spontaneously, although simple conservative measures are sufficient.

Chronic anal fissures need careful evaluation to decide what therapy is suitable [4].

The initial approach in treatment of anal fissure is non operative, and include Medical therapy with warm baths, stool softeners, laxatives, analgesics, topical anesthetics, and Glyceryl trinitrate oint and calcium channel blockers [9].

Most chronic anal fissures are associated with a raised anal pressure and reduced vascular perfusion at the base of the fissure due to hypertonicity of internal anal sphincter. Current treatment has aimed at reducing resting anal pressure by diminishing sphincter tone and improving blood supply at the site of fissure, thus promoting healing [4].

LIS is the surgical treatment of choice for chronic anal fissure after failure of conservative measures. Sometimes, longstanding chronic Anal fissure do not heal even with an adequate sphincterotomy and an anal advancement flap must be performed to cover the defect in the mucosa [12].

This study was conducted to compare the outcomes of open lateral sphincterotomy alone versus V-Y advancement flap combined with Tailored Open Lateral internal sphincterotomy in treatment of chronic Anal fissure.

This study revealed that there was a statistically significant difference regarding operative time with (p=0.005) (mean time was 15.6 minutes in Group (A) and 33.1 minutes in Group (B). This was in accordance to the study by Jahan et al., who showed that the mean operative time was 9 minutes in open lateral sphincterotomy alone [15].
Other study by Farid et al., 2010, showed that the mean operative time was (44 minutes) longer in Group B where combined lateral internal sphincterotomy and V-Y advancement flap was done [16].

This study showed that there was less severe postoperative pain and less time interval and doses for analgesia in Group B. The difference was statistically significant ($p=0.005$). This was in compliance with other studies as Tayyab M. et al., 2010 who found on a study conducted on 45 patients that severe pain was a major complaint in 31 patients (68.8%) with open lateral internal sphincterotomy alone [17].

Wang et al., 2011 on the other hand found less severe pain on those with V-Y advancement flap combined with Lateral internal sphincterotomy [18].

The severe pain in Group A was attributed to the raw area.

As regard the rate of healing of fissure and the timing of complete healing, this study showed rapid rate of healing and earlier complete healing of the raw area in Group B. The difference was statistically significant ($p=0.0055$).

This was in compliance with other studies, as Filigeri et al., [19]. Who showed no healing occurring in the first week for all patients undergoing only open lateral internal sphincterotomy and by the end of 2nd week only 50% of patients showed healing and by the end of 4th week all patients showed complete healing. Also Jaleel F. et al., [20] showed complete healing of the fissure till 6 weeks postoperatively in 97% of patients and two patients remained unhealed after 6 weeks. Giordano et al., [21] and Chamber et al., [14] in their study on patients with combined lateral internal sphincterotomy and V-Y advancement flap showed complete healing in 98% of patients by the end of first week.

As regard postoperative bleeding, our study showed statistically significant difference as regard bleeding ($p=0.0005$) with more bleeding occurring in Group A during the first two weeks as the raw area and granulation tissue are easily bleeding during defecation and in Group B, only one case in the first week, showed bleeding in which flap dehiscence on one side was present.

Tayyab et al., [17] and Jaleel et al., [20] noticed immediate post operative bleeding for patients with internal lateral sphincterotomy and Wang et al., [18] showed no bleeding in patients undergoing combined lateral internal sphincterotomy and V-Y advancement flap.

As regard soiling and fecal incontinence our study showed statistically significant difference ($p=0.005$) as all patients in Group A had soiling in first and second week post operatively and one patient with faecal incontinence and in Group B, only three patients had soiling on the first day postoperative and no soiling afterwards.

Giordano et al., [21] showed no soiling or incontinence in patients with V-Y advancement flap and lateral internal sphincterotomy.

Jahan et al., [15] and Tayyab et al., [17] showed minor faecal incontinence and soiling in 3% and 9% of the patients in their study.

The results of this study show that the technique of combined tailored lateral internal sphincterotomy and V-Y advancement flap can be applied to chronic anal fissures with success as a primary therapy as it shows excellent and rapid rates of healing of fissure with rapid relieve of pain and minor complications.

References


Salivary Gland Neoplasms
A Histopathological and Statistical Study

The Department of Pathology, Faculty of Medicine, Cairo University, Egypt

Abstract
Salivary gland neoplasms represent only about 3 to 4% of all head and neck tumors. This study shows a registry of cases showing salivary gland neoplasms received by the department of Pathology, Kasr El Aini Hospital, during the period from January 2006 till December 2010. This work also comprises the determination of the frequency and distribution of these tumors as a group in a sample of Egyptian patients. Statistical evaluation of clinical and patient data and that of the pathological findings available in the request sheet is done to analyze these data.

Material and Methods: This work includes 140 cases of salivary gland neoplasms obtained through collection of all archived cases of salivary gland tumors during the period from January 1, 2006 till December 31, 2010 from the Pathology Department, Faculty of Medicine, Cairo University, Kasr El Aini Hospital. The available archived H&E. Glass slides were obtained and revised. The paraffin blocks were cut 5u thick and stained by hematoxylin and eosin for histopathological examination.

Results: 140 cases were collected. The patient’s age ranged from 6 to 84 years with male predominance 76 (54%), 99 cases (70.7%) were of parotid origin, 30 cases (21.4%) were of minor salivary gland origin and 11 cases were submandibular in origin (7.9%). The predominant histopathologic type was pleomorphic adenoma 61 cases (43.6%).

Conclusion: Many variations were seen between the results of salivary gland neoplasms in Egyptian patients and those obtained by other studies in different parts of the world. However, many clinical characteristics of the diseases were in agreement with most studies all over the world.

Key Words: Salivary gland neoplasms – Histopathological – Statistical.

Introduction
SALIVARY gland tumors are specific neoplasms in the oral and maxillo-facial area. However, morphological heterogeneity and low frequency make it considerably difficult to histologically classify these tumors [1,2].

The frequency of salivary gland neoplasms varies from 0.4 to 13.5 annual cases per 100,000 inhabitants in various populations.

Of all diagnosed neoplasms, salivary gland tumors constitute 3% of those in the head and neck area [3].

Salivary gland tumors are more frequent in adults’ than in children [4].

Among all salivary gland tumors, the most frequently reported benign tumor is pleomorphic adenoma [1,3].

Considering the malignant salivary gland neoplasms, reports of the most commonly seen tumors varies but, in general, mucoepidermoid carcinoma [5-7] and adenoid cystic carcinoma [8-10] were reported. While tumors of the salivary glands can appear at any age, the maximum incidence is in the fourth decade of life for benign lesions and in the fifth decade for malignant tumors [7,11].

Classically, these lesions have been reported to be more frequent in women, although the proportion varies according to the histological type of tumor [12].

Material and Methods
This work included 140 cases of salivary gland neoplasms obtained through collection of all archived cases of salivary gland tumors during the period from January 1, 2006 till December 31, 2010 from the Pathology Department, Faculty of Medicine, Cairo University, Kasr El Aini Hospital.

Data obtained from pathology sheet are:
- Age and sex of patient.
- Symptoms and duration.
- Site of salivary gland tumors.
Diagnosis of salivary gland tumors and histopathologic subtypes.
- Gross pathological features e.g. size, tumor ulceration and lymph node status.

**All collected cases were:**
- The available archived H&E. glass slides.
- The paraffin blocks which were cut of 5μ and stained by hematoxylin and eosin stains for histopathological examination.

**Histopathological evaluation:**
Revision of all available slides were done, reclassified according to the most recent staging systems and photographed with a digital camera attached an Olympus microscopic model BX5 1.

**Statistical methods:**
Data were analyzed using SPSS (Statistical program for social science version 17). Numerical data were summarized as means and standard deviations (SD) or medians and ranges as appropriate. While qualitative data were described as frequencies and percentages.

**Results**
140 cases of salivary gland tumors received by Pathology Department at Kasr El Aini Hospital during the last five years (2006-2010) were included in this study.

The median age at presentation was 47 years with a range of (6-84) years with male predominance of 76 patients in a ratio 1.2:1 respectively (M: F ratio).

Sex distribution of salivary gland tumors showed male predominance (54%).

The mean age of the benign cases was 44.6 years±13 compared to 44.7 years for malignant cases±8.2.

Of the 140 patients collected, 96 patients had a benign pathology with a male predominance of 64.6%. On the other hand, 68.2% of malignant cases were females.

The peak incidence of both benign and malignant tumors was in the fifth decades followed by six decades.

Benign tumors outnumbered malignant tumors in all decades except in females in 1st, 2nd, 7th and 8th decades.

**Presentation:**
Most of the patients presented with more than one symptom. A mass was the main presenting symptom (95%) followed by palpable lymph nodes (33.6%). Pain was common feature (17.1%). Table (1.3).

Facial nerve paralysis was found in (27.3%) of malignant cases whereas it was not noted in patients of benign tumors, skin infiltration was noted in (22.7%) of malignant cases. Pain was a common feature in (34.1%) of malignant tumors, whereas only (9.4%) of patients of benign tumors had pain. (Table 1.4).

**Tumor site:**
Concerning the tumor site, the majority were in parotid gland (70.7%), followed by minor salivary glands (21.4%).

Benign tumors were more commonly encountered in the parotid while malignant tumors were most common in minor salivary glands.

Minor salivary gland tumors affected females more than males (19/30 cases), also in females the malignant tumors in minor salivary glands were predominant (73.7%).

The most common site of minor salivary gland tumors was palate (16 cases).

Twenty cases showed recurrence of the tumor, further analysis revealed (16.2%) were benign (12 cases were pleomorphic adenoma and 4 cases were warthin’s tumor) while (9.1%) in malignant cases (3 cases adenoid cystic carcinoma and one case acinic cell tumor).

**Pathological data:**
Perineural invasion was seen in 7/44 cases of malignant tumors (15.9%). Four cases of mucoepidermoid carcinoma, two cases of adenoid cystic carcinoma and one case of acinic cell carcinoma.

Dissected lymph nodes were positive for metastasis in 8/35 cases only (22.9%).

Pleomorphic adenoma was the commonest benign salivary gland tumors (43.6%) followed by warthin’s tumor (21.4%) while the most common malignant salivary gland tumor was mucoepidermoid carcinoma (11.4%) followed by adenoid cystic carcinoma (8.6%). Table (1.5).

The most common benign tumor in parotid was pleomorphic adenoma (45.6%) while Mucoepidermoid carcinoma was the commonest malignant tumor (10%) while adenoid cystic carcinoma was the commonest malignant tumor in minor salivary gland (36.7%). Table (1.5).
### Table (1.1): Age, Sex and tumor nature of cases of salivary gland tumors.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Benign</th>
<th>Malignant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>0-10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>2 (3.2%)</td>
<td>1 (2.9%)</td>
<td>3 (3.1%)</td>
</tr>
<tr>
<td>21-30</td>
<td>8 (12.9%)</td>
<td>7 (20.6%)</td>
<td>15 (15.6%)</td>
</tr>
<tr>
<td>31-40</td>
<td>10 (16%)</td>
<td>4 (11.8%)</td>
<td>14 (14.6%)</td>
</tr>
<tr>
<td>41-50</td>
<td>19 (30.6%)</td>
<td>16 (47.1%)</td>
<td>35 (36.5%)</td>
</tr>
<tr>
<td>51-60</td>
<td>16 (25.8%)</td>
<td>5 (14.7%)</td>
<td>21 (21.9%)</td>
</tr>
<tr>
<td>61-70</td>
<td>3 (4.8%)</td>
<td>0</td>
<td>3 (3.1%)</td>
</tr>
<tr>
<td>More than 80</td>
<td>0</td>
<td>1 (2.9%)</td>
<td>1 (1.1%)</td>
</tr>
</tbody>
</table>

Total 62 34 96 14 30 44

### Table (1.2): Frequency of presenting symptoms of salivary gland tumors.

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>133</td>
<td>95</td>
</tr>
<tr>
<td>Pain</td>
<td>24</td>
<td>17.1</td>
</tr>
<tr>
<td>Facial nerve paralysis</td>
<td>12</td>
<td>8.6</td>
</tr>
<tr>
<td>Firm induration</td>
<td>12</td>
<td>8.6</td>
</tr>
<tr>
<td>Skin infiltration and ulceration</td>
<td>10</td>
<td>7.1</td>
</tr>
<tr>
<td>Palpable lymph nodes</td>
<td>47</td>
<td>33.6</td>
</tr>
</tbody>
</table>

### Table (1.3): Correlation of the main presenting symptoms with the tumor nature.

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Benign (n=96)</th>
<th>Malignant (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>92 (95.8%)</td>
<td>41 (93.2%)</td>
</tr>
<tr>
<td>Pain</td>
<td>9 (9.4%)</td>
<td>15 (34.1%)</td>
</tr>
<tr>
<td>Facial nerve paralysis</td>
<td>0</td>
<td>12 (27.3%)</td>
</tr>
<tr>
<td>Firm induration</td>
<td>3 (3%)</td>
<td>9 (20.5%)</td>
</tr>
<tr>
<td>Skin infiltration and ulceration</td>
<td>0</td>
<td>10 (22.7%)</td>
</tr>
<tr>
<td>Palpable lymph nodes</td>
<td>12 (12.5%)</td>
<td>35 (79.5%)</td>
</tr>
</tbody>
</table>

*p*-value=0.000

### Table (1.5): Frequency of different tumor types according to site of 140 salivary gland tumors.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Parotid gland</th>
<th>Submandibular gland</th>
<th>Minor salivary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benign tumors:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleomorphic adenoma</td>
<td>45 (45.6%)</td>
<td>6 (54.5%)</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Warthin’s tumor</td>
<td>28 (28.4%)</td>
<td>2 (18.2%)</td>
<td>0</td>
</tr>
<tr>
<td>Myoepithelioma</td>
<td>0</td>
<td>0</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haemangioma</td>
<td>0</td>
<td>1 (9.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Basal cell adenoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Malignant tumors:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>10 (10%)</td>
<td>1 (9.1%)</td>
<td>5 (16.7%)</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>Carcinoma ex pleomorphic adenoma</td>
<td>2 (2%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>3 (3%)</td>
<td>0</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Adenocarcinoma (NOS)</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Epithelial-myoepithelial carcinoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>3 (3%)</td>
<td>1 (9.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Metastatic renal cell carcinoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total 99 (100%) 11 (100%) 30 (100%)
Fig. (1): Pleomorphic adenoma in submandibular gland (H & E x100).

Fig. (2): Warthin’s tumor in parotid gland showing Luminal layer of palisaded oncocytic cells (H & E x200).

Fig. (3): Adenoid cystic carcinoma, showing nests of cells with cylindromatous microcystic spaces, some filled with hyaline material (H & E x400).

Fig. (4): Mucoepidermoid carcinoma showing mixture of epidermoid and intermediate cells, with tiny cystic space (H & E x400).

Fig. (5): Squamous cell carcinoma in parotid gland, the cells showing nuclear pleomorphism and central keratinization in a desmoplastic stroma (H & E x400).

Fig. (6): Acinic cell tumor showing acinar cells with basophilic granular cytoplasm and eccentric round nucleus (H & E x200).
Discussion

Salivary gland neoplasms represent only about 3 to 4% of all head and neck tumors. The salivary glands stand out as the tissue with probably the most diverse pathology in the human body. The WHO salivary gland tumour classification lists at least 38 subtypes of epithelial tumours alone, as well as several stromal types [13].

Regardless of this diversity, salivary gland tumours are rare; and most of them occur in the major glands (75-91%), [11]. The literature reports that the site of the tumours is distributed mainly between the parotid gland (about 80%) and submandibular gland (20%), while rarely are tumours found in the sublingual gland. Minor salivary gland tumours of the lips, oral cavity, pharynx, larynx, trachea, nasal mucosa, and paranasal sinuses are reported as uncommon (9-23%), [14].

The present study (received in the Pathology Department, Kasr El Aini Hospital) included 140 salivary gland tumors during the period between 2006-2010.

In our study, it was observed that benign tumors were the most frequent (68.6%), similar to most published series (6,15). Parotid major salivary glands were most commonly affected both in benign and malignant neoplasias, which is reported in the majority of the scientific series [3,15,16].

According to WHO, the average ages of patients with benign and malignant tumours are 46 and 47 years, respectively and the peak incidence is in the sixth and seventh decades [13].

In this study, the mean age was 44.7; the average age of patients with benign and malignant tumours was 44.6 and 44.7 years, respectively. The difference was statistically insignificant and the peak incidence was in fifth and sixth decades. The mean age is in accordance with Al-Khateeb et al., [9] (40 years), Li et al., [1] (41.38 years), Subhashraj, [10] (46 years), Vargas et al., [5] (48 years), and Kamulegeya et al., [17] (34 years).

In our study, there was a slight overall male predominance, with a male to female ratio of 1.2:1; males. This finding coincides with some reports [7,10], but differs from the majority of studies that showed a higher frequency among females [1,5,9,17].

In our study, there was predominance of male in the fifth decades of life, which is different from studies by [7,18,19,20] which were in fourth decades.

As regard the relation of sex to the nature of the tumor, in our study, benign tumors had a male predominance of 64.6%. On the other hand, 68.2% of malignant cases were females, which is highly significant.

Although benign tumors were found to be more common in females [3,15,21], a few studies found an association between the male sex and benign tumours and the female sex and malignant tumours [18,22].

Concerning the aetiology of salivary gland tumours, the prevalence of pleomorphic adenoma and acinic cell carcinoma in women with a distinct peak in the 3rd decade of life, signalises a hormone influence, some studies have been performed in this subject [21], but without conclusive results. Among others, ethnicity and geographic locations have been proposed as having an effect on the frequency of occurrence of salivary gland tumours [24,25]. However, the data presently expressed, show similarities among those encountered all over the world [26-28], especially in relation to the sex,
mean age, and location. These findings may imply that common aetiologic agents might be acting regardless of ethnical and geographic location.

Concerning the presenting symptoms, in our study, firm induration was found in 20.5% of patients with malignant lesions while 3% in benign cases, facial nerve palsy founded in 12% of the patients with malignant lesions and not detected in benign tumours. Skin ulceration was seen in 22.7% of malignant lesions. Thus, in agreement with [29], pain and firm induration were significant indicators of malignancy.

Of all salivary gland tumors included in this study, 70.7% were located in the parotid gland, 7.6% in the submandibular gland, and 21.4% in the minor salivary glands. This result is similar to findings of most of the published reports [1,9,10].

In the study by Ito et al., [21], the parotid gland was the most frequent site, representing 70% of the cases, followed by the minor salivary glands and the submandibular gland with 22% and 8% of tumours, respectively. All large series of salivary gland tumours showed similar results [11,30].

However, according to Vargas’s et al., the submandibular gland is the most frequent site.

In our study, 80.2% of benign tumors were in the parotid gland. In almost all published studies, the parotid gland was the predominant area of the benign tumors [1,3].

52% of malignant tumours were in the parotid gland followed by 43% in minor salivary glands. The most frequent malignant tumour of the parotid gland is mucoepidermoid carcinoma (30-50%), followed by adenoid cystic carcinoma (25%), carcinoma ex-pleomorphic adenoma (15%), and acinic cell carcinoma (5%) [21,31].

In our study, as regard the frequency of benign tumors, pleomorphic adenoma was the most commonly encountered type of salivary gland neoplasm (43.6%) followed by Warthin’s tumor (21.4%), which is in agreement with findings from all of the published literature from all over world [1,3]. The incidence of pleomorphic adenoma has been reported to range from between 40.8% and 70% [9].

On the other hand, the most common malignant salivary gland tumor was mucoepidermoid carcinoma 11.4% followed by adenoid cystic carcinoma 8.6%. The reports according to the prevalence of malignant salivary gland tumors were variable. Some authors (Yih et al., [6], Otoh et al., [7] & Vargas et al., [5]) have reported that mucoepidermoid carcinoma is the most frequent malignant salivary tumor, similar to our study, while adenoid cystic carcinoma was the most frequent malignant tumor found in other reports [8-10,31,32].

In parotid gland, the present study confirms the findings of Wang et al., [3] and Li et al., [1] that the pleomorphic adenomas (45.6%) are the most frequently seen tumor in parotid gland and representing 59.2% of benign parotid gland tumors.

The most frequent malignant tumor in parotid gland was mucoepidermoid carcinoma representing 10/23 cases, (43.5%) followed by acinic cell carcinoma 3/23 cases (13%) of malignant parotid tumors.

Although there is no consensus in the literature according to parotid gland malignancies, the majority of reports found that mucoepidermoid carcinoma is the most frequent parotid gland malignancy [2,5,10].

According to Al-Khateeb and Ababneh, [9], the frequency of mucoepidermoid carcinoma and adenoid cystic carcinoma originating from the parotid gland was equal. On the other hand, other series have reported adenoid cystic carcinoma [32], carcinoma ex pleomorphic adenoma [11], squamous cell carcinoma [7], and acinic cell carcinoma as the most common parotid gland malignancy.

In the submandibular gland, we found that the most common benign tumor was pleomorphic adenoma 66.7%, which is consistent with the all of the previously published reports [5,7,32]. In this study, only two malignant cases were seen in this area, including, mucoepidermoid carcinoma, and lymphoma one case each. The adenoid cystic carcinoma is the most frequent malignant tumor of the submandibular gland by Al-Khateeb and Ababneh, (2007) [9], Li LJ et al., (2008) [1] and Subhashraj, (2008) [10]. The small number of cases in this site in our study may be the cause of such discrepancy.

In our study, pleomorphic adenoma was the most frequent tumor of the minor salivary glands (33.3%), in agreement with Wang et al., [3] & Li et al., [1]. Adenoid cystic carcinoma was the most commonly encountered malignant tumor in the minor salivary glands 11/19 case (57.9%) followed by mucoepidermoid carcinoma 5/19 (26.3%).

Although a great number of reports [1,7-10] corroborate our report, mucoepidermoid carcinomas
were the most common malignant minor salivary gland tumors by Yih et al. [6] and Vargas et al., [5].

In the studies from outside of Japan, it has been reported that the most common malignant minor salivary gland tumours are adenoid cystic carcinomas and mucoepidermoid carcinomas. The incidence of adenoid cystic carcinomas and mucoepidermoid carcinomas has been reported to account for 7.7-48% and 6.5-41.3% of all tumours (11.5-51.1% and 16.7-73.6% of malignant tumours), respectively (18, 33). Similar to these findings, in our study adenoid cystic carcinoma represent 36.7% of all malignant salivary gland tumors followed by mucoepidermoid carcinoma 16.7% (57.9% and 26.3% of malignant tumors) respectively. The discrepancies existing in the frequency and distribution of these tumours is possibly influenced by race and geographic location of the population in question.

References


Preemptive Use of Intravenous Acetaminophen, Ketamine or Their Combination in Patients Undergoing Elective Open Abdominal and Urological Surgeries: Effects on Intraoperative and Postoperative Analgesic Requirements

MAHMOUD M. AMER, M.D.; DOAA A. RASHWAN, M.D. and DOAA M. SAYEM, M.Sc.
The Department of Anesthesiology, Faculty of Medicine, Beni Suef University, Egypt

Abstract

Study Objective: This study was designed to evaluate the effect of preemptive use of intravenous acetaminophen, ketamine or their combination on intraoperative and postoperative analgesic requirements in patients undergoing elective open abdominal and urological surgeries under general anesthesia.

Setting: Beni Suef University Hospital, Egypt.

Patients and Interventions: 80 ASA I-II patients undergoing elective open abdominal and urological surgeries under general anesthesia were randomly allocated into four equal sized groups:

Group I (n=20): Control group, received IV normal saline 20cc as placebo over 15 minutes IV before induction of anesthesia.

Group II (n=20): Received 1 gram acetaminophen over 15 minutes IV before induction of anesthesia.

Group III (n=20): Received IV 0.5mg/kg ketamine diluted in 20cc normal saline 15 minutes before induction of anesthesia.

Group IV (n=20): Received IV 1 gram acetaminophen and 0.5mg/kg ketamine diluted in 20cc normal saline 15 minutes before induction of anesthesia.

Measurements and Main Results: Intraoperative. Heart rate and mean arterial blood pressure: Preinduction, after induction of anesthesia every 15 minutes, intraoperative fentanyl requirements (ug).

Postoperative time to first request of analgesia (minutes), postoperative pain at rest measured at 1,8,16, and 24h postoperatively using (VAS), systolic, diastolic arterial blood pressure, heart rate at 1,8,16, and 24h and analgesic requirements of tramadol 50mg im were recorded.

Intraoperative and postoperative analgesic requirements were statistically significantly lower, and the time to first request of analgesia was statistically significantly longer in group IV than groups I,II and III.

Postoperative pain at rest (VAS) was statistically significantly lower in group IV than groups I and II.

Postoperative heart rate, systolic and diastolic arterial blood pressure showed no clinical significant differences between the studied groups.

Conclusion: Preemptive use of intravenous combination of IV acetaminophen 1g and 0.5mg/kg ketamine decreased intraoperative and postoperative analgesic requirements and pain score more than the use of preemptive intravenous acetaminophen or ketamine alone or palcebo in patients undergoing elective open abdominal and urological surgeries under general anesthesia.

Key Words: Preemptive analgesia – Acetaminophen – Ketamine.

Introduction

POSTOPERATIVE pain control has been improved due to more understanding of pain mechanisms and physiology and the development of new analgesic techniques [1].

A recent meta-analysis has documented that coadministration of non-steroidal anti-inflammatory drugs (NSAIDs) and morphine reduces opioid-related side effects [2] but NSAIDs have numerous contraindications [3]. Acetaminophen has a few contraindications and is relatively free from side effects at clinical doses [4].

Previous studies documented that the use of perioperative acetaminophen has a morphine-sparing effect [5-8], intravenous acetaminophen is used widely for postoperative pain control, very limited evidence supports it is use in the preoperative period [9].

In total abdominal hysterectomy, preemptive iv acetaminophen (paracetamol) 1 g provided adequate postoperative pain control, and decreased consumption of morphine [10].
Ketamine a N-methyl-d-aspartic acid (NMDA) receptor antagonist used for perioperative analgesia although there are conflicting results about its efficacy as preemptive analgesic some studies have documented a preemptive effect [11-14] and others have not [15-19].

The aim of this study was to evaluate the effect of preemptive use of acetaminophen and ketamine or their combination compared to placebo (normal saline) on intraoperative and postoperative analgesic requirements in patients undergoing elective open abdominal and urological surgeries under general anesthesia.

Patients and Methods

This study was carried out at Beni Suef University Hospital from Jan. 2013 – Sep. 2013 after the approval of institutional review board and ethical committee and obtaining a written informed consent from each patient before operation.

80 patients of both sex undergoing elective open abdominal and urological surgeries under general anesthesia at Beni Suef University Hospital enrolled in this study.

Inclusion criteria:
- American Society of Anesthesiologist (ASA) Physical status I-II.
- Age from 18 to 60 years.

Exclusion criteria:
- Morbid obese patients.
- A known allergy to the one of study drugs.

Preparation of the patients:

The study protocol and the visual analogue scale (VAS) for pain were explained to the patients preoperatively.

Premedication:

The patients did not receive any premedication in either the surgical floor or the operation room.

Monitoring:

Electrocardiogram, pulse oximetry, and non-invasive arterial blood pressure at 5 minutes intervals were applied.

Patients were randomly assigned into four equal size groups using a closed envelop technique as follows:

Group I (n=20): Control group, received IV normal saline 20cc as placebo over 15 minutes iv before induction of anesthesia

Group II (n=20): Received 1 gram acetaminophen (perfalgan) over 15 minutes iv before induction of anesthesia.

Group III (n=20): Received IV 0.5mg/kg ketamine diluted in 20cc normal saline 15 minutes before induction of anesthesia.

Group IV (n=20): Received IV 1 gram acetaminophen (perfalgan) and 0.5mg/kg ketamine diluted in 20cc normal saline 15 minutes before induction of anesthesia.

Anesthetic technique:

General anesthesia was induced in all patient with i.v. 2-3mg/kg propofol, 2ug/kg fentanyl, 0.5mg/kg atracurium, oral cuffed endotracheal tube, anesthesia was maintained with oxygen 100%, isoflurane 1-2%, additional doses of 0.1mg/kg atracurium, mechanical ventilation with maintenance of endtidal carbon dioxide 35-40mmHg.

At the end of surgery, inhalational anesthetic was discontinued, neuromuscular blockade were reversed with neostigmin 0.04mg/kg and 0.02mg/kg atropine IV, the trachea was extubated when the patient respond to commands, all patient were transferred to PACU where they were monitored for 1 hour. Face oxygen masks were applied and their pain was assessed using the VAS If patient reported a VAS at rest of 2 or higher, tramadol 50mg im was given then 50mg im prn.

The following parameters were evaluated and recorded:

- Patients’ characteristics: Age, sex, ASA, height, weight.
- Heart rate and mean arterial blood pressure: Preinduction, after induction of anesthesia every 15 minutes intraoperative.
- Intraoperative fentanyl requirements (ug).
- Postoperative time to first request of analgesia (minutes).
- Postoperative pain at rest measured at 1,8,16, and 24h postoperatively using (VAS) where zero score corresponds to no pain and 10 to the maximum or worst pain.
- Postoperative systolic, diastolic arterial blood pressure, heart rate at 1,8,16, and 24h.
- Postoperative analgesic requirements: Tramadol 50mg im prn.

Statistical analysis:

Data are presented as mean and standard deviation (SD) or numbers as appropriate. Student $t$-
test: Was used for comparison between means of each two groups, ordinal data were analyzed by Mann-whitney U test. \( p \)-values <0.05 was considered statistically significant.

Sample size was calculated based on a previous study \cite{20} the power of the this study is calculated and was found to be more than 95% using G *power 3.1.5 program, and the a-error level was fixed at 0.05.

**Results**

All patients completed the study. There was no statistically significant differences between the studied groups as regards to patient characteristics and operative data (Table 1).

As regards to intraoperative heart rate, no statistically significant differences between group I and group II.

It was a statistically significant higher in group I than group III from 15 to 60 minutes. It was a statistically significant higher in group I than group IV from preinduction time to 75 minutes, 120 minutes and at 165 minutes (Table 2). It was a statistically significant higher in group II than group III from 15 to 60 minutes.

It was a statistically significant higher in group II than group IV from 15 to 90 minutes and at 120 and 165 minutes. It was a statistically significant higher in group III than group IV at 30,75,105,120 minutes and 165 minutes (Table 2).

As regards to intraoperative mean arterial blood pressure, there was no statistically significant differences between group I and group II except at 105 minutes intraoperative.

It was statistically significant higher in group I than group III at 15 minutes and was statistically significant higher in group I than group IV from preinduction to 105 minutes intraoperative.

It was a statistically significant higher in group II than group IV at 15,45,60,75,90,105 minutes. It was a statistically significant higher in group III than group IV at 15,60,75,105 minutes (Table 3).

There was no statistically significant differences between group I and group II as regards to intraoperative fentanyl requirements and time to first request of analgesia but postoperative tramadol requirements was statistically significantly lower in group II compared to group I.

Intraoperative and postoperative analgesic requirements were statistically significantly lower and time to first request of postoperative analgesia was statistically significantly longer in group III compared to group I.

Intraoperative and postoperative analgesic requirements were statistically significantly lower and time to first request of postoperative analgesia was statistically significantly longer in group IV compared to group I (Table 4).

Intraoperative and postoperative analgesic requirements were statistically significantly higher and time to first request of analgesia was statistically significantly shorter in group II compared to group IV.

Intraoperative and postoperative analgesic requirements were statistically significantly higher and time to first request of analgesia was statistically significantly shorter in group III compared to group IV (Table 4).

There was no statistically significant differences in postoperative heart rate between group I and both group II and III, and there was statistically significant differences between group I and group IV at 8 and 16 hours postoperative, Table (6) and there was a statistically significant differences between group II and group IV at 16 hours, and between group III and group IV at 8 and 16 hours.

There was no statistically significant differences in postoperative systolic arterial blood pressure between group I and group II.

But it was statistically significant lower in group III and group IV compared to group I from 8 to 24 hours postoperative, and was statistically significant higher in group II than group IV at 8 and 16 hours, no statistically significant differences between group III and group IV (Table 7).

There was no statistically significant differences in postoperative diastolic arterial blood pressure between group I and group II, but it was statistically significant lower in group III and group VI compared to group I at 16 hours postoperative and was statistically significant lower in group IV compared to group II and III at 16 hours (Table 8).
Table (1): Patient characteristics and operative data in the studied groups. Data presented as Mean ±SD, numbers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.0±13.6</td>
<td>40.2±13.0</td>
<td>41.3±10.8</td>
<td>43.3±11.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.5±4.6</td>
<td>162.0±5.5</td>
<td>160.2±5.8</td>
<td>160.3±5.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.9±11.0</td>
<td>80.8±10.0</td>
<td>75.0±11.9</td>
<td>76.3±11.1</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>7/13</td>
<td>6/14</td>
<td>7/13</td>
<td>8/12</td>
</tr>
<tr>
<td>ASA (I/II)</td>
<td>8/12</td>
<td>6/14</td>
<td>7/13</td>
<td>5/15</td>
</tr>
<tr>
<td>Operation (stone kidney/cholecystectomy/simple nephrectomy)</td>
<td>8/120</td>
<td>7/11/2</td>
<td>9/11/0</td>
<td>8/10/2</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>123.75±25.69</td>
<td>124.00±19.7</td>
<td>120.00±19.46</td>
<td>119.25±20.34</td>
</tr>
</tbody>
</table>

Group I: Control group.
Group II: 1 gram acetaminophen IV.
Group III: Ketamine IV 0.5 mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5 mg/kg ketamine.
No statistical significant differences between the studied groups, p-values >0.05.

Table (2): Intraoperative heart rate (Bpm). Data presented as Mean±SD.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinduction</td>
<td>83.1±8.8</td>
<td>86.7±8.9</td>
<td>88.3±7.5</td>
<td>90.4±8.0*</td>
</tr>
<tr>
<td>15 min</td>
<td>89.3±8.6</td>
<td>89.94±5.3</td>
<td>83.2±5.4*</td>
<td>80.4±6.6*</td>
</tr>
<tr>
<td>30 min</td>
<td>86.04±6.2</td>
<td>87.2±8.9†</td>
<td>81.3±7.0*</td>
<td>76.2±7.2*#§</td>
</tr>
<tr>
<td>45 min</td>
<td>87.7±8.0</td>
<td>85.1±6.4†</td>
<td>81.0±6.3*</td>
<td>77.6±8.6*‡</td>
</tr>
<tr>
<td>60 min</td>
<td>85.08±8.6</td>
<td>83.8±7.6†</td>
<td>78.6±6.6*</td>
<td>78.2±8.0*‡</td>
</tr>
<tr>
<td>75 min</td>
<td>84.1±5.5</td>
<td>83.8±8.2</td>
<td>80.7±5.5</td>
<td>74.0±8.1*§</td>
</tr>
<tr>
<td>90 min</td>
<td>83.1±6.5</td>
<td>82.6±6.9</td>
<td>79.8±6.4</td>
<td>76.4±7.2‡</td>
</tr>
<tr>
<td>105 min</td>
<td>81.3±8.3</td>
<td>81.5±6.8</td>
<td>82.4±6.4</td>
<td>76.7±7.7‡§</td>
</tr>
<tr>
<td>120 min</td>
<td>81.5±8.4</td>
<td>82.8±7.6</td>
<td>82.7±7.9</td>
<td>74.6±5.6*§§</td>
</tr>
<tr>
<td>135 min</td>
<td>82.7±6.9</td>
<td>73.8±21.9</td>
<td>82.0±3.1</td>
<td>75.3±6.6‡</td>
</tr>
<tr>
<td>150 min</td>
<td>82.2±6.8</td>
<td>82.5±7.9</td>
<td>83.0±2.6</td>
<td>77.5±10.6</td>
</tr>
<tr>
<td>165 min</td>
<td>79.5±6.4</td>
<td>76.3±5.5</td>
<td>82.0±0.0</td>
<td>64.0±0.0*‡§</td>
</tr>
</tbody>
</table>

Group I: Control group.
Group II: 1 gram acetaminophen IV.
Group III: Ketamine IV 0.5mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5mg/kg ketamine.
* Statistically significant differences compared to group I.
† Statistically significant differences compared to group III.
‡ Statistically significant differences compared to group II.
§ Statistically significant differences compared to group III.
Bpm = Beat per minute.

Table (3): Intraoperative mean arterial blood pressure (mmHg). Data presented as Mean±SD.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinduction</td>
<td>102.3±12.9</td>
<td>98.4±7.8</td>
<td>96.8±7.6</td>
<td>93.5±6.4*</td>
</tr>
<tr>
<td>15 min</td>
<td>103.5±8.6</td>
<td>102.8±8.6</td>
<td>94.6±5.5*</td>
<td>88.7±6.4*§§</td>
</tr>
<tr>
<td>30 min</td>
<td>93.1±12.0</td>
<td>90.2±12.5†</td>
<td>89.8±9.4</td>
<td>86.1±8.0*</td>
</tr>
<tr>
<td>45 min</td>
<td>95.8±16.0</td>
<td>97.4±11.4</td>
<td>91.4±13.5</td>
<td>85.4±6.3*‡§</td>
</tr>
<tr>
<td>60 min</td>
<td>92.9±10.7</td>
<td>96.5±12.3</td>
<td>94.4±11.7</td>
<td>85.7±7.4*§§</td>
</tr>
<tr>
<td>75 min</td>
<td>97.0±10.7</td>
<td>101.0±8.7†</td>
<td>91.7±7.8</td>
<td>86.2±5.9*§§</td>
</tr>
<tr>
<td>90 min</td>
<td>99.4±11.2</td>
<td>97.1±10.0</td>
<td>92.7±10.7</td>
<td>87.0±6.8*§§</td>
</tr>
<tr>
<td>105 min</td>
<td>93.0±8.5</td>
<td>99.5±9.8*</td>
<td>93.8±10.9</td>
<td>85.1±5.8*§§</td>
</tr>
<tr>
<td>120 min</td>
<td>95.5±8.0</td>
<td>98.8±13.0</td>
<td>91.1±10.0</td>
<td>91.6±7.0</td>
</tr>
<tr>
<td>135 min</td>
<td>93.9±10.8</td>
<td>95.4±10.1</td>
<td>93.4±9.0</td>
<td>90.8±2.9</td>
</tr>
<tr>
<td>150 min</td>
<td>88.2±12.4</td>
<td>96.7±8.3†§</td>
<td>86.7±7.2</td>
<td>95.0±0.0</td>
</tr>
<tr>
<td>165 min</td>
<td>92.5±20.5</td>
<td>99.0±6.0</td>
<td>93.0±5.8</td>
<td>95.0±1.3</td>
</tr>
</tbody>
</table>

Group I: Control group.
Group II: 1 gram acetaminophen IV.
Group III: Ketamine IV 0.5mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5mg/kg ketamine.
* Statistically significant differences compared to group I.
† Statistically significant differences compared to group III.
‡ Statistically significant differences compared to group II.
§ Statistically significant differences compared to group III.

Table (4): Intraoperative and Postoperative analgesic requirements, TFA. Data presented as Mean±SD.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl (ug)</td>
<td>200±16.2</td>
<td>185±23.5†</td>
<td>150±42.9*</td>
<td>122.5±38.0*§§</td>
</tr>
<tr>
<td>TFA (min)</td>
<td>19.5±13.2</td>
<td>19.3±7.7</td>
<td>27.3±1.0†</td>
<td>43.5±7.6*§§</td>
</tr>
<tr>
<td>Tramadol (mg)</td>
<td>160±26.2</td>
<td>125±30.3*</td>
<td>122.5±30.2*</td>
<td>82.5±33.5*§§</td>
</tr>
</tbody>
</table>

Group I: Control group.
Group II: 1 gram acetaminophen.
Group III: Ketamine IV 0.5mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5mg/kg ketamine.
* Statistically significant differences compared to group I.
† Statistically significant differences compared to group III.
‡ Statistically significant differences compared to group II.
§ Statistically significant differences compared to group III.

Table (5): Postoperative visual analogue scale, data presented as Mean±SD.

<table>
<thead>
<tr>
<th>VAS</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>4.8±1.1</td>
<td>4.1±0.8*</td>
<td>3.5±0.9*</td>
<td>3.0±0.9*‡</td>
</tr>
<tr>
<td>8h</td>
<td>3.6±0.5</td>
<td>2.9±0.6*</td>
<td>2.5±0.5*</td>
<td>2.4±0.7*§</td>
</tr>
<tr>
<td>16h</td>
<td>2.7±0.5</td>
<td>2.3±0.7</td>
<td>2.0±0.8*</td>
<td>1.6±0.7*§</td>
</tr>
<tr>
<td>24h</td>
<td>2.6±0.7</td>
<td>2.2±0.7†</td>
<td>1.3±0.4*</td>
<td>1.2±0.4*‡</td>
</tr>
</tbody>
</table>

Group I: Control group.
Group II: 1 gram acetaminophen.
Group III: Ketamine IV 0.5mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5mg/kg ketamine.
* Statistically significant differences compared to group I.
† Statistically significant differences compared to group III.
‡ Statistically significant differences compared to group II.
Table (6): Postoperative heart rate (Bpm). Data presented as Mean±SD.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>81.4±10.2</td>
<td>79.6±8.3</td>
<td>81.5±7.3</td>
<td>76.6±9.2</td>
</tr>
<tr>
<td>8h</td>
<td>82.8±11.3</td>
<td>81.3±8.4</td>
<td>82.4±6.6</td>
<td>76.0±6.8*§</td>
</tr>
<tr>
<td>16h</td>
<td>81.7±9.2</td>
<td>80.5±8.6</td>
<td>80.4±8.1</td>
<td>73.5±6.3*‡§</td>
</tr>
<tr>
<td>24h</td>
<td>76.8±8.3</td>
<td>76.9±6.8</td>
<td>77.2±7.1</td>
<td>75.0±4.3</td>
</tr>
</tbody>
</table>

Group I: Control group.
Group II: 1 gram acetaminophen.
Group III: Ketamine IV 0.5mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5mg/kg ketamine.

*p-values <0.05 is statistically significant.
† Statistically significant differences compared to group I.
‡ Statistically significant differences compared to group II.
§ Statistically significant differences compared to group III.

Bpm = Beat per minute.

Table (8): Postoperative diastolic arterial blood pressure (mmHg) Data presented as Mean±SD.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>79.5±10.1</td>
<td>80.3±8.0</td>
<td>79.5±11.0</td>
<td>80.8±6.9</td>
</tr>
<tr>
<td>8h</td>
<td>83.4±7.0</td>
<td>83.3±7.7</td>
<td>81.5±6.3</td>
<td>80.5±4.6</td>
</tr>
<tr>
<td>16h</td>
<td>84.0±7.3</td>
<td>80.8±6.7</td>
<td>79.3±6.3*</td>
<td>74.5±4.6*‡§</td>
</tr>
<tr>
<td>24h</td>
<td>76.9±6.9</td>
<td>77.0±5.9</td>
<td>76.3±8.3</td>
<td>73.5±5.8</td>
</tr>
</tbody>
</table>

Group I (n=20): Control group.
Group II: 1 Gram acetaminophen.
Group III: Ketamine iv 0.5mg/kg.
Group IV: IV 1 gram acetaminophen and 0.5mg/kg ketamine.

*p-values <0.05 is statistically significant.
† Statistically significant differences compared to group I.
‡ Statistically significant differences compared to group II.
§ Statistically significant differences compared to group III.

Discussion

The result of the present study showed that preemptive use of IV acetaminophen 1g alone did not decrease intraoperative analgesic requirements compared to placebo but it decreased the postoperative analgesic requirements and pain score, while preemptive use of intravenous 0.5 mg/kg ketamine with IV acetaminophen 1g decreased intraoperative and postoperative analgesic requirements and pain score more than the preemptive use ketamine 0.5mg/kg alone or intravenous IV acetaminophen 1g alone in patients undergoing elective open abdominal and urological surgeries under general anesthesia.

Preemptive analgesia is a strategy for administration of analgesics before the surgical stimulus to attenuate postoperative pain [21].

Acetaminophen (Paracetamol) is a non-opioid agent, it acts on the central nervous system by inhibition of central cyclooxygenase, It also suggested to inhibit both isoforms of cyclooxygenase enzymes, it also has an indirect effect on the serotonergic system [10].

Previous studies showed conflicting results about the efficacy of IV acetaminophen as a preemptive analgesic agent, Its effective for relief of somatic pain as in orthopedic surgeries more than the visceral pain of abdominal surgeries which can be explained by the fact that large component of visceral pain consists of afferent fibers that are normally unresponsive to stimuli and become activated only in the presence of inflammation but it has less pronounced anti-inflammatory effects [9].

A study done by Arici et al. [10] showed that IV acetaminophen 1g given 30 minutes before induction of anesthesia provided adequate postoperative analgesia in patients undergoing total abdominal hysterectomy.

It was reported that preemptive IV acetaminophen 15mg/kg given half an hour preoperatively in adult patients undergoing lower limb surgery under spinal anesthesia, reduced postoperative 24 hours meperidine consumption and resulted in a lower pain scores than in patients given preventive IV acetaminophen 15mg/kg and 100mL of IV normal saline before skin closure and longer time to initial analgesic requirement in the preemptive and preventive acetaminophen groups than the control group who received 100mL of intravenous normal saline as a placebo [22].

In contrast, preemptive use of 1g intravenous paracetamol 30 minutes before incision in abdominal surgery does not reduce the analgesic consumption or postoperative pain intensity [23], also in patients underwent lumbar disc surgeries the administration of 1g i.v. paracetamol 15 minutes
before the induction or 15 minutes before the end of operation has no preemptive analgesic effect [24].

Noxious inputs may cause N-methyl-D-aspartate (NMDA) glutamate receptor activation and central sensitization, analgesic intervention before the noxious stimulus may attenuate sensitization and acute pain [25].

In laparoscopic gynecologic surgery, preoperative IV ketamine (0.15mg/kg) has a preemptive analgesic effect [11].

Preemptive ketamine 0.5mg/kg ketamine followed by a ketamine infusion (10 micrograms/kg-1.min-1) decreased postoperative analgesic consumption in patients underwent abdominal surgery [14].

While in patients underwent total mastectomy preoperative ketamine 0.15mg/kg has no preemptive analgesic effect [16].

It was reported that IV ketamine 0.4mg/kg failed to demonstrate a preemptive analgesic effects in patients underwent abdominal hysterectomy [17]. Also preoperatively 1mg/kg ketamine did not reduce postoperative pain compared to 1mg/kg dose of ketamine given at the end of surgery [18] and preoperative ketamine 0.5mg/kg in patients underwent cesarean section had no preemptive analgesic effect [26].

In contrast, a subanesthetic dose of intravenous ketamine bolus of (0.25-0.5mg/kg) followed by an infusion of (0.125-0.25mg/kg per h) reduced mechanical hyperalgesia and improved postoperative analgesia in patients scheduled for rectal adenocarcinoma surgery under combined epidural/general anesthesia [27], also intravenous ketamine 0.15mg/kg, 30 minute before surgery followed by ketamine 2mcg/kg/min infusion decreased postoperative pain and morphine consumption after open renal surgery [28]. It was reported that intravenous ketamine 0.5mg/kg before incision followed by 24-h infusion (2 microg xkg (–1) x min(–1)) reduced postoperative morphine consumption after total hip arthroplasty [29].

A study in patients undergoing laparoscopic cholecystectomy showed that the combination of low-dose ketamine 0.15-mg/kg plus diclofenac sodium 1mg/kg diluted in 100-mL isotonic saline i.v. 20 minutes before the induction of anesthesia had a significantly lower pain score and longer time to postoperative analgesic request compared with patients receiving placebo and ketamine 0.15mg/kg alone [20].

**Preemptive Use of Intravenous Acetaminophen**

**Conclusion:**

Preemptive use of intravenous combination of iv acetaminophen 1g and 0.5mg/kg ketamine decreased intraoperative and postoperative analgesic requirements and pain score more than the use of preemptive intravenous acetaminophen or ketamine alone or placebo in patients undergoing elective open abdominal and urological surgeries under general anesthesia.

**References**


Impact of Obesity on Ovulatory Functions in Polycystic Ovarian Syndrome

AHMED AL-SAWAF, M.D. and EMAN A. HUSSEIN, M.D.
The Department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University

Abstract

Background: Indeed, the impact of obesity on female infertility is a topic of increasing importance in modern gynecological practice and extensive research is needed to reach well-defined guidelines. In this current work, the effect of obesity on ovulatory functions in PCO was evaluated in 80 PCO patients attending the infertility outpatient clinic of Kasr Al-Aini Hospital.

Patients and Methods: 80 PCO patients were selected according to restricted inclusion and exclusion criteria from Kasr Al-Aini infertility clinic in the period from July 2011 to March 2012, then divided into three groups according to BMI: Group (A) 38 patients with BMI <30 kg/m², Group (B) 24 patients with BMI 30-35 kg/m², Group (C) 18 patients with BMI 35-40 kg/m². Ovulatory function in the three groups was evaluated by folliculometry and day 21 progesterone. Basal hormonal profile on day 2 of the cycle (FSH, LH, E2, PRL and TSH) was also evaluated and statistically analyzed.

Results: The results showed a significant difference in the level of basal LH and prolactin (p-value <0.05) between the three groups; there was an inverse correlation between obesity and basal LH. PRL level was also significantly higher in the non-obese groups. There was no significant difference in the level of basal FSH, E2 nor TSH. As regards to ovulatory functions; non-obese group showed better ovulatory performance especially when monitored by day 21 progesterone (18.4% compared to 5.5% in the morbidly obese group).

Conclusion: Obesity in PCO seems to increase the risk for anovulation most probably including and excluding the disruption of HPO axis by hyperandrogenism, and/or insulin resistance. Leptin may play a role through its direct effect on folliculogenesis. Weight reduction in PCO may improve ovulatory performance. Monitoring ovulation by folliculometry alone may not reflect accurate ovarian response and addition of day 21 progesterone should be considered to improve evaluation. Further studies and RCTs are needed to justify this conclusion.

Key Words: Obesity – PCO – Hormonal profile – Follicular maturation.

Introduction

OBESITY is thought to result from a combination of environmental and genetic factors [1]. There are several methods for estimating body fat in adults; these include total body weight, waist circumference and hip circumference. Several indices of obesity have been derived from these measurements, including body mass index (BMI). BMI is the most widely used index of body fat in relation to body weight and height [2].

According to the WHO classification of obesity, increased BMI over 35, could be associated with serious health risks [1].

Obesity affects fertility through the presence of functional hyperandrogenism, hyperinsulinemia and insulin resistant state, leading to increased free androgen availability, alterations of granulosa cell functions and follicular development [3].

Many studies have shown that many women with polycystic ovarian syndrome PCOS (between 38% and 88%) are overweight or obese [4]. Obesity is suggested to play a specific pathophysiological role in the development of PCOS [5].

The clinical features of PCOS are heterogeneous and may change throughout the lifespan, starting from adolescence to post-menopausal age [3]. Many definitions were used to describe PCO syndrome, however, the most commonly used in clinical practice is the ROTTERDAM CRITERIA which was used in the current study. In 2003 in Rotterdam, Netherlands, a consensus meeting between the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) “The Rotterdam ESHRE/ASRM Consensus Workshop” defined PCOS; Affected individuals must have two out of the following three criteria: 1- Oligo- and/or ano-
vulation, 2- Hyperandrogenism (clinical and/or biochemical), and 3- Polycystic ovaries on sonographic examination with one ovary being sufficient for diagnosis [6].

Several studies have demonstrated higher rates of in-vitro fertilization IVF cycle cancellation prior to oocyte retrieval in overweight and obese women especially obese PCO [7-9]. A higher cancellation rate of 25.3% was found in the morbidly obese group compared to 10.8% in the normal weight and overweight women [10].

The precise mechanisms for increased gonadotrophins requirements and higher cancellation rate in obese women undergoing IVF remain unclear. However it has been suggested that abnormal LH and FSH levels may result in impaired ovarian response and decreased follicular maturation in this population [9].

Indeed the impact of obesity on female infertility is a topic of increasing importance in modern gynecological practice and extensive research is needed to reach well defined guidelines. In this current work the effect of obesity on ovulatory functions in pco was evaluated in 3 groups of women attending the infertility outpatient clinic of Kasr Al-Aini Hospital: (Group A: BMI <30kg/m², Group B: BMI between 30-34.9kg/m², Group C: BMI between 35-40kg/m²).

Patients and Methods

Place:
Kasr AL-Aini infertility Outpatient Clinic, Cairo University, Egypt.

Design:
This is a prospective comparative study carried out on 80 PCO patients who underwent folliculometry and hormonal profile to assess their ovulation in the period from July 2011 to March 2012 at Kasr Al-Aini Infertility Outpatient Clinic.

Inclusion criteria:
• Age group: 20-35 years old.
• Parity: Variable.
• PCO patient according to Rotterdam criteria.
• Agreement for participation in the study.

Exclusion criteria:
• Age: <20 years and >35 years old.
• Any history of diseases that may affect ovulatory functions as thyroid, hepatic or renal problems.
• Patients who had previous operations on the ovary as cases with ovarian drilling.

Written consents were taken from all cases according to the code of clinical research ethics of Kasr Al-Aini Hospitals.

Methods:
All participants were subjected to the following:
• Full history taking with special emphasis on; age, parity, recent medications with specialty drugs that may affect ovulatory functions and special habits of medical importance e.g. smoking.
• Full clinical examination including weight, height and BMI which was calculated as weight (in kilograms) divided by the height in meters squared [1].

Patients were divided into 3 groups according to BMI:
• Group A: 38 cases with BMI<30kg/m².
• Group B: 24 cases with BMI between 30 and 35kg/m².
• Group C: 18 cases with BMI between: 35 and 40kg/m².

All participants were given induction of ovulation by clomiphene citrate 50mg twice daily for 5 days on day 2 or 3. Metformin 500mg tds was also given for one month.

Ovulation was monitored in the 3 groups by serial sonography (Toshiba Famio 5 6MHz) starting from day 9 or 10 and serum progesterone day 2 1. Ovulation was positive when the follicle size reached 16mm or more (16-21mm) or day 21 progesterone exceeded 12ng/ml.

Basal hormones (FSH, LH, E2, PRL and TSH) were also evaluated in the 3 groups and statistically analysed using computer programs Microsoft Excel version 7 (Microsoft corporation, NY, USA) and SPSS (Statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Statistical analysis of the data:
The results were analyzed by ANOVA.

Results

The demographic data of the cases included in the study is presented in Table (1). It is evident that there is no significant difference between the three groups regarding their age or parity.
The basal hormonal profile on day 2 of the cycle is shown in Table (2). The results showed significant difference in the level of basal LH and prolactin ($p$-value <0.05). No significant difference was observed regarding basal FSH, E2 nor TSH ($p$-value >0.05).

Table (3): U/S evidence of ovulation in the three groups according to follicular size.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>9 (23.6%)</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>5 (20.8%)</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>18 (22.5%)</td>
</tr>
</tbody>
</table>

Upon studying ovulation in the three groups of patients with different BMI (Table 3), it was found that ovulation was better in the non obese group, however, this result did not show a statistical significance ($p$-value >0.05).

Upon studying ovulation by day 21 progesterone (Table 4), it was obvious that ovulation was better in the non obese groups and this result was statistically significant.
Table (4): U/S evidence of ovulation according to day 21 progesterone

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ovulation</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31</td>
<td>7 (18.42%)</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>4 (16.66%)</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>1 (5.55%)</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>12 (15%)</td>
<td>80</td>
</tr>
</tbody>
</table>

Discussion

Most of the studies about obesity and infertility have been controversial and this may be attributed to a vast array of factors such as type of the study, sample size, variation in methodology and statistical analysis [11].

The present study was carried on 80 patients attending the infertility outpatient clinic of Kasr Al-Aini Hospitals. They were divided according to their BMI into three groups: First with BMI <30, second with BMI=30-35 and third group with BMI=35-40. Regarding the patient demographic data, there was no significant difference among the three groups included for their age and parity i.e. patients were matched as regards to age and parity.

Regarding the basal hormonal profile, there was no significant difference in the levels of day 2 FSH, E2 nor TSH between the three groups, but there was a statistically significant increase regarding day 2 LH and PRL in the non obese group having BMI>30.

This inverse correlation between BMI and basal LH was observed in similar studies; this result agrees with Pagán et al., [12] who studied twenty-four women with PCOS across a spectrum of BMIs with frequent blood sampling, IV administration of GnRH (75ng/kg), and SC administration of the NAL-GLU GnRH antagonist (5 gg/kg) in the General Clinical Research Center at an Academic Hospital coming out with results that illustrates the inverse correlation between BMI and mean LH, LH/FSH, and LH pulse amplitude. They also suggested that the effect of BMI on LH is mediated at a pituitary and not a hypothalamic level in PCOS as they noticed that the pituitary response to a weight-based dose of GnRH is inversely related to BMI in PCOS.

This conclusion was also obtained by Bohlke et al., [13] who assessed the relationship between body mass index (BMI) and basal LH and the LH- FSH ratio revealing the same inverse association between BMI and basal LH levels but the study was on normally menstruating women and not on PCO patients.

However, on the other hand Micah et al., [14] and Sathya et al., [15], who studied the impact of BMI on the outcome of ICSI cycles (they classified their patients only according to their BMI without focusing on pco patients), have stated in their results that the level of basal LH did not differ between obese and lean women.

Our results also revealed significantly lower levels of prolactin in the obese group compared to the non-obese candidates. This finding is also an important secondary outcome observed in this study.

As for basal FSH, E2 and TSH; results showed no significant difference between the three groups with different BMI. This result agrees with the findings observed by Sathya et al., [15]. In their study which focused on impact of obesity on IVF outcome, they recorded non significant difference in basal hormones with different BMI.

On the contrary our findings may disagree with other studies as Loveland et al., who performed retrospective analysis on one hundred thirty-nine women <40 years old undergoing IVF cycles with fresh embryo transfers. They found that there is a decrease in basal FSH levels in the obese patients compared to non obese partners [16].

Also Giovanni et al., found lower basal FSH and estradiol levels in obese patients in their study that was carried on 22 patients with BMI >30 to document the hormonal differences in obese women [17].

In the second part of our work ovulation in the three groups was monitored by direct demonstration of a growing follicle using the trans-vaginal U/S. Although ovulation seemed to be better in non obese group (23.6%), yet, this result was not statistically significant. When ovulation was monitored by day 21 progesterone; ovulation was much better in the non obese group (18.3%), compared to the other groups. Ovulation was worst in the morbidly obese group (5.5%).

Our results may agree with a prospective cohort study carried by Walter et al., These authors found that; in anovulatory women with PCOS resumption of ovulation was associated with early and consistent loss of intra-abdominal fat (12.4 versus 5.0% at 3 months and 18.5 versus 8.6% at 6 month) [18].
There are also several studies that agree with our results such as those carried by Norman et al., who concluded that there is a definite relationship between anovulation and body BMI [19].

Our results may be attributed to abnormal leptin levels in obese patients which is believed to play a role in ovarian folliculogenesis. Human leptin is a protein of 167 amino acids. It is manufactured primarily in the adipocytes of white adipose tissue, and the level of circulating leptin is directly proportional to the total amount of fat in the body [20].

Leptin participates in regulation of ovarian folliculogenesis indirectly via control of LH and FSH secretion. More recent evidence suggests that leptin also has direct regulatory actions on the developing follicle. The presence of leptin receptors on follicular cells, including oocytes, and early pre-implantation embryos suggests that leptin may play a direct physiologic role in follicular maturation, oocyte development [21]. Because circulating leptin levels are directly related to body adiposity, elevated leptin concentrations associated with obesity may partly explain the negative impact of obesity on ovulation through its effect on FSH secretion and its direct effect on the follicles.

There are several biological mechanisms through which obesity may increase the risk for anovulation. These mechanisms center on the HPO axis, which regulates both the menstrual cycle and ovulatory function through a complex hormonal regulation system. The two main disturbances to the HPO axis occur through either hyperandrogenism or insulin resistance [22].

Hyperandrogenism is a biological condition where there is an excess production or secretion of androgens, which include sex hormones [3]. Adipose tissue has been shown to have the potential to alter the secretion of sex hormones, given its essential role in both androgen production and in the conversion of androgens into other sex hormones [22].

In the same direction, insulin resistance and hyperinsulinemia can lead to disturbance in ovulatory function. The ovary is a target organ for insulin to stimulate the production of sex hormones [22]. This increased insulin level will negatively affect follicular maturation through increasing free insulin-like growth factor 1.

In conclusion:

There is a biological evidence supporting the hypothesis that obesity increases the risk for anovulation in PCOS through the disruption of HPO axis by hyperandrogenism, and/or insulin resistance [22] or through abnormal levels of leptin [21]. Therefore weight reduction in PCOS may improve ovulatory functions and should be advised complementary to induction protocols. However, further research studies are still required to elucidate the role of individual members of the complex endocrine system that controls these relationships.

References


12- PAGÁN Y.L., SROUJI S.S., JIMENEZ Y., EMERSON A., GILL S. and HALL J.E.: Inverse relationship between...


Congenital Inguinal Hernia: Results of 2207 Procedures

MOHAMED KORANY, M.D.; MOHAMED A. OSMAN, M.D.; GAMAL MAKHLOUF, M.D. and MAHMOUD A. MAHMOUD, M.D.*

The Department of Surgery, Faculty of Medicine, Assiut University Hospital and South Valley University*

Abstract

Aim of Work: To evaluate the type, prevalence, presentation, and complications of congenital inguinal hernia in children at Assiut University Hospital and South Valley University.

Patients and Methods: Between 2000 and 2011, at Pediatric Surgery Unit, Assiut University Hospital and South Valley University, a retrospective study of 1957 patients with congenital inguinal hernia, the medical records were reviewed for patient data including age, sex, type of presentation, side of hernia, operative findings, type of intervention, and post-operative complications.

Results: From 2000 to 2011; more than 2207 procedures for 1959 patients with congenital inguinal hernia had been done. The male to female ratio was 1588 (81.1%) to 371 (18.9%); age at presentation was 322 (16.4%) neonates, 975 (49.8%) infants, and 665 (33.7%) children. 1055 right sided hernia, 652 left sided, and 252 bilateral. 345 cases have different type of complications. The sac was complete in 1454. Irreducibility was common complication, and more common in male. Incarceration was the second complication.

Conclusion: Congenital inguinal hernia is commonest hernia facing pediatric surgeons. It may have a different demography all over the world.

Key Words: Congenital – Inguinal – Hernia.

Introduction

An inguinal hernia is usually only intermittently detectable swelling, becoming most obvious on straining or crying. It is a type of ventral hernia that occurs when an intra-abdominal structure, such as bowel or omentum, protrudes through a defect in the abdominal wall. The cause of inguinal hernia in children is probably congenital in nature, due to simple opening of the peritoneal vaginal canal. However, they always require surgical treatment for the definitive occlusion of the orifice.

Inguinal hernia repair is the most common operation performed in pediatric surgical practice. In children, the quintessential step of the standard surgical repair procedure is ligation of the inguinal hernia sac at the internal ring either through a standard inguinal incision or using laparoscopy. This is a simple surgical procedure, which has given successful results in the treatment of hernias [1].

In children the quintessential step of the standard surgical repair procedure is ligation of inguinal hernia sac at the internal ring either through a standard inguinal incision or using laparoscopy. Examination of the child in both supine and standing position is important step for diagnosis of inguinal hernia. Differentiation between a hernia and a hydrocele is not always easy. Complication may be the first presentation, particularly irreducibility or strangulation.

Patients and Methods

A retrospective study was carried out, at Pediatric Surgical Unit, Children University Hospital, Assiut, over a period from 2000 to 2011. 1957 patients with 2207 inguinal hernias were admitted to our unit. All 2207 inguinal hernias were repaired surgically, after carrying out simple routine investigations. Medical records were analyzed regarding the following data; age at presentation, type of presentation, side of hernia, type and time of surgery, type of sac (complete or not), intra or post-operative complications, and recurrences. All these data was tabulated and analyzed.

All patients were examined in both supine and standing positions for diagnosis. Most of the children were presented by a palpable inguinal swelling. All patients were subjected to simple routine investigations as CBC; prothrombin time and concentration, and sometime to additional investigations if recommended. Sometime, diagnosis was obtained from parents, in addition to the palpation of the thick cord of the affected side. All operations have been done under general endotracheal intubation anesthesia.
Results

Our patients were segregated into three groups according to the age, and the hernias were distributed as (322) 16.4% in neonates, (975) 49.7% in infants, and (665) 33.9% in children.

The rate of hernia in male patients in our study was (1588) 81.1%, and their side distribution was (957) 60.2% on right, (461) 29% on left, and (170) 10.7% bilateral.

The rate of hernia in female patients was (371) 18.9%, and the side was (98) 26.4% on the right, (191) 51.4% on the left, and (82) 22.1% bilateral.

The right side hernia in neonates was (268) 83.2%, (500) 51.2% in infants, and (287) 43.4% in children. Whatever; the left side was (45) 16.77%, (337) 34.56%, and (259) 39.29%, in neonates, infants, and children respectively. The rate of bilateral hernia in neonates was zero, 7% in infants, and 5.8% in children.

The relation between presentation and age in our study was distributed as; Reducible hernia (262) 81.6% for neonates, (811) 83.2% for infants, and (539) 81.6% for children. Irreducible hernia (56) 17.4%, (127) 13%, and (96) 14.5% for neonates, infants, and children respectively. Incarceration was distributed as; (2) 0.6%, (23) 2.4%, and (17) 2.6% for neonates, infants, and children respectively. However; the strangulated hernia was (2) 0.6%, (14) 1.4% and (8) 1.2% in neonates, infants, and children respectively.

The relation between presentation and age in our study was distributed as; Reducible hernia (262) 81.6% for neonates, (811) 83.2% for infants, and (539) 81.6% for children. Irreducible hernia (56) 17.4%, (127) 13%, and (96) 14.5% for neonates, infants, and children respectively. Incarceration was distributed as; (2) 0.6%, (23) 2.4%, and (17) 2.6% for neonates, infants, and children respectively. However; the strangulated hernia was (2) 0.6%, (14) 1.4% and (8) 1.2% in neonates, infants, and children respectively.

The table shows presentation related to gender; Reducible hernia was (337) 91% in female and (1275) 80.3% in male. Irreducible hernia was as (24) 6.5%, (257) 16.2% for female and male respectively.

The relation of postoperative complications to gender of patients was (385) 24.2% in male, and (9) 2.4% in female. Regarding age the postoperative complications were (67) 20.8% in neonates, (199) 20.4% in infants, and (127) 19.2% in children. The main postoperative complication was cord edema and its rate was (299) of total cases 15.3%, and represent 54.3% of all complicated cases. Whatever; the age relation to postoperative complication was (67) 20.8% in neonates, (199) 20.4% in infants, and (127) 19.2% in children.

The rate of recurrence was (41) 2.1% of all cases and represented 9.6% of total complications, and distributed as 4.5% of children, 4.5% of infants, and 0.1% in neonates. The rate of infection was (10) 0.5% of total cases, and represented 2.5% of complicated cases; it was distributed as 0.5% in neonates, 1.2% in infants, and 0.76% in children. The scrotal edema was present in (38) 2% of all patients and represented. The rate of urgent procedure was 225 of 1959 (11.4%), and elective procedure was (1734) 88.5%. This rate was less than that of irreducible cases due to response of some cases to conservative treatment. The rate of urgent procedure was 54.2% for irreducible cases, 31.3% for incarceration, and 14.5 for strangulation. The total urgent procedure for male cases was 202 and 23 for female cases. Laparoscopic hernia repair had carried out for (120) 6.1%.

The rate of recurrence was (41) 2.1% of all cases and represented 9.6% of total complications, and distributed as 4.5% of children, 4.5% of infants, and 0.1% in neonates. The rate of infection was (10) 0.5% of total cases, and represented 2.5% of complicated cases; it was distributed as 0.5% in neonates, 1.2% in infants, and 0.76% in children. The scrotal edema was present in (38) 2% of all patients and represented. The rate of urgent procedure was 225 of 1959 (11.4%), and elective procedure was (1734) 88.5%. This rate was less than that of irreducible cases due to response of some cases to conservative treatment. The rate of urgent procedure was 54.2% for irreducible cases, 31.3% for incarceration, and 14.5 for strangulation. The total urgent procedure for male cases was 202 and 23 for female cases. Laparoscopic hernia repair had carried out for (120) 6.1%.

The complete sac was 74.3% and incomplete sac 25.7%. Congenital inguinal hernia usually associated with other anomalies, the common anomalies were undescented testis (8.2%), hydrocephalus (1.5%), hypospadias (1.2%), and bladder extrophy (0.6%).

Table (1): Collected data of the studied cases.

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
<th>Neonate</th>
<th>Infant</th>
<th>Children</th>
<th>Urgent procedure</th>
<th>Elective procedure</th>
<th>Type of sac</th>
<th>Associated anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1588</td>
<td>371</td>
<td>322</td>
<td>579</td>
<td>660</td>
<td>225</td>
<td>1734</td>
<td>Complete Sac</td>
<td>1454 Bladder extrophy 12</td>
</tr>
<tr>
<td>Side</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Incomplete Sac</td>
<td>505 Hydrocephalus 30</td>
</tr>
<tr>
<td>Lt 957</td>
<td>Lt 98</td>
<td>Lt 268</td>
<td>Lt 500</td>
<td>Lt 287</td>
<td></td>
<td></td>
<td>Undescend T. 162</td>
<td>Hypospadias 25 Others</td>
</tr>
<tr>
<td>Lt 461</td>
<td>Lt 191</td>
<td>Lt 337</td>
<td>Lt 259</td>
<td>Lt 138</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi 170</td>
<td>Bi 82</td>
<td>Bi 114</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complicated:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incarceration</td>
<td>36</td>
<td>6</td>
<td>2</td>
<td>23</td>
<td>17</td>
<td>180</td>
<td>990</td>
<td></td>
</tr>
<tr>
<td>Irreducible</td>
<td>257</td>
<td>24</td>
<td>56</td>
<td>127</td>
<td>97</td>
<td>120</td>
<td>990</td>
<td></td>
</tr>
<tr>
<td>Strangulation</td>
<td>20</td>
<td>4</td>
<td>2</td>
<td>14</td>
<td>8</td>
<td>18</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Noncomplicated</td>
<td>1275</td>
<td>337</td>
<td>255</td>
<td>776</td>
<td>533</td>
<td>120</td>
<td>990</td>
<td></td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>10</td>
<td>72</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Postoperative complications</td>
<td>585</td>
<td>9</td>
<td>96</td>
<td>199</td>
<td>127</td>
<td>990</td>
<td></td>
</tr>
</tbody>
</table>

Congenital Inguinal Hernia: Results of 2207 Procedures
Discussion

Inguinal hernia is one of the most common disorders in infants and children and its exact incidence is unknown. In USA the reported incidence ranges from 1-5%. Sixty percent of hernias occur on the right side. The incidence is higher in neonates. Premature infants are at increased risk for inguinal hernia, with incidence rates of 2% in females and 7-30% in males. Approximately 5% of all males develop a hernia during their lifetime [2,3]. The incidence of pediatric inguinal hernia is highest during the first year of life and then gradually decreases thereafter. One-third of children undergoing surgery for hernia are less than 6 months of age [4].

In our series the incidence in neonates is 16.4%, less than other studies; it seems to us that our tertiary hospital draining a large area that is not suitable for families to transfer their children in neonatal age. Infant and children incidence are more or less equal to other studies. The male-to-female ratio in our study is 5:1 ranges from 3:1 to 10:1 in related literatures [5,6].

The incidence rates regarding side are similar to published literatures. The rate of right side hernia in male is more than that in female.

The incidence of irreducibility of our study was 14.3%, incarceration 3.8%; it was slightly more in female, and strangulation 1.2%.

All postoperative complications (ranged from mild edema to recurrence) were 17.45%. The main postoperative complication was cord edema and its rate was 13.5%. The rate of recurrence was 1.8% of all cases. The rate of infection was 0.45%. The scrotal edema was present in 1.7%.

In Africa; complication rates for large tertiary centers are low, as reported by Abantanga, who noted a 0.7% recurrence rate after repairing of 396 hernias [7]. Often, however, the operation may be delegated to less experienced or junior surgeons in smaller surgical centers who may not have an appreciation for how significantly pediatric hernias differ to adult hernias.

The reported incidence of incarceration in large series of pediatric hernias ranges from 10-13% in western countries [8,9] and from 4-8% in African reports [10-12].

In our series, complication rate more or less is similar in all age group (20%) Neonatal inguinal hernias have a number of characteristics which seem quite unique. They almost exclusively occur in males. There was only one female patient in this entire series. In this age group, inguinal hernias were predominantly right-sided. The 87% incarceration rate is approximately 3 times that of the 31% reported in patients less than 1 year [13]. The high incidence of incarceration in neonatal hernia indicated that this can be potentially life-threatening in the small infant. Our study showed high significant results for complicated cases in relation of female to male, and also to the age group. Furthermore it was high significant for the relation of the side of hernia to age group.

References

Cardiac Resynchronization Therapy May Avoid Dilated Rather Than Ischemic Cardiomyopathy Patients the Need for Primary Prevention Defibrillator Implantation

SALAH ATTA, M.D.*; MOHAMED BASHANDY, M.D.** and SHERIF ZAKI, M.D.***
The Department of Cardiology, Faculty of Medicine, Assiut University*; The Cardiology Department, Faculty of Medicine; The Alhabtain Cardiac Center, Al-Dammam, KSA*; * and Department of Critical Care, Cairo University***

Abstract

The influence of the aetiology of systolic heart failure (HF) on the potential benefit of cardiac resynchronization therapy (CRT) is still unclear.

We aimed at comparing the response to CRT among patients with dilated (DCM) versus ischemic cardiomyopathy (ICM) and checking the possibility that CRT implantation may avoid some patients the need for defibrillator (ICD) implantation.

Patients and Methods: This prospective observational study included patients with advanced systolic HF who had CRT implantation and were followed-up for at least 6 months.

Results: The 1st group included 51 patients aged 51.94 ± 10.84 years who had DCM. The 2nd group included 17 patients aged 52.71 ± 10.61 years who had ICM. During the follow-up period, 42 patients (82.4%) of group 1 showed good response to CRT vs. 4 patients (23.5%) of group 2 (p=0.00). About 69% of the responders showed improvement of EF to >35%; all belonged to group 1. They had wider QRS than other patients in group 1 (157.06 ± 19.74 vs. 137.65 ± 18.38; p=0.001). Sustained ventricular tachycardia and ICD shocks happened in 2 patients (4.1%) of responders to CRT vs 6 patients (31.6%) of non responders (p=0.005), and occurred more among ICM (35.3%), p=0.002.

Conclusion: Patients with DCM may benefit more than ICM patients from CRT implantation. The significant EF improvement after CRT implantation may avoid some patients with DCM the need for Iry prevention ICD, specially patients with a significantly wide QRS before implantation. This may not be the case for ICM patients.

Key Words: Cardiac Resynchronization therapy – Implantable defibrillator – Dilated cardiomyopathy – Ischemic cardiomyopathy.

Introduction

CARDIAC resynchronization therapy (CRT) is a therapy of proven benefit in patients with advanced heart failure (HF). Identifying potential responders remains challenging, and whether the aetiology of the HF is related to the potential hemodynamic benefit and long-term outcome of CRT is unclear [1]. According to the current guidelines, primary prevention defibrillator (ICD) implantation is nearly indicated for all patients for CRT unless having a contraindication. In the COMPANION study [2], survival curves between CRT-D and CRT-P were parallel beyond 9 months, suggesting that the incremental benefit of ICD may be short-lived. Furthermore, CRT-P improves left ventricular function and potentially reduces the risk of subsequent sudden cardiac death (SCD). This is consistent with data from CARE-HF suggesting that CRT-P per se reduces SCD as well as total mortality [3]. CRT-D is also more expensive that CRT-P, and the addition of ICD backup is associated with increased morbidity [4].

Aim of the Work:

The aim was to compare the response to CRT among patients with dilated (DCM) versus ischemic cardiomyopathy (ICM) with advanced systolic heart failure and standard indications to CRT. Also, we aimed at checking the possibility that CRT implantation may avoid patients with advanced systolic dysfunction the need for Iry prevention ICD implantation and whether this is similar among patients with DCM and ICM.

Patients and Methods

Our study included 68 patients with advanced systolic HF despite optimal medical therapy subjected to CRT implantation in the electrophysiology (EP) laboratory of Saud Al-Babtain Cardiac Center, Al-Dammam, KSA in the period between May
2009 and July 2012. The patients were divided according to the presence of significant coronary artery disease (CAD) documented with coronary angiography) CAG into two groups: Group 1 included patients with DCM and group 2 included patients with ICM. All patients met the standard criteria of CRT implantation.

**Inclusion criteria:** Included age >18 years, sinus rhythm, NYHA class III-IV heart failure despite optimal medical treatment for at least 3 months, LV ejection fraction less than 35%, standard indicator of dyssynchrony (standard electrical criteria in the form of left bundle branch block (LBBB) and wide QRS complex >150ms, or 120-150ms with echo evidence of dyssynchrony).

**Exclusion criteria:** Patients with any of the following were excluded from our study: Patients with hypertrophic, restrictive or obstructive cardiomyopathy, organic and hemodynamically significant structural valve disease (defined as valvular stenosis greater than mild, and organic valvular regurgitations), constrictive pericarditis, primary pulmonary hypertension, uncorrected congenital heart disease, patients candidates for heart surgery, patients with acute myocardial infarction, severe unstable angina and stroke that occurred within 6 weeks prior the study enrolment, pregnant women, patients with RBBB and patients with end-stage co-morbidities affecting their expected survival were not included in this study.

All included patients were subjected to: Full history and clinical evaluation, 12 lead electrocardiogram (ECG), chest X-ray and 24 hours ECG (Holter) monitoring. Patients received all appropriate treatments for HF, which included a diuretic, an ACE-inhibitor, or an angiotensin receptor blocker, a beta-blocker and if needed digitalis. Doses of these background medications were kept maximized during the follow-up period.

**Echocardiographic assessment protocol:**

Standard echocardiography, including Doppler studies, was performed using a Vivid 7, Vingmed echocardiographic machine (General Electric, USA).

**Measurements:** LV size was evaluated measuring the end-diastolic and end-systolic diameters (EDD, ESD in cm) in 2D parasternal short-axis view images. The LV volumes and ejection fraction were assessed by using the apical 4-chamber and 2-chamber views calculating the end-diastolic and end-systolic volumes (EDV, ESV in ml) using Simpson formula. LV systolic function was evaluated using both the ejection fraction (EF,%) calculated from volumes [5].

**MR severity evaluation:**

The severity of mitral regurgitation was graded semi-quantitatively from colour-flow Doppler in the conventional parasternal long-axis and apical 4-chamber images. Mitral regurgitation was characterized as: Mild = 1+ (jet area/left atrial area <10%), moderate = 2+ (jet area/left atrial area 10-20%), moderately severe = 3+ (jet area/left atrial area 20-45%), and severe = 4+ (jet area/left atrial area >45%). In addition, grading according to mitral regurgitant volume was calculated by volumetric method to confirm and compare its amount quantitatively. Mitral regurgitation was characterized as: Mild = 1+ (regurgitant volume <30ml/beat), moderate = 2+ (regurgitant volume 30-44ml/beat), moderately severe = 3+ (regurgitant volume 45-59ml/beat), and severe = 4+ (regurgitant volume >60ml/beat) [6,7].

**Dysynchrony indexes were calculated as follows:**

**M-mode index:**

Septal-to-posterior wall motion delay (cut-off value = 130ms): The measurement was obtained from the M-mode tracing of the LV as the time interval between the maximal inward motion of the septum and the left posterior wall [8].

**Assessment of inter-ventricular dysynchrony:**

The aortic pre-ejection time was measured from the beginning of QRS complex to the beginning of the aortic flow velocity curve recorded by pulsed-wave (PW) Doppler in apical 5-chamber view. The pulmonary pre-ejection time was measured from the beginning of QRS complex to the beginning of the pulmonary flow velocity curve recorded in the left parasternal view. The difference between the two values determined the inter-ventricular mechanical delay (IVMD); an IVMD >40ms was considered as the cut-off value for inter-ventricular dyssynchrony [9].

**TDI time intervals and indexes [10]:**

For TDI analysis, the digital cine loops were analyzed using commercial software (Echopac 6.1, General Electric-Vingmed, Milwaukie, WI, USA) by 2 observers, blinded to the clinical condition. The sample volume was placed in the LV basal portions of the anterior, inferior, septal and lateral walls (using the 2- and 4-chamber images), a high frame rate of 150 frames/second (fps) was used and per region, the time interval between the onset
of the QRS complex and the peak systolic velocity were derived as follows:

Time to peak velocity, including the post-ejection period: The interval from onset of the QRS to the maximum positive velocity, including the period after aortic valve closure. Lateral and septal basal segments in the apical four-chamber view are compared and dyssynchrony is present when the delay in activation of opposite walls is more than 65ms. Aortic valve closure is automatically driven from the time/velocity curves at the basal septal segment very close to the aortic valve and it is positioned at the end of the negative spike after ejection (The time point closest to AVC is the time point of zero velocity after the initial negative velocities at end-systole) [11].

**Speckle tracking dysynchrony analysis [12]:**

For speckle tracking analysis, standard gray scale 2D images were acquired in the 2- and 4-chamber apical views as well as the parasternal short-axis views at the level of the papillary muscles. Special care was taken to avoid oblique views from the mid-level short-axis images and to obtain images with the most circular geometry possible. All of the images were recorded with a frame rate of at least 40 fps to allow for reliable operation of the software (EchoPac 6.1).

Speckle-tracking analysis for each of the 6 segments of the mid-ventricle short-axis view was done and the time to peak radial strain was measured. Then, the radial strain dyssynchrony index was calculated as the difference between earliest and latest time to peak strain and a cut-off value=130ms was considered in our study as diagnostic of significant dyssynchrony [13].

We categorized patients as having echocardiographic evidence of dyssynchrony only with strong and at least two echocardiographic parameters denoting dyssynchrony if QRS width was 120-150ms.

**Biventricular device implantation:**

Biventricular devices were implanted endovascularly via left or right subclavian veins in all except two patients where an epicardial left ventricular lead was inserted. The LV pacing lead was inserted into either the lateral or posterolateral cardiac vein. In only one case, it was positioned in the anterolateral position. The two patients in whom an epicardial left ventricular lead was inserted, this was due to inability to cannulate their coronary sinus and one of them had dextrocardia.

Of the included 68 patients, 59 patients received an Attain system (over-the-wire, Medtronic Inc), 33 of them received biventricular cardiac defibrillators (CRT-D) (InSync Marquiz, ICD, Medtronic), 5 received St. Jude Inc. and 4 received the Easylrek over-the-wire lead (Guidant Inc.). Patients with Holter evidence of significant ventricular arrhythmias (non sustained ventricular tachycardia (VT)) were given CRT-D (38 patients) while the rest of the patients received CRT-P.

**Programming and follow-up:**

The biventricular pacemaker was programmed in DDD mode with biventricular pacing. Adjustment of the atrio-ventricular delay was performed to ensure the longest possible atrio-ventricular filling time evaluated from pulsed Doppler analysis of trans-mitral LV filling to maximize LV filling and ejection times. The inter-ventricular interval was programmed at 0 interval basically and later optimized according to ECG and Echo evidence of synchronization. Before CRT implantation, after 24 hours, one month, 3-month and at 6-month follow-up, the clinical status, including NYHA functional class was assessed and echocardiographic assessment of LV volumes and EF was performed. Response to CRT was assessed after 6 months of CRT as follows:

**CRT response:** Defined as NYHA class improvement by at least one grade and echocardiographic evidence of LV reverse remodelling at 6 months. Successful LV reverse remodelling was defined as a reduction of LV end-systolic volume of >15%, and increase in EF by >10%.

**CRT non-response:** Death from cardiac causes or failure to reach the above pre-specified NYHA class and echocardiographic changes.

The study protocol was approved by the scientific committee, and written informed consents were obtained from all the patients.

**Statistical analysis:**

Continuous variables were expressed as Mean ±SD. Categorical data were summarized as frequencies and percentages. Differences in continuous variables between the two groups were analyzed using independent sample "t" test and x² or Fisher’s exact tests were used for dichotomous variables as appropriate. A p-value of ≤0.05 was considered statistically significant. For skewed data, we determined median values and we ran a Mann-Whitney’s U test. Logistic regression analysis was used to determine factors predicting response to CRT.
Results

This prospective observational study included 68 patients (54 males), with cardiomyopathy, EF less than 35% and advanced systolic heart failure who had CRT implantation in our centre. The patients were divided into 2 groups according to the presence of significant CAD (documented with CAG). The 1st group included 51 patients (39 males) aged 51.94±10.84 years who had DCM. The 2nd group included 17 patients (15 males) aged 52.71±10.61 years who had ischemic cardiomyopathy. All patients were in functional class III/IV and on optimal medical treatment for at least 3 months. The baseline QRS width of the included patients was not significantly different between the two groups (150.59±21.23 vs. 144.71±19.80, p=0.3) (Table 1).

At 6 months post implantation follow-up, 42 patients (82.4%) of group 1 showed clinical response to CRT compared to 4 patients (23.5%) of group 2 (p=0.00). There was no statistically significant difference between the 2 groups as regards pre-CRT functional class (3.18±0.39 vs 3.12±0.33; p=0.5). There was statistically significant difference between the 2 groups as regards post-CRT functional class (2.2±0.72 vs 2.88±0.6; p=0.00 1). There was statistically significant difference between the 2 groups as regards functional class improvement after CRT (0.98±0.58 vs. 0.24±0.44; p=0.00) (Table 2).

Regarding echocardiographic response, 72.1% (49 patients) of all patients showed good response to CRT. Evidence of echocardiographic response to CRT was observed in 45 patients (88.2%) of DCM (group 1) patients versus 4 (23.5%) patients of ICM patients (as shown in Fig. 1). Reduction of LVESV was highly more significant (17.68±10.84% in group one than group 2 (7.72±12.84% p=0.003). Also improvement of EF in group 1 from 19.92±5.96% before to 34.37±7.52% after CRT was significantly more than in group 2 (21.50±4.64% before, to 25.7±5.96% after CRT) p<0.00 1 (Table 2).

The medians of percentage of reduction of LVESV in the 2 groups were 20.45 and 8.15ml respectively. We ran a Mann-Whitney’s U test which showed that there was a statistically significant difference between the 2 groups; U=240, Z=–2.74, p=0.006. The medians of EF improvement in the 2 groups were 15.20 and 5.53 respectively. We ran a Mann-Whitney’s U test which showed that there was a statistically significant difference between the 2 groups; U=1 13, Z=–4.54, p=0.00 (Table 2).

MR grade before CRT implantation were 2.24±0.51 in group 1 vs. 2.35±0.49 in group 2 (p=0.4) while at 6 months post implantation, the MR grade became 1.18±0.77 in group 1 vs. 2.1±0.53 in group 2 (p=0.002). The medians of MR grade improvement in the 2 groups were 1.0 and 0 respectively. We ran a Mann-Whitney’s U test which showed that there was statistically significant difference between the 2 groups; U=202, Z=–3.46, p=0.001. The medians of MR volume reduction in the 2 groups were 20 and 5ml respectively. We ran a Mann-Whitney’s U test which showed that there was statistically significant difference between the 2 groups; U=168.5, Z=–3.76, p=0.00. (Table 2). Logistic regression analysis of pre-procedural factors predicting response to CRT, showed that cardiomyopathy type and QRS width are predictors of response to CRT with the type of cardiomyopathy being a stronger predictor (β coefficient=4.94 and 0.14) (p<0.001 and 0.002 respectively).

Among patients with good echocardiographic response to CRT, 34 patients (69.4% of the responders and 66% of the DCM patients) showed improvement of EF to >35%; all of them belonged to group 1, while none of the patients with ICM showed improvement of EF to >35%, p=0.00. Among patients with DCM, comparing the patients who had a post CRT EF improvement to more than 35% with the patients who had less improvement in EF with still ≤35% showed that they had basically wider QRS than the other patients in the same group (157.06±19.74 vs. 137.65±18.38; p=0.001). These patients also had less LVESV before and after CRT, LVEDV before and after CRT, and more EF after CRT but no significant difference in EF before CRT. However, using logistic regression analysis, it was found that QRS width was the only predictor of this super response to CRT (β coefficient=0.07) that is statistically significant (p=0.005) (Table 3).

During the follow-up period, none of the CRT responders of the DCM group had sustained VT or appropriate ICD shocks, while two patients of the DCM non responders (3.9% of group 1) had sustained VT, one of them who initially had a CRT-P required upgrading to a CRT-D. vs. 6 patients of ICM group (35.3%) (4 non responders and two responders) (p=0.002). So, Sustained VT and ICD shocks happened in 2 patients (4.1%) of echocardiographic responders to CRT vs 6 patients (31.6%) of non responders (p=0.005). Three patients died during the follow-up period. The three patients were non responders to CRT and belonged to the ICM group.
Table (1): Demographic Data and pre-implantation parameters of the included patients in both groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (DCM) (5 1)</th>
<th>Group II (ICM) (17)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean±SD) years</td>
<td>51.94±10.84</td>
<td>52.71±10.61</td>
<td>0.1</td>
</tr>
<tr>
<td>Male sex</td>
<td>39 (76.5%)</td>
<td>15 (88.2%)</td>
<td>0.4</td>
</tr>
<tr>
<td>NYHA functional class pre-implantation</td>
<td>3.18±0.39</td>
<td>3.12±0.33</td>
<td>0.5</td>
</tr>
<tr>
<td>QRS width (Mean±SD) ms</td>
<td>150.59±21.23</td>
<td>144.71±19.80</td>
<td>0.3</td>
</tr>
<tr>
<td>HTN</td>
<td>22 (43.1%)</td>
<td>8 (47.1%)</td>
<td>0.7</td>
</tr>
<tr>
<td>DM</td>
<td>32 (62.7%)</td>
<td>12 (70.6%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Dyslipidemic</td>
<td>26 (51%)</td>
<td>10 (58.8%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Smokers</td>
<td>31 (60.8%)</td>
<td>13 (76.5%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Obesity</td>
<td>16 (31.4%)</td>
<td>6 (35.3%)</td>
<td>0.7</td>
</tr>
<tr>
<td>COPD/BA</td>
<td>11 (21.6%)</td>
<td>2 (11.8%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Family history</td>
<td>10 (19.6%)</td>
<td>5 (29.4%)</td>
<td>0.5</td>
</tr>
<tr>
<td>BB</td>
<td>42 (82.4%)</td>
<td>17 (100%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Diuretics</td>
<td>40 (78.4%)</td>
<td>17 (100%)</td>
<td>0.054</td>
</tr>
<tr>
<td>ACE</td>
<td>41 (80.4%)</td>
<td>17 (100%)</td>
<td>0.056</td>
</tr>
<tr>
<td>ARBs</td>
<td>7 (13.7%)</td>
<td>0 (0%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>33 (64.7%)</td>
<td>11 (64.7%)</td>
<td>1.0</td>
</tr>
<tr>
<td>NTG</td>
<td>0 (0%)</td>
<td>8 (47.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ivalbradin</td>
<td>14 (27.5%)</td>
<td>7 (41.2%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Digoxin</td>
<td>9 (17.6%)</td>
<td>4 (23.5%)</td>
<td>0.7</td>
</tr>
</tbody>
</table>


Table (2): Comparison between the 2 groups as regards clinical and echocardiographic data and response to Cardiac resynchronization therapy (CRT).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (DCM) (5 1)</th>
<th>Group II (ICM) (17)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical responders; No. (%)</td>
<td>42 (82.4%)</td>
<td>4 (23.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NYHA functional class post implantation</td>
<td>2.2±0.72 vs</td>
<td>2.88±0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Echo Responder; No. (%)</td>
<td>45 (88.2%)</td>
<td>4 (23.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MR improvement; No. (%)</td>
<td>38 (74.5%)</td>
<td>7 (41.2%)</td>
<td>0.018</td>
</tr>
<tr>
<td>LVESV before CRT (Mean±SD)</td>
<td>206.75±43.63</td>
<td>226.18±36.70</td>
<td>0.1</td>
</tr>
<tr>
<td>LVESV after CRT (Mean±SD)</td>
<td>170.25±44.43</td>
<td>209.29±48.14</td>
<td>0.003</td>
</tr>
<tr>
<td>Reduction in LVESV (%) (Mean±SD)</td>
<td>17.68±10.84</td>
<td>7.72±12.84</td>
<td>0.003</td>
</tr>
<tr>
<td>LVEDV before CRT (Mean±SD)</td>
<td>259.18±55.52</td>
<td>290.00±54.83</td>
<td>0.051</td>
</tr>
<tr>
<td>LVEDV after CRT (Mean±SD)</td>
<td>257.98±50.24</td>
<td>281.55±50.26</td>
<td>0.11</td>
</tr>
<tr>
<td>EF before CRT (Mean±SD)</td>
<td>19.92±5.96</td>
<td>21.50±6.46</td>
<td>0.32</td>
</tr>
<tr>
<td>EF after CRT (Mean±SD)</td>
<td>34.37±7.52</td>
<td>25.70±5.96</td>
<td>0.000</td>
</tr>
<tr>
<td>EF improvement (Mean±SD)</td>
<td>14.45±7.34</td>
<td>4.20±5.44</td>
<td>0.000</td>
</tr>
<tr>
<td>MR grade Before CRT</td>
<td>2.24±0.51</td>
<td>2.35±0.49</td>
<td>0.4</td>
</tr>
<tr>
<td>MR grade After CRT</td>
<td>1.18±0.77</td>
<td>2.15±0.53</td>
<td>0.002</td>
</tr>
<tr>
<td>MR Grade Improvement</td>
<td>1.06±0.81</td>
<td>0.11±0.86</td>
<td>0.003</td>
</tr>
<tr>
<td>MR volume Before CRT (ml)</td>
<td>39.71±8.62</td>
<td>40.29±7.19</td>
<td>0.8</td>
</tr>
<tr>
<td>MR volume After CRT (ml)</td>
<td>21.90±13.86</td>
<td>37.82±8.07</td>
<td>0.007</td>
</tr>
<tr>
<td>MR volume Difference (ml)</td>
<td>17.12±15.00</td>
<td>0.76±12.84</td>
<td>0.001</td>
</tr>
</tbody>
</table>

No : Number. MR : Mitral regurgitation. LVESV : Left ventricular end systolic volume. LVEDV : Left ventricular end diastolic volume. EF : Ejection fraction.
The better response to CRT among patients with DCM compared to ICM in our study is concordant with previous reports in this field. Mcleod et al., [1] reported that at median follow-up of 7.1 months after CRT implantation, the DCM group experienced greater improvement in left ventricular ejection fraction \((8.3\% \pm 10\% \text{ vs } 6.2\% \pm 10\%, \ p=0.05)\) and left ventricular end-diastolic volumes than did those with ICM \((-28.2\% \pm 53\text{ml vs } 15.3\% \pm 46\text{ml}, \ p=0.024)\). Survival estimates at 4 years were 55\% for ICM and 77\% for DCM groups \((p<.001)\), respectively, whereas no significant difference in the incidence of appropriate/inappropriate ICD shocks was observed. The ICM group remained at higher risk for death compared to the DCM group after controlling for pre-implant variables (hazard ratio 1.6, 95\% confidence interval 1.1-2.3, \(p=0.008\)). According to these findings, they concluded that compared to those with an ischemic cardiomyopathy patients with a non-ischemic cardiomyopathy appear to achieve greater clinical outcome and improved cardiac indices, independent of gender, age, and LV lead position. In our study, we had similar findings with less response and higher mortality among the ICM patients but our patients showed also a higher incidence of appropriate ICD shocks.

Many explanations were raised for this finding including the presence of trans-mural scars that can’t be improved by CRT specially if the scar is extensive or in the posterolateral wall [14]. A higher number of viable segments at baseline was associated with a higher probability of response; vice-versa, a higher total scar score was associated with a lower probability of response. The subendocardial or transmural scar nevertheless can impact the activation wavefront exit and potentially interfere with CRT effect thus a transmural scar tissue in the region of the LV pacing lead may prohibit response [15].

Another possible factor is the degree of improvement of MR. Functional mitral regurgitation previously has been shown to improve with CRT, and it has also been suggested that patients with worse mitral regurgitation may respond better and the degree of improvement of MR may influence the outcome after CRT [16,17]. The improvement of MR among DCM patients in our study was substantially more than among the ICM patients and this may have contributed to the differential response in the two groups to CRT.

In the updated guidelines for 1ry prevention ICD implantation, the current cut-off value of EF that is considered as an indication for primary
prevention ICD implantation for non-ischemic DCM is ≤35% with NYHA Functional Class II or III while for ICM the guidelines state as a class I A indication for an ICD implantation the existence of LVEF ≤35 % due to prior MI (at least 40 days post-MI) and NYHA Functional Class II or III or LVEF ≤30%, and NYHA Functional Class I [18]. According to the guidelines, initially all patients included in the study could be considered indicated for 1ry prevention defibrillator implantation in addition to CRT.

However, by the end of 6 months post implantation, 66% of our patients with DCM included in the study became non indicated for primary prevention ICD by having an EF of >35% vs non of the ICM patients. These patients characteristically had a wider QRS at baseline which may predispose them to a better response to CRT and logestic regression analysis showed that the QRS width was the only predictor of this super response to CRT among the DCM patients. These findings are concordant to most of the previous large trials in the literature. A significant treatment interaction with QRS duration was observed in MADIT-CRT and RAFT, and a trend toward an interaction was observed in REVERSE QRS subgroup findings [19].

On the otherhand, this doesn't mean that non of the DCM may need ICD as these patients are still at risk of life threatening ventricular arrhythmias. In our study one patient of the DCM patients who initially had a CRT-P implantation, required upgrading to CRT-D later and another patient who had a CRT-D received appropriate shocks. Importantly, the two patients were non-responders to CRT which may support the assumption that good responders may be in less need of an ICD which is consistent with data from CARE-HF suggesting that CRT-P per se reduces SCD as well as total mortality [2].

Thus, giving a chance for CRT-P to show its full effect before considering ICD implantation may avoid some patients with DCM (specially who have significantly wide QRS) the possible morbidity inherent in ICD implantation like inappropriate shocks and lead problems in addition to the expected cost benefit of avoiding unrequired defibrillator implantation. However, for ischemic cardiomyopathy patients, this was not the case as the response to CRT was significantly less than the DCM patients and non of the ICM patients had an improved EF to >35%, in addition to the more frequent VT and ICD shocks and the three mortalities in our study that happened among the ICM group.

Conclusions:

Patients with DCM may benefit more than ICM patients from CRT implantation. The significant EF improvement after CRT implantation may avoid some patients with DCM the need for 1ry prevention ICD implantation, especially if they have a significant widening of the QRS before implantation. However, this may not be the case for patients with ICM.

References


9- ROULEAU F., MERHEB M., GEFROY S., BERTHELOT J., CHALEIL D., DUPUIS J.M., VICTOR J. and...


A Structured Review of Outcome Measures Post Aerobic Training for Chronic Obstructive Pulmonary Disease (COPD) Patients

AMANY R. MOHAMED, Ph.D.* and MARWA M. SHABAN, M.D.**
The Departments of Physical Therapy, Critical Care, Cairo University Hospitals* and Chest Disease, Faculty of Medicine, Cairo University

Abstract
The purpose of this study was to evaluate the benefits of aerobic training program for patients with COPD in combination with pharmacological treatment through investigation of outcome measures for these patients. Thirty COPD patients with age >50 years old, participated in this study. They were clinically stable COPD, on optimized medical treatment all over the study, patients were randomly divided into two groups of 15 patients for each, study and control group. Patients of study group received hospital-based supervised aerobic training program in addition to medical treatment, while patients of control group received optimized medical treatment only. The exercise program was conducted three times/week for successive 6-8 weeks for the study group patients. Assessment included: Body mass index (BMI), pulmonary function test, modified medical research council (MMRC), six minute walk test (6-MWT), and BODE index were conducted at the starting and at the end of study. Comparing the baseline data with data after treatments, the results showed that: Within the control group, there was a statistically significant improvement in 6-m.w. distance with an increase of 15 meters with non statistically significant difference in Mean ± SD of BMI or spirometric parameters. While within the study group, there was a statistically significant improvement in 6-m w. Distance with an increase of 78 meters and also a significant increase difference in Mean ± SD of FVC% and FEV1/FVC as (85.87 ± 17 vs 73.4 ± 20% and 46.27 ± 12.3 vs 49.2 ± 12.7) with significant improvement in BODE score with greater number of patients who improved within the study group. The present study concluded that short term aerobic training program has the capacity to break the vicious circle of dyspnea, increasing inactivity and exercise intolerance and improve some components of bode index supporting the use of this multi dimensional index to evaluate the effect of training program for COPD patients.

Key Words: Outcome measures – Aerobic training – COPD patients.

Introduction
CHRONIC obstructive pulmonary disease (COPD) is a preventable and treatable disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles of gases, primarily caused by cigarette smoking. Patients with COPD typically show a decrease in both FEV1 and FVC. The presence of airflow limitation is defined by a post-bronchodilator FEV1/FVC <0.70. Assessment of airflow limitation is based on the patient’s level of symptoms, the severity of the spirometric abnormality, and the presence of complications such as respiratory failure, right heart failure, weight loss, and arterial hypoxemia [1].

Recent COPD guidelines such as GOLD (Global Initiative for Chronic Obstructive Pulmonary Disease), NICE (National Institute for Health and Clinical Excellence) and BTS (British Thoracic Society) underline the importance of pulmonary rehabilitation (PR) as a part of an integrative multidisciplinary approach regardless the stage of disease [2].

The American Thoracic Society and the European Respiratory Society have recently adopted the following definition of pulmonary rehabilitation: Pulmonary rehabilitation is an evidence-based multidisciplinary, and comprehensive intervention for patients with chronic respiratory diseases who are symptomatic and often have decreased daily life activities. Integrated into the individualized treatment of the patient, pulmonary rehabilitation is designed to reduce symptoms, optimize functional status, increase participation, and reduce health care costs through stabilizing or reversing systemic manifestations of the disease [3].

One of the important functions of pulmonary rehabilitation is to help selection of appropriate patients for surgery and to ensure that patients
make a truly informed choice about treatment options. Some patients may improve sufficiently after rehabilitation and choose to defer or delay the decision to pursue surgical options [4].

Pulmonary rehabilitation programs monitor outcomes partly as indicators of performance and to ensure quality, but also because many third-party payers now require such assessments to qualify for reimbursement [5,6].

Outcome measures usually include a functional assessment. Many programs use the 6-min walk test or the 10-m shuttle test. Both are widely applied tests of functional endurance that are of prognostic value. The shuttle walk test is an incremental test, but both it and the 6-min walk test are effort-dependent and subject to non respiratory limitations such as weakness, pain, or arthritis [6-8].

Some programs perform maximal cardiopulmonary exercise tests that test maximal capacity rather than endurance. These tests are usually combined with a dyspnea assessment as a rough gauge of effort, such as the Borg score or rating on a visual analog scale. Many programs also use dyspnea scales such as the Baseline and Transitional Dyspnea indices that assess dyspnea as related to function, effort, and task [9,6]. In addition, most programs use questionnaires to assess overall quality of life (or health status). The Short Form-36 is a commonly used proprietary instrument that tests overall health status. Disease-specific questionnaires such as the St. George’s Respiratory Questionnaire (SGRQ) [6,10], or the Chronic Respiratory Disease Questionnaire (CRQ) are also commonly used [5,6].

A composite index has recently been described that combines body mass index (B), severity of airway obstruction (O), dyspnea index (D), and exercise capacity (E) (BODE index) and correlates with prognosis of patients with COPD [11,6].

The aim of this study was to evaluate the benefits of Outpatient Hospital-Based Exercise Program in addition to pharmacological treatment for patients with COPD using several validated methods to measure outcome as 6 MWT, MRC dyspnea score, BODE index and pulmonary function tests.

Patients and Methods

Thirty COPD patients presenting to Critical Care Department Kasr El-Aini Hospital during 2011-2012. All patients are ≥50 years of age; ≥10 pack-year history of cigarette smoking, they are clinically stable COPD (not suffering from a recent respiratory tract infection). The selected patients on Optimized medical therapy according to Global Initiative for Chronic Obstructive Lung Disease [12], and had not been engaged in any exercise-training program before participating in the study [13]. Patients with Other chronic diseases that may contribute to exercise limitation such as: Cardiac, renal, and liver diseases, metabolic, mental and neurological disorders were excluded from the study. Malignancies and those with Chronic hypoxemia at rest requiring continuous oxygen support (PaO₂ <7.3kPa), those with Lack of motivation, Non-adherence were also excluded. Participants in the study were divided into two groups: Group I (Control group): 15 patients on medical treatment including short-acting bronchodilators, methyl-xanthines, sometimes inhaled short acting B2-agonist; inhaled glucocorticosteroïd combined with a short acting B2-agonist or inhaled short acting anticholinergic combined with short acting B2-agonist. Group II (Training group): 15 patients subjected to hospital based supervised aerobic training program in addition to medical treatment. All patients subjected to: Full history taking including medical and smoking history, clinical examination and Plain Chest X-ray (P-A view). Baseline & post study body mass index, Pulmonary function test: (Flow/volume loop) using body plethysmography with highly transparent box; Sensor-medics V max series, 2130 Spirometer, V6200 Autobox, 6200DL, Six-Min Walk Test using Electrical treadmill (Schiller Quinton 4000) that has no inclination and BODE index were assessed.

Methods:

1- The body mass index (BMI), or Quetelet index: It is defined as the individual’s body mass divided by the square of his height-with the value universally being given in units of kg/m² [14].

2- Pulmonary function test: (Flow/volume loop): Spirometry indices are reported comparing the individual’s value along with the predicted values [15]. The Forced vital capacity (FVC), the forced expiratory volume in the first second (FEV1), the ratio of FEV 1 to FVC and the average of forced expiratory flow at 25-75 % of forced vital capacity (FEF 25-75%) were measured. The presence of a post bronchodilator FEV1 <80% predicated together with an FEV1/FVC <0.70 confirm the presence of airflow limitation that is not fully reversible [14]. Bronchodilator Reversibility Tests: Reversible airway obstruction characterized by increase in FEV1 that is both greater than 200ml (absolute change) and 12% (% change) above the pre-bronchodilator FEV1 is considered significant [1].
3- Modified medical research council: MMRC dyspnea questionnaire for assessing the severity of breathlessness [1].

4- Treadmill six-minute walk test: Use of a treadmill to determine the 6MWD allow constant monitoring during the exercise. Standardized instructions and encouragement similar to those for the corridor walk were given, according to ATS guidelines [16]. Patients were instructed to walk “as far as possible” during the time that is, as fast as possible. They were told that they could slow down or even stop if necessary. There was no warming up before test. The initial treadmill speed was zero, and the test began when treadmill activated and the patient started walking. The patient controlled the treadmill speed during the test and could stop to rest at any time, as in the hallway test. Before, during, or after treadmill walk test, the walk testing was discontinued if the patient had thoracic pain, intolerable dyspnea, cramps, dizziness, staggering, diaphoresis, pallor, or an SpO2 <90% [16].

5- BODE index: Variables and point values used in the BODE index [11]. Table (1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>BODE score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index body weight (in kg) / height (in meters)</td>
<td>&gt;21 ≤21 – –</td>
</tr>
<tr>
<td>Airway obstruction: Forced expiratory volume at 1 second (as percentage of predicted)</td>
<td>&gt;65 50-65 35-49 &lt; 35</td>
</tr>
<tr>
<td>Dyspnea: MMRC</td>
<td>0 or 1 2 3 4</td>
</tr>
<tr>
<td>Exercise capacity: 6 minute walk test distance (meters)</td>
<td>≥350 250-349 150-249 ≤149</td>
</tr>
</tbody>
</table>

(N.B): All the previous measurements were done for all patients before and at the end of the study.

6- Exercise Prescription: An Outpatient Hospital-Based Exercise Program for a period of 8 weeks of aerobic training as graduated treadmill exercises was initiated upon the results of the exercise testing, so the exercise prescription was individually designed. Frequency of sessions: 3 times per week that are equally spaced throughout the week. Regular supervision of exercise sessions: Aiming to achieve optimal physiologic benefits. Duration of each session: 20-30 minutes. Intensity of exercise: An adequate training intensity for endurance conditioning usually targeted 70%-85% of the individually determined maximal heart rate [17]. The maximal heart rate can be estimated from the formula (220 minus age) [18]. The exercise session was subdivided into: Warm up phase: 5-10min of both light muscular stretching & inspiratory muscle training to avoid muscle strains and injuries. Exercise phase: 9-13min. Of treadmill exercise using Electrical treadmill (Schiller Quinton 4000) with different speeds & inclination grades. Cool down phase: This relaxation period after the exercise session ensures that body not experience any muscular problems. It includes 5-7min. Of continued exercise with a speed 1 km/h with inclination grade 0.0%.

Progression of exercise includes:
- First week of training should be at 60-70% of the individually determined maximum heart rate to allow for the development of motor skills and musculoskeletal conditioning.
- As patient’s tolerance for exercise improved, the duration of walking increase gradually and the target is increased by 5-10% of the maximum heart rate.
- After 4 weeks of training, exercise intensity achieves a level of 80% of maximum heart rate, as it was increased gradually every 2 weeks.

Statistical analysis:

Data were statistically described in terms of Mean±Standard deviation (±SD), median and range. Comparison between the both groups was done using Student t-test for independent samples. Within group comparison was done using paired t-test. p-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

When compare baseline data with data after 8 weeks within each group it showed that: Within control group, there was a statistically significant improvement in 6MWD with an increase of 15 meters while no statistically significant difference in the Mean±SD of BMI and spirometric data as shown in Table (2). While training group: There was a statistically significant improvement in 6MWD with an increase of 78 meters and also a significant increase in the Mean±SD of FVC% and FEV1/FVC with no statistically significant in the Mean±SD of BMI and other spirometric data as shown in Table (3). As regards BODE index: Within both control and study group there was a statistically significant improvement in BODE score as shown in Tables (4,6), with greater number of
patients improved within study group as shown in Tables (5, 7).

When compare characteristics between control and study groups after training program: There was a statistically significant improvement in the Mean±SD of FEV1 & FVC% and 6MWD of study group higher than control group after training program as shown in Table (8). As regards BODE index, there was a statistically significant improvement in Mean±SD of BODE index of study group more than control group after training program as shown in Table (9).

Table (2): BMI, Functional characteristics in control group baseline and after 6-8 weeks of medical treatment.

<table>
<thead>
<tr>
<th>Characteristics within control group (n=15)</th>
<th>Baseline data</th>
<th>After 6-8 weeks of medical treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m2) Mean±SD</td>
<td>25.75±5.09</td>
<td>25.72±5.11</td>
<td>0.317</td>
</tr>
<tr>
<td>FVC% Mean±SD</td>
<td>62.27±19.86</td>
<td>60.87±20.31</td>
<td>0.581</td>
</tr>
<tr>
<td>FEV1% Mean±SD</td>
<td>34.80±14.05</td>
<td>34.67±13.70</td>
<td>0.788</td>
</tr>
<tr>
<td>FEV1/FVC% Mean±SD</td>
<td>41.76±8.30</td>
<td>44.77±11.18</td>
<td>0.125</td>
</tr>
<tr>
<td>FEF25-75% Mean±SD</td>
<td>11.67±6.73</td>
<td>12.20±6.95</td>
<td>0.875</td>
</tr>
<tr>
<td>6min walk Distance (in meters) Mean±SD</td>
<td>36.07±7.88</td>
<td>51.40±19.98</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*P-value <0.05 statistically significant.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).

Table (3): BMI, Functional characteristics in study group baseline and after 6-8 weeks of aerobic training program.

<table>
<thead>
<tr>
<th>Characteristics within aerobic group (n=15)</th>
<th>Baseline data</th>
<th>After 6-8 weeks of training program</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m2) Mean±SD</td>
<td>25.54±3.93</td>
<td>25.54±3.93</td>
<td>1.00</td>
</tr>
<tr>
<td>FVC% Mean±SD</td>
<td>73.40±20.70</td>
<td>85.87±17.01</td>
<td>0.001*</td>
</tr>
<tr>
<td>FEV1% Mean±SD</td>
<td>48.40±20.95</td>
<td>51.00±18.97</td>
<td>0.127</td>
</tr>
<tr>
<td>FEV1/FVC% Mean±SD</td>
<td>49.20±12.69</td>
<td>46.27±12.29</td>
<td>0.033*</td>
</tr>
<tr>
<td>FEF25-75% Mean±SD</td>
<td>24.40±19.62</td>
<td>24.33±19.56</td>
<td>0.972</td>
</tr>
<tr>
<td>6min walk Distance (in meters) Mean±SD</td>
<td>41.53±9.55</td>
<td>119.53±27.39</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*P-value <0.05 statistically significant.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).

Table (4): BODE index in control group baseline and after 6-8 weeks of medical treatment.

<table>
<thead>
<tr>
<th>BODE index within control group (n=15)</th>
<th>Baseline</th>
<th>After 6-8 weeks of medical treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODE index Mean±SD</td>
<td>7.07±1.33</td>
<td>6.47±1.41</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*P-value <0.05 statistically significant.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).

Table (5): Change in BODE index after 6-8 weeks of medical treatment.

<table>
<thead>
<tr>
<th>Change in BODE index after 6-8 weeks of medical treatment</th>
<th>Improved*</th>
<th>Not improved</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (% within the group) (n=15)</td>
<td>9 (60%)</td>
<td>6 (40%)</td>
</tr>
</tbody>
</table>

*Improvement: At least one unit change in the BODE index.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).

Table (6): BODE index in study group baseline and after 6-8 weeks of training program.

<table>
<thead>
<tr>
<th>BODE index within aerobic group (n=15)</th>
<th>Baseline</th>
<th>After 6-8 weeks of training program</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODE index Mean±SD</td>
<td>6.80±1.32</td>
<td>5.00±1.73</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*P-value <0.05 statistically significant.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).

Table (7): Change in BODE index after 6-8 weeks of study training program.

<table>
<thead>
<tr>
<th>Change in BODE index after 6-8 weeks of training program within aerobic group (n=15)</th>
<th>Improved*</th>
<th>Not improved</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (% within the group) (n=15)</td>
<td>13 (86.6%)</td>
<td>2 (13.33%)</td>
</tr>
</tbody>
</table>

*Improvement: At least one unit change in the BODE index.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).

Table (8): Comparison of BMI, functional characteristics between control and study Group after 6-8 weeks of training program.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group (n=15)</th>
<th>Aerobic group (n=15)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m2) Mean±SD</td>
<td>25.72±5.11</td>
<td>25.54±3.93</td>
<td>0.66</td>
</tr>
<tr>
<td>FVC% Mean±SD</td>
<td>85.87±17.01</td>
<td>85.87±17.01</td>
<td>0.002*</td>
</tr>
<tr>
<td>FEV1% Mean±SD</td>
<td>51.00±18.97</td>
<td>51.00±18.97</td>
<td>0.006*</td>
</tr>
<tr>
<td>FEF25-75% Mean±SD</td>
<td>24.33±19.56</td>
<td>24.33±19.56</td>
<td>0.065</td>
</tr>
<tr>
<td>6min walk Distance (in meters) Mean±SD</td>
<td>119.53±27.39</td>
<td>119.53±27.39</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*P-value <0.05 statistically significant.

BMI: Body mass index.

FVC: Forced vital capacity.

FEV1: Forced expiratory volume in 1st second.

FEF25-75: The average of forced expiratory flow at 25-75% of forced vital capacity.

Table (9): Comparison of BODE index between control and study group after 6-8 weeks of training program.

<table>
<thead>
<tr>
<th>BODE index after 6-8 weeks of training program</th>
<th>Control group (n=15)</th>
<th>Aerobic group (n=15)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODE index Mean±SD</td>
<td>6.47±1.41</td>
<td>5.00±1.73</td>
<td>0.017*</td>
</tr>
</tbody>
</table>

*P-value <0.05 statistically significant.

BODE: The body-mass index (B), degree of airflow obstruction (O), functional dyspnea (D) and exercise capacity (E).
Discussion

The quality of life for a person suffering from COPD diminishes as the disease progresses. American Lung Association (ALA) survey revealed that half of all COPD patients (51%) say their condition limits their ability to work. It also limits them in normal physical exertion (70%), household chores (56%), social activities (53%), sleeping (50%), and family activities (46%). According to an ALA survey, at least half of COPD patients are expected to benefit from rehabilitation [19].

This study was designed to evaluate benefits of Outpatient Hospital-Based Exercise Program for people with COPD, as a non-pharmacological treatment method, using several validated instruments to measure outcomes after Pulmonary rehabilitation as, 6 MWT, MRC dyspnea score, BODE index and pulmonary function tests. While previous studies had established the efficacy of pulmonary rehabilitation for COPD: A randomized controlled trial by Griffiths et al., [20] showed that, an outpatient pulmonary rehabilitation program was cost-effective and was likely to result in financial benefit to the health service. Kozora et al., [21], found reductions in patients with COPD with anxiety and depression after pulmonary rehabilitation compared with matched control subjects.

To clarify the effect of aerobic training, the present study conducted a training program of 6-8 weeks. Most previous studies were fully consistent with this. Green et al., [22] who compared 4 weeks with 7 weeks of rehabilitation and concluded that 4 weeks of rehabilitation was less effective. While Sneed & Paul et al., [23] found that longer rehabilitation programs (6 months or longer) yield significantly greater effects, concluded that although measurable physiological changes may occur within weeks, behavioral changes may require longer time periods.

The present study implemented moderate to high intensity training program that targeted 80-85% of the individually determined maximal heart rate in-order to maximize the training effects. Initially the intensity should be at 60-70% for the first 3-4 sessions. As patient’s tolerance for exercise improved, the duration of walking increase gradually and the target is increased by 5-10% of the maximum heart rate. After 4 weeks of training, exercise intensity achieve a level of 80% of maximum heart rate. Similarly Gimenez et al., [24] confirmed that high training intensity is required to elicit physiologic training effects. While previous studies were not fully consistent with this, Clark and colleagues [25] examined the efficacy of low-intensity isotonic exercises of the upper and lower extremities performed at home in a group of 40 patients with COPD. They demonstrated a dramatic improvement in treadmill walking time and suggested that their program would be applicable in patients with COPD with a wide range of functional defects.

The present study demonstrated that short term aerobic training program (6-8 weeks) didn’t show a statistically significant improvement in BMI due to while Stav and co-workers [26], found that prolonged pulmonary rehabilitation program (three years) may improve BMI. Measurement of BMI may not accurately reflect changes in body composition in these patients and that measurement of FFM (fat free mass) may be required to estimate body cell mass [27].

The present study showed that, there was a statistically significant improvement of both FEV1 & FVC physiological parameters of study group
when compared with control group. Gohar [28],
was fully consistent with this, he found that there
was a significant improvement of both FVC and
FEV1 parameters in COPD patients undergo lower
limb exercise for 6 weeks. While Stav and co-
workers [26], demonstrated that outpatient pro-
longed pulmonary rehabilitation program (three
years) didn’t improved FEV1, but has an impor-
tant beneficial impact on the rate of FEV1 decline. In
addition, it increased endurance time and work.
As the result PR should be considered as a disease
modifier [29].

The present study showed that 6MWD increased
by 78 meters after 6-8 weeks of aerobic training
program. This improvement was statistically sig-
nificant. Redelmeir et al., [30] suggested that the
minimal clinically meaningful increase in the
6MWD is about 54 meters. While De-Torres et al.,
[31] demonstrated that the mean improvement in 6
MWD was 65m after 6-8 weeks of PR. Other study
noted an increase of 78.41m in 6 MWD after six
months of outpatient and home-based program
[32].

The present study used BODE index to evaluate
the effect of aerobic training. It demonstrated that
13 patients or 86.6% showed a statistically signif-
icient improvement in BODE score after 6-8 weeks
of training program in contrast, control group
showed 9 patients or 60% improvement after 6-8
weeks of optimal medical treatment. This finding
supports the use of this multi-dimensional index
to evaluate the effects of pulmonary rehabilitation,
as has been in study done by Cote & Celli [33].

Although PR has minimal effect on lung func-
tion, it improves dyspnoea [34], exercise capacity,
and healthcare resource utilization [35]. Two of
these outcomes, dyspnoea and exercise capacity,
are components of the BODE index. As such, the
BODE index could be used to evaluate the effect
of PR. For this Cote & Celli [33]. Defined one unit
change in BODE as being clinically significant,
because it implies a change in any of its component
of a magnitude large enough to influence clinical
outcomes. Indeed, one unit change in the Modified
Medical Research Council scale predicts mortality
[36]. Likewise, one unit change in the 6MWD in
the BODE score far exceeds the 50m considered
to be clinically significant changes for this test
[30]. Similarly, one unit change in the FEV1 com-
ponent of the BODE index reflects the thresholds
that have been accepted by the ATS/European
Respiratory Society and Global Initiative for Chron-
ic Obstructive Lung Disease (GOLD) as the basis
for the physiological staging of COPD [37,38].

In conclusion the present study found that short-
term aerobic training program has the capacity to:
Break out the vicious circle of dyspnea, increasing
inactivity and exercise intolerance-the hallmark
features of COPD patients and improve some com-
ponents of BODE index supporting the use of this
multi-dimensional index to evaluate the effects of
pulmonary rehabilitation. This findings suggested
that even if the program duration does not exceed
8 weeks, it can still benefit patients with COPD.
Further studies are needed to compare the respon-
siveness of used outcome measures after pulmonary
rehabilitation, detect the best practical tools to
evaluate the responsiveness to PR, and to detect
the best predictor of survival following pulmonary
rehabilitation.

References
1- Global Initiative for Chronic Obstructive Lung Disease
(GOLD) update: Global strategy for the diagnosis, man-
agement and prevention of chronic obstructive pulmonary
disease, 2008.
2- CIOBANU L., PESUT D., MILOSKOVIC V. and PETRO-
VIC D.: Current opinion on the importance of pulmonary
rehabilitation. Chinese Medical Journal, 120 (17): 1539-
1543, 2007.
3- American thoracic society/european respiratory society:
ATS/ERS statement on pulmonary rehabilitation. Am. J.
4- RIES A.L., MAKE B.J. and REILLY J.J.: Pulmonary
rehabilitation in Emphysema. The proceedings of the
5- GUYATT G.H., BERMAN L.B., TOWNSEND M., PUGS-
LEY S.O. and CHAMBERS L.W.: A measure of quality
of life for clinical trials in chronic lung disease. Thorax.,
6- HILL N.S.: Pulmonary rehabilitation. The Proceedings
7- SINGH S.J., MORGAN M.D.L., SCOTT S., WALTERS
D. and HARDMAN A.E.: Development of a shuttle walk-
test of disability in patients with chronic airways
8- GUYATT G., KELLER J., SINGER J., HALCROW S.
and NEWHOUSE M.: Controlled trial of respiratory
muscle training in chronic airflow limitation. Thorax.,
10- JONES P.W., QUIRK F.H., BAVEYSTOCK C.M. and
LITTLEJOHNS P.: A self-complete measure of health
status for chronic airflow limitation the St. George’s 2
Respiratory Questionnaire. Am. Rev. Respir. Dis., 145:
11- CELLI B.R., COTE C.G., MARIN J.M., CASANOVA
C., MONTES DE OCA M., MENDEZ R.A., PINTO
PLATA V. and CABRAL H.J.: The body-mass index,
airflow obstruction, dyspnea and exercise capacity index
in chronic obstructive pulmonary disease. N. Engl. J.


Upper Gastrointestinal Mucosal Changes in Patients with Congestive Heart Failure

ZAIN E.A. SAYED, M.D.1; MOHAMMAD ABDEL-GHANY, M.D.2; LOBNA ABDEL-WAHID, M.D. 1 ELHAM A. HASSAN, M.D.3 and KHALED M. ATTALLAH, M.D. 4

The Departments of Internal Medicine1, Cardiology2, Tropical Medicine3, Assiut University and Tropical Medicine & Gastroenterology4, National Liver Institute, Menoufia University, Egypt

Abstract

Introduction: Congestive heart failure increases systemic venous pressure which is transmitted to the inferior vena cava and the hepatic veins, this may induce gastro-intestinal changes. This research aimed to study gastro-intestinal tract changes in patients with congestive heart failure.

Aims and Methods: 120 patients with congestive heart failure (CHF) presenting with gastro-intestinal symptoms underwent upper endoscopy. All patients underwent echocardiography to determine the ejection fraction and the degree of tricuspid regurgitation and pulmonary hypertension. Abdominal ultrasound was done to measure the diameters of the hepatic veins, the inferior vena cava, and the portal vein for which pulsatility index was assessed.

Results: Gastric mucosal changes were present in 106 (88.4%), duodenal mucosal changes in 71 (59.2%), and esophageal mucosal changes in 3 (2.5%) patients. Gastric mucosal changes were the following: Mosaic-like pattern (n=92, 76.7%), punctate spots (n=73, 60%), thickened folds (n=20, 16.7%), watermelon stomach (n=8, 6.7%), and telangiectasia (n=35, 29.2%). Duodenal mucosal changes were the following: Mosaic-like pattern (n=58, 48.4%), thickened folds (n=17, 14.2%), and telangiectasia (n=7, 5.9%). Gastrointestinal symptoms were significantly associated with gastropathy and duodenopathy (<0.001). There was a positive correlation between the degree of gastro-intestinal symptoms and gastropathy and duodenopathy (Gamma=0.6, p=0.03 and 0.5, p=0.04 respectively). Patients with gastropathy and duodenopathy had higher mean inferior vena cava (IVC) and hepatic vein diameters than those without gastropathy and duodenopathy. Low EF was associated with increased portal vein, IVC and hepatic vein diameters, (p=0.02, 0.008, 0.002) respectively. Moreover it was associated with gastro-intestinal symptoms, gastropathy and duodenopathy (p<0.001). There was a positive correlation between the ejection fraction and severity of gastro-intestinal symptoms (r=0.6, p<0.001). Tricuspid regurgitation was associated with gastro-intestinal symptoms, stomach gastropathy, diameter of hepatic vein and IVC (p=0.007, 0.019, <0.001, <0.001). Mean pulsatility index in patients in the present study was 0.7±0.53 and there was positive correlation between pulsatility index and Pulmonary Artery Systolic Pressure (PASP) (r=0.61, p=0.02). Patients with low ejection fraction have a higher pulsatility index than patients with higher ejection fraction (0.7±0.67, 0.6±0.18, p=0.26).

Conclusion: CHF is associated with gastro-intestinal changes which are significantly associated with the severity of congestive heart failure.

Key Words: Congestive heart failure – Duodenopathy – Gastropathy – Pulsatility index.

Introduction

CONGESTIVE changes in the gastric mucosa were first described by McCormack et al., in patients with portal hypertension (portal hypertensive or congestive gastropathy) [1]. Similar congestive mucosal changes also have been described in the duodenum, jejunum and colon [2]. Chronic heart failure (CHF) is a multi-organ disease with increasing evidence for the involvement of the gastrointestinal (GI) system in this syndrome. In CHF, the increased systemic venous congestion is readily transmitted to the inferior vena cava (IVC) and the hepatic veins (HV) and hepatic sinusoids [3]. Increased sinusoidal pressure, leads to an increase in the resistance to blood flow in the portal vein (PV) and hereby to congestion in its draining territories this leads to congestion of GIT mucosa with several structural changes [4]. The aim of this study to define gastro-intestinal tract changes in patients with CHF, assessed with upper endoscopy and abdominal ultrasound.

Patients and Methods

This was a cross-sectional prospective study of patients with CHF that were selected from Assiut University Hospitals’ based Gastroenterology and Cardiology departments during a period from March 2012 to March 2013. The study was ap-
proved by the regional Ethical Committee of AUH and informed consent was obtained from all the participants before enrollment.

**Study population:**

Adult 120 patients with CHF and having gastrointestinal (GI) symptoms (e.g. abdominal pain, heartburn, nausea, vomiting, severe anorexia or weight loss) assessed by the Patient Assessment of Gastrointestinal Disorders- Symptom Severity Index (PAGI-SYM) (reference) were included in the study [5].

Heart failure defined as patients with symptoms typical of heart failure: (Breathlessness at rest or on exercise, fatigue, tiredness, ankle swelling), signs typical of heart failure: (Tachycardia, tachypnoea, orthopnoea, paroxysmal nocturnal dyspnea, pulmonary rales, pleural effusion, raised jugular venous pressure, peripheral oedema, hepatomegaly) and objective evidence of a structural or functional abnormality of the heart at rest (cardiomegaly, third heart sound, cardiac murmurs, abnormality on the echocardiogram) [6].

Patients with haemodynamic instability, positive hepatitis markers (HBsAg, HCVAb), chronic liver disease of non cardiac origin, H. pylori infection and those received medications affecting GIT mucosa e.g. NSAIDs were excluded from this study.

All patients were subjected to the following:

- Full clinical history and examination.
- Transthoracic echocardiography was done to diagnose any structural heart disease, assess LV dimensions and function, assess degree of tricuspid regurgitation (TR) and PASP.
- Trans-abdominal ultrasonography to assess the following:
  - Maximum width (in mm) of the distal part of the main trunk of the left, the middle, and the right HVs, just proximal to their entry into the IVC (during shallow inspiration after at least 5 minutes of rest).
  - Maximum transverse diameter of the IVC just above its confluence with the HVs (with breathing held in expiration).
  - Maximum width of the PV at the porta-hepatis.
- Assessment of liver and spleen status and the presence of vascular collaterals.
- Portal vein Pulsatility index (PI) calculated as follows: (Peak maximum velocity) - (Peak minimum velocity) / (Peak maximum velocity) using ultrasonic Doppler.

**Upper Endoscopy:**

Endoscopic changes were classified by using a modification of the grading system for portal hypertensive gastropathy [7]: Grade 0, normal-appearing mucosa; grade 1 (mild), mosaic like pattern and/or fine punctate speckling of the mucosa; grade 2 (moderate), blunted and thickened folds and/or telangiectasia; and grade 3 (severe), cherry red spots and/or diffusely or linearly hyperaemic mucosa.

**Statistical analysis:**

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS-version 17). Continuous data were expressed as Means ± Standard deviation (SD) and compared using Student t-test. Categorical variables were expressed as percentage and compared using chi-square (χ²) test. Correlations were assessed by the Spearman rank correlation coefficient among continuous variables and Gamma statistics for ordinal variables. p-values of less than 0.05 were considered significant.

**Results**

**Characteristics of the study population:**

The baseline demographic and clinical characteristics of 120 studied patients with CHF are summarized in table 1 where the mean age of the patients was 33.4±8.8 years and males constituted 61.7%. The majority of cardiac patients presented with severe GIT symptoms (41.7%). Rheumatic heart disease was the commonest cause for these cardiac patients (44.2%) and 38.3% of them had severe degree of tricuspid regurge.

| Table (1): Demographic and clinical characteristics of the study patients. |
|-----------------------------|-----------------------------|
| Patients (n=120)            | No (%)                     |
| Male                        | 74 (61.7)                  |
| Age                         | 33.4±8.8                   |
| Git symptoms *:             |                             |
| Mild                        | 21 (17.5)                  |
| Moderate                    | 49 (40.8)                  |
| Severe                      | 50 (41.7)                  |
| Causes of heart failure:    |                             |
| Dilated cardiomyopathy      | 13 (10.9)                  |
| Ischemic cardiomyopathy     | 49 (40.8)                  |
| Peripartum cardiomyopathy  | 5 (4.2)                    |
| Rheumatic heart disease     | 53 (44.2)                  |
| Degree of tricuspid regurge:|                             |
| No regurge                  | 6 (5)                      |
| Mild                        | 40 (33.3)                  |
| Moderate                    | 28 (23.3)                  |
| Severe                      | 46 (38.3)                  |

* Assessed by the patient assessment of gastrointestinal disorders-symptom severity index (PAGI-SYM).
Endoscopic findings:
The endoscopic findings were listed in Table (2) and Fig. (1). Gastric mucosal changes (gastro- 
opathy) were present in 106 (88.4%) patients, where, mosaic like pattern lesions were the com-
monest endoscopic changes. In addition, duodenal mucosal changes (duodenopathy) were found in 
71 (59.2%) patients, and esophageal mucosal changes in 3 (2.5%) patients that were the least 
endoscopic changes. Some patients had more than one endoscopic lesion.

Table (2): Endoscopic findings in the study patients.

<table>
<thead>
<tr>
<th>Endoscopic lesions*</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophageal changes:</td>
<td></td>
</tr>
<tr>
<td>Linear veins in distal third</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Varices</td>
<td>0</td>
</tr>
<tr>
<td>Gastric changes:</td>
<td></td>
</tr>
<tr>
<td>Mosaic-like pattern</td>
<td>92 (76.7)</td>
</tr>
<tr>
<td>Thickened folds</td>
<td>20 (16.7)</td>
</tr>
<tr>
<td>Punctuate spots</td>
<td>73 (60)</td>
</tr>
<tr>
<td>Watermelon stomach</td>
<td>8 (6.7)</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td></td>
</tr>
<tr>
<td>Duodenal changes:</td>
<td></td>
</tr>
<tr>
<td>Mosaic-like pattern</td>
<td>58 (48.4)</td>
</tr>
<tr>
<td>Thickened folds</td>
<td>17 (14.2)</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>7 (5.9)</td>
</tr>
</tbody>
</table>

* The patient may have one or more endoscopic lesion(s).

Patients with moderate and severe upper-GI symptoms had significantly more gastropathy and 
duodenopathy than those with mild symptoms (p<0.00 1 for both). In addition, there was a positive 
correlation between the degree of severity of upper-GI symptoms and severity of gastropathy and 
duodenopathy (Gamma=0.6, p=0.03 and 0.5, p=0.04 respectively).

Ultrasonographic (dopplar) and Echocardio-
graphic findings:
The mean PI in patients in our patients was 0.7±0.53. There was positive correlation between 
PI and PASP (r=0.61, p=0.02). Patients with low EF have a higher PI than patient higher EF but 
without statistical significance (0.7 ±0.67 vs. 0.6±0.18, p=0.26).

Comparison between GIT symptoms, 
endoscopic lesions and ultrasonographic and echocardiographic findings:
Cardiac patients with moderate and severe upper-GI symptoms had higher mean inferior vena 
cava and hepatic vein diameters than those with mild symptoms indicating that hepatic vein and 
IVC diameters were significantly associated with the severity of upper-GI symptoms (p<0.001 for 
both). Despite portal vein diameter was not significantly altered it showed a tendency toward in-
creased values with severity of upper-GI symptoms in patients with CHF Table (3).

Additionally, cardiac patients with gastropathy and duodenopathy had higher mean inferior vena 
cava and hepatic vein diameters than those without gastropathy and duodenopathy. Tables (4,5).

Regarding echocardiographic changes, low EF was associated with increasing severity of upper-
GI symptoms and the presence of gastropathy and duodenopathy (p<0.001 for all). Moreover, it was 
associated with larger portal vein, IVC and hepatic vein diameters, (p=0.02, 0.008, 0.002) respectively. 
On the other hand, Tricuspid regurgitation was associated with upper-GI symptoms, stomach 
changes, and increased hepatic vein and IVC diameters (p=0.007, 0.019, <0.001, <0.001 respec-
tively).

Table (3): Relation between GIT symptoms and ultrasonog-
raphic findings.

<table>
<thead>
<tr>
<th>Patients with mild symptoms (n=21)</th>
<th>Patients with moderate and severe symptoms (n=99)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC diameter</td>
<td>13.4±1.4</td>
<td>19.3±4.9</td>
</tr>
<tr>
<td>Hepatic vein diameter</td>
<td>8.3±1.5</td>
<td>9.7±1.7</td>
</tr>
<tr>
<td>Portal vein</td>
<td>9.3±1.5</td>
<td>9.4±1.5</td>
</tr>
</tbody>
</table>

Table (4): Ultrasonographic findings in patients with and 
without gastropathy.

| Patients with Patients without gastropathy |
|-------------------------------------------|------------------------------------------|
| Gastropathy (n=106) | Gastropathy (n=14) | p |
| IVC diameter | 19.2±4.8 | 13.4±1.3 | <0.001 |
| Hepatic vein diameter | 9.8±1.7 | 8.4±1.6 | 0.004 |

Table (5): Ultrasonographic findings in patients with and 
without duodenopathy.

| Patients with Patients without duodenopathy |
|---------------------------------------------|---------------------------------------------|
| Duodenopathy (n=71) | Duodenopathy (n=49) | p |
| IVC diameter | 20.4±4.8 | 16.5±4.3 | <0.001 |
| Hepatic vein diameter | 10.2±1.8 | 8.9±1.5 | <0.001 |
Fig. (1-A): Esophageal linear veins.  

Fig. (1-B): Gastric mosaic like pattern.  

Fig. (1-C): Thickened gastric folds.  

Fig. (1-D): Gastric telangiectasia.  

Fig. (1-E): Watermelon stomach.  

Fig. (1-F): Duodenal mosaic-like pattern.  

Fig. (1-G): Duodenal thickened folds.  

Fig. (1-H): Duodenal telangiectasia.  

Fig. (1): Endoscopic view showing different aspects of congestive mucosopathy.
Discussion

The present study assessed the frequency of upper gastrointestinal mucosal changes in patients with CHF, where gastropathy were the most common finding followed by duodenopathy.

With agreement with Raja et al., [8] we found no patient had esophageal varices; only 3 patients (2.5%) have dilated veins that do not meet the criteria of esophageal varices Previous studies showed that low frequency of esophageal varices in patients with CHF ranged from 1.2% to 6.7% [9-11].

The absence of cardiac cirrhosis may explain the lack of esophageal varices in the present study patients since high porto-systemic pressure gradient is required for the formation of esophageal varices that is not present in cardiac cirrhosis [8].

Gastric mucosal changes observed in the present study were a mosaic-like pattern, thickened mucosal folds, watermelon stomach, punctate spots and telangiectasia. These results were compatible with previous studies that demonstrated several abnormalities of gastric mucosa in mosaic pattern including antral vascular ectasia, mucosal thickening and areas of telangiectasias [8,12]. These changes were similar to portal hypertensive gastropathy [13,14].

In this study, the duodenal findings were mild changes (mosaic-like pattern) that were the most common findings, followed by moderate changes (thickened mucosal folds, telangiectasia) and none had severe changes. Duodenopathy was less frequent than gastropathy. This might be clarified by the compact mucosal layer of the stomach with interruption of the area gastricae appearance with congestion as mosaic like. Similar changes are infrequent in the duodenum because of the supporting tissue in the lamina propria and the absence of the area gastricae. These results were consistent with Raja et al., [8] who found the common duodenal changes were mild but less frequent than gastropathy.

In our study, these changes “congestive mucosopathy” in patients with CHF were similar to those caused by portal hypertension. However, a portosystemic venous pressure gradient exists in CHF, is much lower than that which occurs with cirrhosis. In congestive heart failure, the increase in systemic venous pressure is transmitted to the portal circulation via the hepatic venous bed and thus mucosal congestion is to be expected [4,15].

Patients with CHF have an increased splanchnic fluid volume, an important factor in maintaining splanchnic venous hypertension [6].

In addition, the low cardiac output state and arterial hypoxemia that occur in CHF might contribute to splanchnic hypoperfusion leading to GI mucosal changes [16].

We found that patients with moderate and severe upper-GI symptoms had significantly higher frequency of gastropathy and duodenopathy. Increasing severity of upper-GI symptoms was associated with increasing gastropathy and duodenopathy severity and increasing diameter of hepatic veins and IVC. However, severity of symptoms was not associated with portal vein diameter in our study. One possible explanation for this observation is that medication received by patients to treat CHF e.g. nitrites may control portal pressure.

In this study, these cardiac patients had higher portal vein pulsatility index (PI) that was positively correlated with PASP. These results agreed with Shih et al., [17] who reported that patients with high right atrial pressure representing right sided cardiac congestion had a high PI. Furthermore, the waveform changes of portal blood flow correlate well with right heart function. So, with the agreement with Hu and colleagues [18], the measurement of portal vein PI change is a simple and non-invasive method to identify right heart failure and is helpful for the diagnosis of stagnant or hepatofugal portal blood flow.

Regarding cardiac changes, degree of severity of tricuspid regurgitation was associated with gastrointestinal symptoms, stomach changes, and increased diameter of hepatic vein and IVC diameters. This can be explained by that with increasing severity of tricuspid regurgitation, the right ventricular systolic pressure is more efficiently transmitted to the systemic veins and the portal circulation; possibly, this leads to more congestive changes [19]. In addition, low EF was associated with larger IVC, hepatic vein and portal vein diameters manifested as systemic and splanchnic congestion thus, a more severe effect on the mucosa and consequently increasing severity of GIT symptoms, gastropathy and duodenopathy.

In Conclusion:

Congestive mucosopathy “gastropathy and duodenopathy” is common in patients with CHF. Severity of this finding is related to severity of hepatic congestion caused by tricuspid regurgitation and or right ventricular dysfunction that is the predominant mechanism. The measurement of
portal vein PI is an indirect non-invasive method of right ventricular dysfunction.

Conflicts of interest:
The authors declare that they had no conflicts of interest concerning this article.

References
The Effect of Early Versus Late Start of Minimal Enteral Nutrition on Clinical Outcomes of Parenterally Fed Preterm/Very Low Birth Weight Infants

RABAB E.H. EL-SAYED, D.N.Sc.
The Department of Pediatric Nursing, Faculty of Nursing, Mansoura University

Abstract

Background: Although parenteral nutrition has been used widely in management of preterm/very low birth weight infants admitted to the Neonatal Intensive Care Units (NICUs), a smooth transition to enteral feedings is desirable, with concerns about nutrient intolerance and the risk of necrotizing enterocolitis (NEC). This study aims to compare the effects of early with late start of minimal enteral feedings (MEN) on specific and non-specific clinical indicators of NEC in parenterally fed preterm/very low birth weight infants, as well as their clinical outcomes; including time to reach full enteral feedings, weight gain, glucose metabolism, use of phototherapy, and length of hospital stay.

Methods: A quasi-experimental design was used. A representative sample of 65 preterm (PT) and very low birth weight (VLBW) infants who admitted to the NICU affiliated to El-Mansoura University Children’s Hospital from May 1st to 31st of October 2008 was randomly divided into intervention and control groups. Parenteral nutrition (PN) is initiated routinely in the first 24 hours of life for both the study two groups. Then, from day 2 to day 7 PNP, PT infants in the intervention group received trophic feeds of 12-24ml/kg/day of expressed breast milk (EBM) or preterm formula in addition to the estimated amount of PN, while infants in the control group received only PN according to the NICU feeding protocol (110-120 Kcal/kg/day). The effects of early (on day 2 NP) and late start (after day 7 PNP) of MEN on the clinical outcomes were compared between the two groups.

Results: Neonates in the control group were significantly more likely to show specific and non-specific clinical indicators of NEC. The mean days to reach full enteral feedings were significantly fewer for the intervention group (p<0.001), which also significantly reduced the time of their hospital stays (p<0.001). Moreover, Only 40% of neonates in the intervention group developed hyperbilirubinemia compared with 60% in the control group, but this difference was not significant (p=0.121).

Conclusion: Early start of MEN for PT and VLBW infants shortens the time to reach full enteral feedings, improves daily weight gain, produces fewer episodes of feeding intolerance, has beneficial effects on glucose and bilirubin metabolism, and shortens the time of hospital stay than late start, even though caloric intake was equivalent among both groups. The study recommended providing PT and VLBW infants with fixed amount of MEN from day 2 to day 7 PNP.

Key Words: Preterm infants – Low birth weight – Minimal enteral nutrition – Parenteral nutrition – Necrotizing enterocolitis.

Introduction

As more very immature preterm infants survive, provision of early nutrition intervention, both parenteral and enteral, is becoming a standard of care in many neonatal intensive care units (NICUs) [1]. However, nutritional practices vary dramatically worldwide among NICUs [2]. Preterm infants have immature gastrointestinal tracts (including immature motility patterns and delayed transit), so enteral feeding is often delayed by days after birth as a protective strategy for fear of feeding intolerance and to reduce the risk of necrotizing enterocolitis. However, some degree of luminal nutrient exposure is essential to prevent intestinal mucosal atrophy [3].

Lack of enteral nutrients may diminish gastrointestinal functional and structural integrity by diminishing hormonal activity, growth of intestinal mucosa, and nutrient absorption. A prolonged delay in starting feeds in preterm neonates may be partly responsible for the common problem of feeding intolerance in these tiny newborns [4]. These problems may then hinder the transition from parenteral to enteral nutrition, and thus prolonged hospital stay [5]. Starvation or the prolonged absence of enteral nutrition disrupts the barrier functions of the gastrointestinal tract resulting in gut atrophy [6,7].
Although many aspects of gastrointestinal function are immature in preterm infants, the ability of preterm neonates to absorb enteral nutrients appears to be adequate to sustain their nutritional needs [1]. Early provision of minimal enteral nutrition (MEN) has been advocated as a supplement to parenteral nutrition in the NICU population to mature the gut faster and make it more receptive to subsequent enteral feeds [8]. There is no well-established definition for MEN, but it generally refers to providing small volume enteral feedings of formula, human milk, or both at the same rate for at least 5 days during the period of parenteral nutrition as a strategy to enhance feeding tolerance and decrease time to reach full enteral feedings. There is no uniform definition for MEN. It is generally refers to small amounts of enteral feedings (5 to 24ml/kg/day) [9,10]. It is also called as Minimal Enteral Feeding (MEF), “TROPHIC”, “NON-NUTRITIVE” feeding, or “PRIMING” because of the role in stimulating many aspects of gut function [11].

Gavage feedings permit full enteral nutrition in preterm infants. However, rapid advancement of feeding by this route can contribute to the development of NEC in many very preterm infants. Thus, the development of complete parenteral nutrition for this age group led to the common practice of withholding enteral nutrition in preterm infants for the first several weeks of life. Subsequent concerns over total parenteral nutrition (TPN) toxicity as well as a growing recognition of the importance of enteral feedings in stimulating growth and development of the gastrointestinal tract prompted a number of studies that demonstrated the benefits and safety of early MEN as a supplement to parenteral nutrition [12].

More studies that are recent have demonstrated the positive direct and indirect trophic effects of MEN on preterm infants, even when administered for brief periods. Direct contact of the gut tissue with milk increases intestinal mass and enhances the synthesis rates of gut hormones. In addition, most of its substances have complex and vital roles in other aspects of gastrointestinal tract function, such as nutrient absorption and digestion [13]. Furthermore, preterm and low birth weight neonates who were fed earlier with minimal feeds were found to have fewer episodes of feeding intolerance and gained weight faster as compared to those who were fed later [14-16]. Despite all these benefits, no previous study has addressed the effect of MEN on preterm infants admitted to the NICUs in Egypt.

**Aim of the study:**

**Therefore, the aims of this study were:**

1- To compare the effects of early and late start of minimal enteral feedings on specific and non-specific clinical indicators of NEC in parenterally fed preterm/very low birth weight infants.

2- To compare the effects of early and late start of minimal enteral feedings on clinical outcomes of parenterally fed preterm infants, including time to reach full enteral feedings, weight gain, glucose metabolism, use of phototherapy, and length of hospital stay.

**Research hypothesis:** Early start of minimal enteral nutrition will improve feeding tolerance, glucose metabolism, and weight gain as well as decrease time to full feedings, use of phototherapy, and length of hospital stay in preterm/very low birth weight infants.

**Material and Methods**

**Research design:**

A quasi-experimental design was used in this study.

**Setting:**

The NICU affiliated to El-Mansoura University Children's Hospital.

**Subjects:**

From May 1st to 31st of October 2008, all preterm infants admitted in level II NICU within the first 24 hours PNP, with a gestational age of 28 to 36 weeks, birth weight from 1000 to 1499 gm, birth weight from 10th to 90th customized centile, and 5-minute Apgar score >5 were eligible to participate in this study.

Exclusion criteria were; major congenital anomalies and infections, signs of genetic syndromes, and gastrointestinal anomalies. Moreover, infants needing exchange transfusion or inotropic drug administration, and those with severe respiratory disorders require ventilation assistance were not included in the study.

**Tools of data collection:**

Data were collected during infants, hospital stays; using medical records, nurses’ charts, enteral nutrition sheet (designed by the researcher), and pulse-oximeter. Gender, medical diagnosis, gestational age (wks), Apgar scores at one and five minutes, birth weight (g), age at MEF initiation (days), time-to-reach full enteral feedings (≥ 1 50ml/kg), type of diet introduced each feed/day (either
EBM or preterm formula), number of gastric residue aspirate through MEN period (>25%), number of infants with significant episode(s) of abdominal girth increment through MEN period that leading to feeding cessation (>2cm), and enteral feeding withheld (hours) were collected. Moreover, preterm infants, exposure to episodes of hypoglycemia or hyperglycemia (glucose metabolism), desaturation, use of phototherapy (bilirubin metabolism), and possible morbidity and mortality related to early feeding (NEC, sepsis episodes), in addition to the duration of hospital stay (days) were documented by the researcher.

**Procedure:**

All preterm/very low birth weight infants entered the study received parenteral nutrition (PN) in the first 24 hours of life (rate=80ml/kg/day glucose 10%). Within 48 hours of life, the infants were randomly assigned to one of two feeding groups. The first group (intervention) received early bolus MEF of expressed breast milk (EBM) or preterm formula; the other group (control) received the same milk type as the intervention group but with bolus delayed (7 days). MEF was administered as 12-24ml/kg/day of full strength milk (20 kcal/30cc) on an interval of 3 hours during the first week of life [17].

Afterwards, feedings were cautiously advanced every 24-h by 15ml/kg, whenever more than 75% of the calculated amount had been tolerated during the previous 24 hours until full enteral feedings (>150ml/kg) were reached [18,19]. Nursing and medical staff were aware of group assignment. All infants received parenteral feeding according to the standard protocol (110-120Kcal/kg/day) [18].

All preterm infants were closely monitored by pulse-oximeter for the non-specific clinical indicators of NEC, which include the incidence of bradycardia and desaturation. Gastric residuals and abdominal distension (specific clinical indicators of NEC) were also monitored as reflection of feeding intolerance. More specifically, gastric residuals were measured every 6-h before the feed by the researcher. At a volume equal to or less than 25% of the previous 6-h feed volume, the scheduled amount was given; otherwise, the difference up to the scheduled amount was given [20].

Feeds were withheld if the volume of gastric residuals exceeded half of the oral intake in the previous 6-h; clinical signs and symptoms suggestive of NEC (sudden increment in the abdominal girth of >2cm, no sufficient amount of stool on a regular basis, or presence of bloody stools in infants’ diaper).

Feedings were temporarily discontinued if sepsis is suspected with abnormal abdominal examination regardless of the gastric residuals. At this time sepsis workup or an abdominal radiograph were performed as appropriate. The feedings were resumed as soon as the abdominal radiograph and sepsis profile was normal. An abnormal abdominal examination was defined as; persistent palpable mass (predominantly in the right lower quadrant); gross abdominal distention; persistent visible bowel loops without peristalsis; abdominal tenderness; decreased abdominal bowel sounds.

The clinical outcome variables including, time to reach full enteral feedings, weight gain, glucose metabolism, use of phototherapy, and length of hospital stay were analyzed to compare between the study two groups.

**Validity and reliability:**

An Expert Panel composed of 3 pediatric medicine professors with their specialty in neonates, including the head of the study setting provided scientific oversight and direction for all aspects of the study protocol. The final research methodology was reviewed by the Expert Panel. On completion of the research plan, the procedure was approved and data collection took its place under Panel supervision and continuous evaluation of the outcomes.

**Pilot study:**

A pilot study will be carried on six preterm/very low birth weight infants (10% of the study sample) who will not be included in the study to test the validity and reliability of the study procedure. Accordingly, any modification will be done to keep the tool more applicable.

**Ethical consideration:**

Because there is no ethics committee of our hospital, all mothers/fathers provided signed informed consent before enrollment. Parents were assured that their infants, participation was voluntary and that they could withdraw from the study at any time without giving any reason. Furthermore, the sample population were equally take the chance of being recruited to the intervention group (if they are admitted in single numbers’ incubators), or to the control group (if they are admitted in paired numbers, incubators).

**Data analysis:**

Statistical analysis was performed using the SPSS software (version 14.0). Variables were presented as number and percent. Continuous variables including, time to reach full enteral feedings, weight
gain, and length of hospital stay were compared by \( t \)-test or by Mann-Whitney U test based on their distribution. The rest of clinical outcomes including, glucose metabolism, and use of phototherapy, were compared by Fisher exact test or chi-square test, based on the cell size. A \( p \)-value ≤0.01 was considered statistically significant.

**Results**

Data collected about 65 preterm/very low birth weight infants were analyzed. Sixty infants were studied, thirty of them (intervention group) received early enteral feeding from day 2 to day 7 PNP (median age: 2 days, range: 1 to 5 days), and another thirty (control group) delayed (median age: 7 days, range: 6 to 14 days) MEF.

Five infants were excluded from the study sample as they died <5 days after birth, two were in the intervention group and three were in the control group.

Patient characteristics were similar in the study two groups, with no statistical significant differences regarding gender, medical diagnosis, gestational age (wks), birth weight (g), Apgar scores at one and five minutes, and age at MEF initiation (days), as \( p = 0.796; p = 0.592; p = 0.863; p = 0.935; p = 0.243; p = 0.190; \) and \( p = 0.03 \) respectively (Tables 1, 2).

![Table 1: Characteristics of the study two groups.](image)

Despite neonates of early and late start groups were fed with similar milk type (EBM/preterm feed = 5/8 and 6/8 respectively) throughout the period of providing MEN, there were statistical significant differences between the study two groups in relation to specific symptoms (abdominal girth increment >2cm, and presence of gastric residue from the previous feed) and non-specific symptoms (incidence of bradycardia, and desaturation) of NEC. Neonates in the control group were significantly more likely to show abdominal girth increment of >2cm once or more through the period of providing MEN than the intervention group (\( \chi^2=14.067, p<0.001 \)). Furthermore, all the studied neonates (100%) of the control group compared with 70% of the intervention group had gastric residue from the previous feed through the period of MEN administration, which revealed a statistical significant difference between the study two groups (\( \chi^2=10.588, p=0.001 \)). In addition, the intervention group were significantly less likely to have desaturation events than the control group, with no statistical significant difference between neonates of both groups regarding occurrence of episodes of
bradycardia throughout the period of providing MEN ($\chi^2=4.043$, $p=0.04$, and $\chi^2=6.667$, $p=0.01$ respectively) (Table 3).

Table (4) shows that neonates of the intervention group who started early MEN had significantly fewer number (X=1.7 ± 1.5) of aspirated gastric residuals of >25% from the previous feed than those of control group (X=5.8 ± 3.8) who started late MEN. Likewise, the mean number of total hours feeding withheld through the period of MEN due to significant aspirate gastric residuals from the previous feed, or abdominal girth increment of >2cm when measured before next feeding given were significantly less for the intervention group ($t=8.075$, $p<0.001$). The mean number of days to reach full enteral feedings were also fewer among neonates of intervention group (X=11.6 ± 5.5) compare with those in control group (X=18.7 ± 7.0), which revealed a statistical significant difference between the study two groups ($t=4.384$, $p<0.001$).

Table (5) presents that, neonates of the intervention group showed more stability concerning glucose metabolism, as they exposed significantly to fewer episodes of hypoglycemia or hyperglycemia throughout the period of providing MEN than the control group ($\chi^2=37.297$, $p<0.001$, and $\chi^2=12.000$, $p=0.001$ respectively). Only 40% of neonates in the intervention group developed hyperbilirubinemia compared with 60% in the control group, but this difference was not significant ($\chi^2=24.000$, $p=0.121$).

Table (6) shows that phototherapy was used for a shorter duration of times in the intervention group (X=2.9 ± 0.8) than the control group (X=3.6 ± 1.3), but this difference was not statistically significant ($t=1.617$, $p=0.117$). Regarding duration needed to regain birth weight, daily weight gain, discharge weight, and duration of hospital stay, there were statistical significance differences between neonates of intervention and control groups ($t=7.847$, $p<0.001$; $t=11.691$, $p<0.001$; $t=4.528$, $p<0.001$; and $t=5.088$, $p<0.001$ respectively).

### Table (3): Effect of early versus late start of MEN with respect to specific and non-specific clinical manifestations of NEC among the study two groups.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (n=30)</th>
<th>Intervention (n=30)</th>
<th>Total (n=60)</th>
<th>$\chi^2$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBM/ preterm feed (n/day)</td>
<td>6/8 75%</td>
<td>5/8 62.5%</td>
<td>NA</td>
<td>NA**</td>
<td>3.034</td>
</tr>
<tr>
<td>Abdominal increment &gt;2 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 60%</td>
<td>4 13.3%</td>
<td>22 36.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12 40%</td>
<td>26 86.7%</td>
<td>38 63.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric residue:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30 100.0%</td>
<td>21 70%</td>
<td>51 85%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0 0.000%</td>
<td>9 30%</td>
<td>15 25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of bradycardia:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 20.0%</td>
<td>1 3.3%</td>
<td>7 11.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>24 80.0%</td>
<td>29 96.7%</td>
<td>53 88.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of desaturation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 33.3%</td>
<td>2 6.7%</td>
<td>12 20.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 66.7%</td>
<td>28 93.3%</td>
<td>48 80.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $p \leq 0.01$.  ** Not applicable.

### Table (4): Comparison between both control and intervention groups regarding MEN-related outcomes (gastric residue, feeding withheld, and reach full enteral feeding).

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (n=30)</th>
<th>Intervention (n=30)</th>
<th>$t$-test</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric residue &gt;25% of the previous meal (n)</td>
<td>5.8±3.8</td>
<td>1.7±1.5</td>
<td>4.754</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Total hours feeding withheld (n)</td>
<td>48.00±31.00</td>
<td>17.00±7.00</td>
<td>8.075</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Days to reach full enteral feeding</td>
<td>18.7±7.0</td>
<td>11.6±5.5</td>
<td>4.384</td>
<td>&lt;0.001 *</td>
</tr>
</tbody>
</table>

* $p \leq 0.01$.  ** Not applicable.
The Effect of Early Versus Late Start of Minimal Enteral

Table (5): Effect of MEN on glucose metabolism and phototherapy of both control and intervention groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of hypoglycemia:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>76.7</td>
<td>0</td>
<td>0.0</td>
<td>23</td>
<td>38.3</td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>23.3</td>
<td>30</td>
<td>100.0</td>
<td>37</td>
<td>61.7</td>
<td>37.297</td>
<td></td>
</tr>
</tbody>
</table>

| Incidence of hyperglycemia: |     |     |     |     |     |     |     |         |
| Yes   | 10  | 33.3| 0   | 0.0 | 10  | 16.7|     | 0.001*  |
| No    | 20  | 66.7| 30  | 100.0| 50  | 83.3| 12.000|         |

| Use of phototherapy: |     |     |     |     |     |     |     |         |
| Yes   | 18  | 60.0| 12  | 40.0| 30  | 50.0| 2.400| 0.121   |
| No    | 12  | 40.0| 18  | 60.0| 30  | 50.0|     |         |

* p≤0.01.

Table (6): Effect of MEN on clinical outcome measures (days under phototherapy, days to regain birth weight, daily weight gain & discharge weight) of both control and intervention groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (n=30)</th>
<th>Intervention (n=30)</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X±SD</td>
<td>X±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean duration under phototherapy (days)</td>
<td>3.6±1.3</td>
<td>2.9±0.8</td>
<td>1.617</td>
<td>0.117</td>
</tr>
<tr>
<td>Mean duration needed to regain birth weight (days)</td>
<td>11.3±3.3</td>
<td>5.3±2.6</td>
<td>7.847</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean daily weight gain (g/day)</td>
<td>15.2±2.9</td>
<td>25.2±3.7</td>
<td>11.691</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean discharge weight (g)</td>
<td>1813.5±51.8</td>
<td>1875.7±54.5</td>
<td>4.528</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean duration of hospital stay (days)</td>
<td>32.9±9.4</td>
<td>22.6±5.8</td>
<td>5.088</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*p≤0.01.

Discussion

Determining the best method for feeding preterm/VLBW infants admitted to the NICUs remains challenging. Preterm infants often experience feeding difficulties primarily because of immaturity of their gastrointestinal systems [20]. It has been well established that inadequate provision of enteral nutrition in very low birth weight neonates leads to suboptimal growth at discharge [21]. Therefore, this study was aimed to demonstrate the potential benefits of early introduction of MEN on stable, preterm/VLBW infants.

Results of the present study suggested that early administration of MEF might not have a significant effect on the incidence of specific and non-specific clinical indicators of NEC or feeding intolerance in the most vulnerable of prematurely born infants. This may be due to the study-setting trend, which encouraged and mainly stressed on the use of maternal EBM in most feeding boluses introduced to preterm infants. Other conducted studies agreed with this opinion, reported that breast milk has been shown to offer protection against NEC [22,23], and the researcher was reassured to observe that even in our early group, more than three fifths of infants received their mother’s breast milk at the initial feed.

On the same context, systematic reviews comparing early versus delayed initiation of progressive enteral feedings indicated that, early enteral feeding may stimulate the premature intestinal tract, promoting intestinal adaptation and consequently improve feeding tolerance later [24,25]. According
to Cobb, Carlo, and Ambalavanann smaller gastric residuals aspirated from the previous feed during the period of providing MEN are indicative of reduced risk of NEC [26].

However, and unlike these studies, findings, the incidence of feeding intolerance between another study’s two groups did not differ [20,27]. Similarly, other studies declared that the gradual recovery of intestinal perfusion during the first days of preterm infants, life provides a sound rationale for a modest delay in enteral feeding [28-30]. Meta-analysis did not demonstrate a significant effect on feed tolerance, weight gain, or NEC [31].

Other related benefits of starting feeds earlier for the intervention group who participated in the present study were a significant shorter time than the control group to establish full enteral feeding independently of parenteral nutrition (p<0.001). This may be related to the fact of a limited number of hours/days that feedings were held due to clinical signs of feeding intolerance. Although one trial reported that trophic feeding reduced the time taken to establish full enteral nutrition [14], this was not confirmed in the other trials [32-34]. Furthermore, early scheduled minimal enteral feedings was related to earlier discharge from the NICU among preterm infants of the intervention group than those of the control group who started MEN lately (p<0.001). This is consistent with many previous studies [35-37], and may be interpreted in relation to the faster growth due to better total caloric intake compared with those who received only PN without enteral feedings.

Similar to the findings of Danielle et al., the early-and late-fed groups in this study did not differ significantly in duration of phototherapy (p=0.117) [38]. The finding of the current study does also agree with the study of Sallakh-Niknezhad et al., who found a decreased incidence of direct and indirect hyperbilirubinemia among preterm infants who experienced early MEN [36].

Another interesting finding is that although the intervention group showed no episodes of hypoglycemia or hyperglycemia during MEN, more than three-fourths and about two thirds of the control infants showed hypoglycemic or hyperglycemic episodes respectively. This is consistent with the findings of Ekblad, Kero & Takala, who found that infants who received earlier nutrition support showed trends toward a lower incidence of elevated serum blood glucose [39]. Donovan, et al., added that limited enteral intake predisposes preterm newborns to hypoglycemia [2]. Moreover, Wang, Dorer, and Fleming found that early initiation of enteral feedings is a successful strategy to maintain stable glucose balance in premature infants [40].

The results showed that, infants in the intervention group regained their birth weight faster than those of the control group, with the results showed a statistical significant difference between the two groups (p<0.001). This is consistent with other previous study [32].

The intervention group showed a significant increase in mean daily weight gain (p<0.001). This translated to heavier discharge weights for survivors, and as a result, this shortens the length of hospital stay. In addition, the early MEN infants experienced positive effects on both discharge weight (1875.7±54.5) compared with only (1813.5±51.8) in delayed MEN group, and significant decline in the duration of hospital stay among the early MEN group compared with delayed MEN group (p<0.001).

Study limitations:

Human milk was not available all the time, so both breastfed and formula-fed were administered formula interchangeably. Furthermore, expressed breast milk should be supplemented with a nutrient fortifier in order to achieve optimal growth in infants weighing less than 1500 g at birth. Unfortunately, breast milk fortifier is not available in Egypt. Moreover, undertaking trials of feeding interventions in preterm/VLBW population is problematic. It is difficult to design a trial in a way that will ensure that caregivers are unaware of the allocated feeding regimen.

Conclusion:

Early start of Minimal enteral nutrition for preterm/very low birth weight infants shortens the time of hospital stay than late start, even though caloric intake was equivalent for both study two groups.

Recommendations:

- Minimal enteral nutrition can be started as early as the second day of life in preterm/very low birth weight infants.
- Future trials should also aim to ensure the participation of extremely low birth weight (ELBW) and extremely preterm infants.
- Further studies about providing minimal enteral nutrition to change trends in feeding policies for ventilated preterm infants in Egypt are needed.
Acknowledgement:
I would like to thank all preterm infants, nurses and the head of the NICU, El-Mansoura University Children’s Hospital, as it would not have been possible to complete this work without their valuable contribution, cooperation, and support.

References


The Protective Role of a Flavonoid “Morin” on the Liver of Streptozotocin-Induced Diabetic Rats

ABEER M. EL-MAHALAWAY, M.D. 1; OLA A. EL-GOHARY, M.D. 2; KHALED ABDULQAWI, M.D. 3 and ODETTE WAHBA, M.D. 4

The Departments of Histology & Cell Biology 1, Physiology 2, Pediatrics 3 and Clinical Pathology 4, Benha and Cairo Universities, Cairo, Egypt

Abstract

Objective: The aim of the this study to evaluate the potential protective effects of morin “a flavonoid” on the liver of streptozotocin-induced diabetic rats subjected to oxidative stress and apoptosis.

Methods: Thirty two healthy young (30 days old) and adult (60 days old) male rats weighing 150-200g were taken for the study and divided into four groups: Group I, control non diabetic group; Group II, morin non diabetic group; Group III, diabetic untreated group and Group IV (diabetic treated group with morin, with each group comprising of 8 rats (n=8). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55mg/kg). Morin was administered orally using an intragastric tube in dose of 30mg/kg daily for 6 weeks. Biochemical estimation of fasting blood glucose, plasma markers of liver function including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and total bilirubin was carried out. The liver of the rats were collected for clinical biochemical analysis, which include measuring the levels of malondialdehyde (MDA) in tissue, and enzymatic antioxidants such as glutathione peroxidase (GSH-Px) and were studied histopathologically by light microscopy as well as by immunohistochemical analysis.

Results: Fasting blood glucose level was highly significantly increased, ALT, AST, ALP and bilirubin level was significantly increased, whereas albumin level was significantly decreased in diabetic untreated group III compared with the control group I. In contrast levels of blood glucos was significantly decreased in morin non-diabetic group II and diabetic group IV treated with morin compared with control group I, whereas the levels of ALT, AST, ALP and bilirubin were significantly decreased and albumin level was significantly increased in diabetic IV treated with morin compared with control group I. The level of MDA was significantly increased, whereas the level of GSH-Px was significantly decreased in diabetic untreated group III compare with the control group I. In contrast the level of MDA was significantly decreased, and the level of GSH-Px was significantly increased in diabetic group IV treated with morin. Histopathological analysis of the liver of Streptozotocin diabetic rats showed pathological changes. However, treatment with morin attenuated the histopathological changes and corrected the biochemical parameters mentioned above.

Conclusion: Morin has a hepatoprotective effect; it has been shown to attenuate the hepatic injury and apoptosis induced by streptozotocin, and has the capacity to scavenge free radicals, protect against oxidative stress, improve antioxidant enzyme activities, and also has antidiabetic efficacy on diabetic rats.

Key Words: Apoptosis – Diabetic rats – Liver – Morin – Streptozotocin.

Introduction

LIVER is a vital organ, has a wide range of functions in the body, including of metabolism of nutrients such as carbohydrates, proteins and lipids, excretion of waste metabolites and detoxification of endogenous and exogenous harmful substance [1]. Diabetes is a common metabolic disorder in humans and characterized by hyperglycemia due to defects in insulin secretion or action or both, which is associated with significant morbidity and mortality, and is a contributor to the development of other diseases [2]. Indirectly or directly, the liver is a major target of insulin action. The onset of diabetes is accompanied by development of major biochemical and functional abnormalities in the liver, including alterations in carbohydrate, lipid, and protein metabolism, and changes in antioxidant status [3]. Diabetes mellitus has high prevalence worldwide and is classified as insulin-dependent and non-insulin dependent diabetes mellitus. Diabetes is induced in experimental animals using drugs such as streptozotocin [4].

Hyperglycemia increases mitochondrial reactive oxygen species (ROS) production, which could represent a key event in the development of diabetes complications [5]. The initial cellular response to high glucose challenge is the generation of ROS,
which rapidly induces apoptotic cell death [6]. Therefore, reinforcing the endogenous and exogenous antioxidant defense system may protect liver injury from oxidative stress [7]. The antioxidant defense can be strengthened with diet rich in antioxidant such as vitamins, flavonoids has been used to prevent the occurrence of many chronic diseases [8,9].

Flavonoids are polyphenolic compounds that can be found in dietary components such as food products, beverages and herbal medicines with different health benefits. Most flavonoids have an antioxidant activity [10].

Morin is one of the naturally occurring bioflavonoids, originally isolated from members of the Moraceae family. It can be found in different herbs and fruits such as onion, seed weeds, mill (Prunus dulcis), fig (Chlorophora tinctoria), almond (P. guajava L.), red wine and Osage orange [11]. Morin exhibited several pharmacological properties including antioxidant [12], anti-inflammatory [13], anticancer [14], and protective effects against chronic disease [15].

The aim of this study to evaluate the potential protective effects of morin “a flavonoid” on the liver of streptozotocin-induced diabetic rats subjected to oxidative stress and apoptosis.

Material and Methods

The present study included thirty-two healthy young (30 days old) and adult (60 days old) male albino rats weighing about 150-200g. It was conducted at Cairo Faculty of Medicine, Cairo University, from October 2011 to May 2012. All rats were kept under normal laboratory environment and fed with a basal diet and liberal supply of water.

Material: Streptozotocin drug (STZ) and morin powder was obtained from Sigma-Aldrich Chemical Co. (St Louis, Missouri, USA). Citrate Buffer (citric acid and sodium citrate in saline at pH 4.5) was obtained from Nile Company (Cairo, Egypt).

Design of the experiments

The animals were divided into four main groups:

Group I: Control nondiabetic group (n=8), in this group the rats were fed a standard rodent chow diet and daily intraperitoneally injected only with citrate buffer [16].

Group II: Morin nondiabetic group (n=8), in which morin was freshly suspended in water and orally administered to rats using an intragastric tube in a dose of 30mg/kg for 6 weeks [10].

Group III: Diabetic untreated group (n=8): Diabetes was induced by a single intraperitoneal injection of 55mg/kg STZ (Sigma-Aldrich Chemical Co.) dissolved in citrate buffer (0.1mol/l, PH: 4.6). The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Fasting blood glucose levels in blood collected from the tail vein were measured 4 days after the streptozotocin injection using a one Touch Glucometer (LifeScan Inc., Milpas, California, USA) to confirm the presence of diabetes. Rats with fasting blood glucose more than 200mg/dl were considered diabetic [17,18].

Group IV: Diabetic group treated with morin (n=8): Five days after induction of diabetes, morin which freshly suspended in water and administered to rats orally using an intragastric tube in dose of 30mg/kg daily for 6 weeks. Morin was initiated 5 days after the administration of streptozotocin, which freshly suspended in water and administered to rats orally using an intragastric tube in dose of 30mg/kg daily for 6 weeks.

At the end of the experiment, the fasted rats were killed by cervical decapitation. The blood was drained from the tail vein using capillary tubes into Eppendorf tubes containing heparin (20ml, 200IU/ml). The plasma was separated by centrifugation (5000 rpm for 5min) and used for biochemical analysis.

Biochemical analysis:

Fasting blood glucose: It was estimated using the glucose oxidase-peroxidase method (GOD-POD kit; Randox Laboratories Ltd, Co. Antrim, Northern Ireland, UK).

Liver function tests: Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and total bilirubin were measured by a standard automated technique using Hitachi Analyzer Model 911 and adequate kits from Roche Co., (Switzerland).

Liver tissues were quickly excised and portions of liver tissues were homogenized in a saline solution (0.9%), centrifuged at 3000 rpm for 15min, and the supernatant was stored at –20 °C and used for the measurement of oxidative stress by determination of malondialdehyde (MDA) [19] and the antioxidant enzymes such as glutathione peroxidase (GSH-Px) [20].

Histopathological evaluation:

Specimens of liver tissues were prepared for both light microscopic and immunohistochemical analysis.
**Light microscopic study:**

Tissue specimens were fixed in 10% neutral buffered formalin embedded in paraffin, and cut in sequential 4-5 µm sections. These sections were stained with hematoxylin and eosin stain (Hx&E) [21] and feulgen reaction for nuclear DNA [22].

**Immunohistochemical analysis:**

Streptavidin peroxidase method for immunohistochemical staining was adopted to test the protein expression of Bcl-xl in the hepatic tissues of the rats. The tissues were cut into slices of 4 µm, and dewaxing hydration was conducted. The first antibody used was the rabbit anti-mouse anti Bcl-xl polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA). The second antibody was labeled by dropping horseradish peroxidase. This was used as a negative control by replacing the first antibody with phosphate buffered saline (PBS). The slices were then coloured with 3,3'-diaminobenzidine (DAB) and then with hematoxylin stain. Finally, the slices were fixed with resin. After Bcl-xl protein expression of hepatic tissues was observed under light microscope, the presence of brown punctate or granular substances in the cells is an indication of positive staining.

**Statistical methods:**

Data were entered on an IBM compatible PC and statistical analysis was carried out using SPSS for Windows (SPSS version 17; SPSS Inc., Chicago, Illinois, USA). Results of descriptive statistical analysis are presented as median and SD. One-way analysis of variance was used to determine significant differences among groups, after which the modified Student’s t-test was used for comparison between individual groups. p-value less than 0.05 was considered statistically significant.

**Results**

Thirty two healthy young (30 days old) and adult (60 days old) male albino rats weighing about 150-200g were included in this study. Table (1) shows the characteristics of the experimental animals, including level of fasting blood glucose and levels of liver injury markers (ALT, AST, ALP and bilirubin), fasting blood glucose level was highly significantly increased in diabetic untreated group III compared with the control group I (p<0.01), but was significantly decreased in morin nondiabetic group II and diabetic group IV treated with morin (p<0.05). ALT, AST, ALP and bilirubin levels were significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but were significantly decreased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III compared with the control group I (p<0.01) but was significantly increased in diabetic untreated group III comparing (TUNEL) staining as described previously [23].
Liver sections from the control group I and morin nondiabetic group II stained with hematoxylin and eosin showed normal appearance of hepatocytes (Fig. 1 A,B). Liver sections from the diabetic untreated group III showed inflammatory cells infiltration in portal area and many hepatocytes having cytoplasmic vacuolization and degenerated nuclei (Fig. 1 C). Liver sections from the diabetic group IV treated with morin showed gradual recovery of hepatocytes with less cytoplasmic vacuolization and degenerated nuclei (Fig. 1D). Liver sections from the control group I and morin nondiabetic group II stained with felugen reaction showed normal vesicular hepatocytes nuclei and numerous intensely stained DNA distributed randomly in the nucleoplasm and along nuclear envelope while the cytoplasm of hepatocytes appeared negatively stained to Feulgen (Fig. 2A). Liver sections from diabetic untreated group III showed hepatocytes nuclei with marked decrease stained DNA in the nucleoplasm and nuclear envelope while cytoplasm of hepatocytes appeared negatively stained to Feulgen (Fig. 2B). Liver sections from the diabetic group IV treated with morin showed most of hepatocytes nuclei with moderately stained DNA distributed randomly in the nucleoplasm and along nuclear envelope while the cytoplasm of hepatocytes appeared negatively stained to Feulgen (Fig. 2C). Liver sections from the control group I and morin nondiabetic group II showed a strong positive immunostaining for Bcl-xl protein of cytoplasm of most hepatocytes (Fig. 3A,B). Liver sections from the diabetic untreated group III showed negative immunostaining for Bcl-xl in cytoplasm of hepatocytes (Fig. 3C). Liver sections from the diabetic group IV treated with morin showed moderate immunostaining for Bcl-xl protein in the cytoplasm of hepatocytes (Fig. 3D). Liver sections from the control group I and morin nondiabetic group II showed no TUNEL positive staining inside hepatocytes nuclei (Fig. 4A,B). Liver sections from the diabetic untreated group III showed a large number of TUNEL positive staining inside hepatocytes nuclei (numerous apoptotic cells) (Fig. 4C). Liver sections from the diabetic group IV treated with morin showed decreased TUNEL positive staining inside hepatocytes nuclei (sparse apoptosis cells) (Fig. 4D).

Fig. (1): Photomicrographs of liver sections stained with hematoxylin and eosin. (1 A) A liver section from the control group and morin nondiabetic group (1B) showing normal appearance of hepatocytes (H). (1C) A liver section from the diabetic untreated group showing inflammatory cells infiltration (I) in portal area and many hepatocytes having cytoplasmic vacuolization and degenerated nuclei (N). (1D) A liver section from the diabetic group treated with morin showing gradual recovery of hepatocytes with less cytoplasmic vacuolization and degenerated nuclei (H). (H&E x400).
Fig. (2): Photomicrographs of liver sections stained with Feulgen reaction. (2A) A liver section from the control group and morin nondiabetic group showing normal vesicular hepatocytes nuclei and numerous intensely stained DNA distributed randomly in the nucleoplasm and along nuclear envelope (arrow) while cytoplasm of hepatocytes appeared negatively stained to Feulgen. (2B) A liver section from the diabetic untreated group showing hepatocytes nuclei with marked decrease stained DNA in the nucleoplasm and nuclear envelope (arrow) while cytoplasm of hepatocytes appeared negatively stained to Feulgen. (2C) A liver section from the diabetic group treated with morin showing most hepatocytes nuclei with moderately stained DNA distributed randomly in the nucleoplasm and along nuclear envelope (arrow), while cytoplasm of hepatocytes appeared negatively stained to Feulgen. (Feulgen reaction x400).

Fig. (3): Immunostaining sections of the liver for Bcl-xL protein (3A) A liver section from the control group and (3B) morin nondiabetic group showing strong positive immunoreaction for Bcl-xL protein of cytoplasm of most hepatocytes (3C) A liver section from the diabetic untreated group showing negative immunoreaction for Bcl-xL in cytoplasm of hepatocytes. (3D) A liver section from the diabetic group treated with morin showing moderate immunoreaction for Bcl-xL protein in cytoplasm of hepatocytes. (Immunostaining of liver Bcl-xL protein x400).
Fig. (4): Photomicrographs of liver sections stained with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). (4A) A liver section from the control group and (4B) morin nondiabetic group showing no TUNEL positive staining inside hepatocytes nuclei. (4C) A liver section from the diabetic untreated group showing large number of TUNEL positive cells (numerous apoptotic cells) (arrow). (4D) A liver section from the diabetic group treated with morin showing a decrease in the number of TUNEL positive cells (sparse apoptosis cells) (arrow). (TUNEL staining x400).

Discussion

The liver is a crucial organ whose functions have been altered in diabetes. Furthermore, liver disease is an important cause of death in individuals with type 2 diabetes [24].

Herbal products and traditional medicines with better effectiveness and fewer side effects, have replaced the synthetically derived drugs in modern allopathic medication system [25].

Morin is one of the naturally occurring bioflavonoids. It can be found in different herbs and fruits and has an useful effect in human health involves prevention of diabetes, prevention of coronary artery diseases, inhibit tumor proliferation and protect human erythrocytes, ventricular myocytes, and saphenous vein endothelial cells and anti inflammatory effect [26].

Our study revealed highly significantly increased fasting blood glucose levels in the diabetic untreated group. Our findings are in agreement with those of previous studies [8,27].

Our study revealed significantly decreased albumin levels and significantly increased ALT, AST, ALP and bilirubin levels in diabetic untreated group.

Liver function tests (LFTs) are commonly used in clinical practice to screen liver diseases, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, and albumin.

The AST, ALT and ALP activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. The increase in the activities of plasma AST, ALT and ALP levels indicated that DM may induce hepatic dysfunction [28,29] (Hamden, 2009 and Kaimal, 2010).

Our findings are in agreement with those of El-Beshbishy, [30] who reported that some chemical agents produce hepatic injury and cause increased plasma ALT, AST levels, which is parallel to the severity of liver damage. Diabetes mellitus induced
by streptozotocin that destroy the pancreatic b-cells, possibly by the production of toxic metabolite free radical, which causes degradation of lipid membranes and damage of the liver and kidney [31].

Alkaline phosphatase is a membrane bound, its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolite [32].

The levels of bilirubin and total protein in serum were related to the function of hepatic cell. A high concentration of bilirubin in serum is an indication for erythrocytes degradation rate caused due to liver injury when treated with hepatotoxin. Diminution of total protein is a further indication of liver damage. The level of total protein will be decreased in hepatotoxic condition due to defective protein biosynthesis in liver [33,34].

Our study revealed a significant increase in the MDA levels, which are an index of lipid peroxidation in the liver of diabetic untreated group. This result could be attributed to excessive generation of free radicals by streptozotocin which was used to induce experimental diabetes [35] (Eliakim-Ikechukwu, 2010). Our findings were in agreement with those of previous studies [36,37].

Our study revealed a significant decrease in levels of GSH-Px in liver of diabetic untreated group.

Li, et al. [38] reported that the decrease GSH-Px level in liver of diabetic animals due to oxidative stress and decrease in antioxidant defenses as result of diabetes and liver damage.

Our study revealed a significant decreased of fasting blood glucose, ALT, AST, ALP and bilirubin levels and a significant increased of albumin levels in diabetic group treated with morin.

Vishnukumar et al., [26] reported that morin treatment for 45 days decreased blood glucose levels in STZ-induced diabetic rats, and this effect due to the regeneration of existing pancreatic β cells in STZ-induced diabetic rats.

Our finding are in agreement with those of previous studies that reported the decreased activities of these enzymes may be due to the prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration [12,39].

Our study revealed a significant decrease in the MDA levels and a significant increase in the GSH-Px levels in the liver of diabetic group treated with morin [26,40].

Our findings are in agreement with those of previous studies that reported morin has a beneficial effects on the liver damage by enhancing antioxidant enzyme activity and help to scavenge superoxide and hydroxyl radicals [26,40,41].

Examination of hematoxylin and eosin and feulgen reaction stained tissue sections from the diabetic untreated group showed inflammatory cells infiltration in portal area and many hepatocytes having cytoplasmic vacuolization and degenerated nuclei. Hepatocytes nuclei appeared with marked decrease stained DNA in the nucleoplasm and nuclear envelope while cytoplasm of hepatocytes appeared negatively stained to Feulgen. Our findings are in agreement with those of previous studies [42,43].

Cells undergoing necrosis swell and their organelles break down. They lose membrane integrity, rupture and spill debris that leads to local inflammation which then results in the death of adjacent cells. Necrosis is morphologically distinct from apoptosis and is defined as cell death accompanied by a rapid efflux of cell constituents to the extracellular space due to a loss of cytoplasmic membrane integrity and it takes place under extremely harmful environmental conditions such as exposure to toxic chemicals and microbial pathogens, and causes inflammation which gives rise to damage to surrounding cells [44].

Oxidative damage induced by streptozotocin resulted in the formation of highly reactive hydroxy radicals, which stimulate lipid peroxidation, enhance urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage and altered gene expression and apoptosis [45,46].

Examination of tissue sections from the diabetic untreated group showed negative immunostaining for Bcl-xI in cytoplasm of hepatocytes and large number of TUNEL-positive cells.

Apoptosis is a highly regulated form of cell death that is characterized by specific morphological, biochemical, and molecular events. It is essential for the normal development of multicellular organisms and is involved in cell turnover in healthy adult tissues [47].

The family of Bcl-2-related proteins plays a key role in the regulation of apoptosis, which are
grouped into Anti-apoptotic Bcl-2 and Bcl-xl localize predominantly at the mitochondria and inhibit apoptosis. Bax, a pro-apoptotic protein, resides in the cytoplasm and stimulates cell death after translocation to mitochondria \[48,49\] (Arash, 2010 and Mitsuol, 2010). Bcl-xL is critically important for the integrity of hepatocytes. Mcl-1 and Bcl-xL are two major Bcl-2 family proteins inhibiting hepatocyte apoptosis \[50\].

Our finding are in agreement with those of previous studies that reported oxidative stress is associated with various diabetic complications leads to apoptotic cell death as evidenced from DNA fragmentation, decrease expression of Bcl-xL in cytoplasm of hepatocytes and increase TUNEL-positive cells \[46,51,52\].

Examination of tissue sections from the diabetic group treated with morin showed gradual recovery of hepatocytes with less cytoplasmic vacuolization and degenerated nuclei, most hepatocytes nuclei with moderately stained DNA distributed randomly in the nucleoplasm and along nuclear envelope while cytoplasm of hepatocytes appeared negatively stained to Feulgen, moderate immunostaining for Bcl-xL protein in cytoplasm of hepatocytes with less cytoplasmic vacuolization associated with various diabetic complications.

Our findings are in agreement with \[26\] Vishnukumar who reported that a flavonoid morin, significantly reduced the pathological alterations in STZ-induced rats.

Khaki and his colleagues \[37\] reported that quercetin, which an isomer of morin have significantly preventive effect on liver cells damages by reducing number of apoptotic cells in liver through reduce oxidative stress.

Kapoor and Kakkar \[40\] found that, morin caused a decline in the release of apoptogenic factors from mitochondria and increased expression of anti-apoptogenic factors like Bcl-2.

**Conclusion:**

Morin has a hepatoprotective effect; it has been shown to attenuate the hepatic injury and apoptosis induced by streptozotocin, and has the capacity to scavenge free radicals, protect against oxidative stress, improve antioxidant enzyme activities, and also has antidiabetic efficacy on diabetic rats.

**Acknowledgements:**

**Conflicts of interest:** There are no conflicts of interest.

**References**


Diagnostic Value of CK19 and HMWCK 34BE12 in Differentiation between Selected Thyroid Neoplasms

TAGHREED ABD EL-SAMEE, M.D.*; RANIA GALAL, M.D.*; NIVEEN TAHOON, M.D.**; MAGDA H. BAKR, M.D.* and HALA A. AGINA, M.D.*

The Department of Pathology, Faculty of Medicine, Benha University* and National Cancer Institute, Cairo University**

Abstract

Thyroid cancer is an uncommon oncological entity, representing about 1% of all malignant neoplasms. In Egypt, malignant thyroid tumors accounting for 1.48% of total malignant cases. Histological findings may not be sufficient to establish a precise diagnosis for some thyroid lesions and consequently can’t predict their clinical course, common dilemma is encountered with encapsulated tumors exhibiting follicular growth pattern with presence or absence of capsular and/or vascular invasion, aiming to distinguish benign from malignant follicular tumors, another challenging situation is encountered when diagnostic nuclear features of papillary carcinoma are not rising to threshold of classic papillary thyroid carcinoma (PTC). CK19 and High molecular weight CK (HMWCK-34BE12) have been used as markers for prognosis in many cancers, but their role in segregating benign thyroid adenomas from atypical nodules and to complete malignant criteria supplied in histopathologically-examined tumors is still unclear.

Aim of the Work: The Purpose of current study is to investigate role of CK19 and HMWCK in separating benign and atypical thyroid nodules from malignant tumors in cases with histopathological criteria not enough to reach accurate diagnosis.

Patients and Methods: This retrospective study was carried upon 70 selected patients with different thyroid lesions including: 12 cases of adenomas (Ad), 9 cases of Atypical nodules (AN), and 49 malignant cases (22 cases of papillary thyroid carcinoma (PTC), 5 cases of anaplastic carcinoma (AC) and 5 cases of Medullary carcinoma (MC). Ten cases of non-neoplastic thyroid lesions were taken as control. Materials included formalin-fixed, paraffin-embedded blocks of thyroid lesions received during period from 2005-2011, where sections were stained with Hematoxylin & Eosin and immunostained with polyclonal antibodies against CK19 and HMWCK.

Results: Benign and atypical cases didn’t show diffuse positivity for CK19 in relation to 47% diffuse positivity in malignant cases which was statistically highly significant, p<0.01, statistically significant higher CK19 diffuse positivity was found in follicular carcinoma cases compared to PTC, while CK19 was not expressed in Medullary carcinomas or anaplastic carcinoma variants, p<0.05. cases of PTC expressed HMWCK while cases of FTC, anaplastic carcinomas and Medullary carcinomas did not express HMWCK which was statistically highly significant, p-value<0.05.

Conclusions: HMWCK and CK19 can be considered as good diagnostic markers for evaluating benign versus malignant thyroid lesions, HMWCK may play an important role to separate benign adenomas from atypical nodules, CK19 can successfully separate atypical encapsulated nodules (completely negative for CK19) from early follicular thyroid carcinomas especially minimally invasive cases (diffusely positive for CK19), and can separate atypical nodules exhibiting incomplete nuclear features supplied for PTC (completely negative for CK19) from cases with ordinary PTC, HMWCK can confirm diagnosis of follicular variant of PTC and successfully segregating them from follicular thyroid carcinomas, CK19 and HMWCK can separate dedifferentiated thyroid carcinoma with minimal follicular pattern from cases with Medullary and anaplastic carcinomas exhibiting follicular arrangement. Further researches may be mandatory to elicit role of HMWCK and CK19 in prognostic purposes regarding out come in patients with malignant thyroid tumors.

Key Words: CK19 – HMWCK – 34BE12 – Thyroid – Neoplasms.

Introduction

THYROID cancer is an uncommon oncological entity, representing about 1% of all malignant neoplasms [1]. In Egypt, malignant thyroid tumors accounts for 1.48% of total malignant cases and 65.3% of total endocrine malignant tumors. Papillary carcinoma is the most common malignant neoplasm of thyroid gland accounting for 67.6% [2].

Histological findings may not be sufficient to establish a precise diagnosis and, consequently, to predict clinical courses of these cases. Objective criteria and identification of markers that permit better characterization of thyroid tumors are therefore required [3].
A common dilemma is encountered with encapsulated tumors showing follicular growth pattern, presence or absence of capsular and/or vascular invasion distinguishes benign from malignant follicular tumors, but identification of this finding can be challenging due to incomplete capsular penetration, equivocal vascular invasion or technical difficulties due to processing or sectioning artifacts, another challenging situation is encountered when some but not all of diagnostic nuclear features of papillary carcinoma are present [3].

Many investigators have focused during last several years on finding immunohistochemical markers to help in distinction between benign and malignant thyroid lesions and subtypes of thyroid cancer [3].

Cytokeratins (CKs) constitute largest intermediate filament protein; Human epidermal keratins can be separated into proteins of different molecular weight. CK19 is one of three main keratins besides CK8 and CK18 expressed in simple or stratified epithelium and in various carcinomas, CK19 has been used extensively as marker of micrometastasis and for detection of circulating tumor cells in many cancers as adenocarcinomas breast, pancreas, and bile ducts as well as in transitional cell carcinomas [4]. Similarly, High molecular weight CK (34BE12), also known as CK903 is cytoplasmic marker corresponding to cytokeratins 1, 5, 10, and 14, which expressed in epithelial and myoepithelial cells [5]. However, their role in evaluating benign thyroid lesion, separating atypical nodules from malignant tumors as well as offering histopathological variant in malignant cases is still unclear and under trial researches. Therefore, it is important to identify a biological marker, independent of the different clinicopathologic factors that can guide the pathologists in accurate diagnosis and subsequently predicating patient’s outcome.

Aim of this work:

This study aimed at evaluating role of CK19 and HMWCK (34BE12) in diagnosis and differentiation between some benign, atypical and malignant thyroid lesions.

Material and Methods

This retrospective study was carried upon selected 70 patients with different thyroid lesions including: 12 cases of adenomas (Ad), 9 cases of Atypical nodules (AN), and malignant lesions consisted of 49 cases that further sub classified into: 22 cases of papillary thyroid carcinoma (PTC), 17 cases of follicular carcinoma (FTC), 5 cases of anaplastic carcinoma (AC) and 5 cases of Medullary carcinoma (MC). Ten cases of non-neoplastic thyroid lesions were taken as control, materials included formalin-fixed, paraffin-embedded blocks of thyroid lesions received during period from 2005-2011, blocks were selected from Early Cancer detection unit, Benha University, Pathology Department of Benha Faculty of Medicine and National Cancer Institute, Cairo University. Three sections of 4 micron thickness were obtained for each case, one section was H & E stained for diagnosis and histopathological reviewing, 2 sections were immunohistochemically stained using monoclonal antibodies against CK19 and HMWCK.

Immunostaining:

Formalin-fixed Paraffin-embedded tissue sections mounted on positive charged slides were heated at 60 degree centigrade for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining, antigen retrieval was done using microwave treatment in 10mM citrate buffer (Neo-markers, cat"AP-9003), Ph 6, Endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 minutes. Sections were washed three times with cold 0.01 phosphate buffered saline (PBS), after blocking with 10% normal rabbit serum, sections were incubated with rabbit anticow polyclonal antibody against CK19 (Santacruz biochemicals, santacruz CA dilution 1:50) and with mouse anti swine monoclonal antibody against HMWCK (Decton Dickenson, san jose Dako) dilution 1:200. Immunohistochemical stain was performed using the avidin-peroxidase complex technique (ABC kit-vector laboratories, Burlinguine, CA). Primary antibody was incubated overnight for CK19 and HMWCK proteins. ABC reaction was developed in presence of freshly prepared Diamino Benzidine supplement with hydrogen peroxide (DAB). Lastly, sections were counterstained with Mayer’s Hematoxylin, dehydrated, cleared and covered by cover slips.

Interpretation of HMWCK (34BE12) and CK19-stained thyroid lesions:

HMWCK and CK19 were expressed in cell cytoplasm with or without membranous accentuation according to Park et al., [6].

Cut off point of positive cells was 10% so:
- Score 1+; staining in 11%-25% of cells.
- Score 2+; staining in 26%-50% of cells.
- Score 3+; staining in 51% to 75% of cells.
- Score 4+; staining in >75%.
- Staining of 1+ or 2+ was defined as focal positive, and staining of 3+ or +4 was defined as diffuse positive [7,8].

**Results**

All control cases showed negative CK19 and HMWCK.

Benign adenomas showed negative CK19 expression in 11 out of 12 cases (91.6%), focal positivity in one out of 12 cases (8.4%) and diffuse CK19 positivity in no cases (0%).

Atypical nodules showed focal CK19 positivity in 3 out of 9 cases (33.3%) and diffuse positivity in no cases.

Malignant cases showed diffuse CK19 positivity in 23 out of 49 (47%) cases.

All examined adenomas showed negative HMWCK (100%), atypical nodules showed diffuse HMWCK positivity in 2 out of 9 (22.2%) cases examined while malignant cases showed diffuse HMWCK positivity in 20 out of 49 (40.8%) cases, Table (1).

<p>| Table (1): Immunostaining for CK19 and HMWCK in benign, atypical and malignant cases. |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunostaining</th>
<th>Benign adenomas</th>
<th>Atypical nodules</th>
<th>Malignant cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>Negative</td>
<td>11 (91.6%)</td>
<td>6 (66.7%)</td>
<td>15 (30.6%)</td>
</tr>
<tr>
<td></td>
<td>Focal positive</td>
<td>1 (8.4%)</td>
<td>3 (33.3%)</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td></td>
<td>Diffuse positive</td>
<td>0</td>
<td>0</td>
<td>23 (47%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>HMWCK</td>
<td>Negative</td>
<td>12 (100%)</td>
<td>4 (44.4%)</td>
<td>27 (55.1%)</td>
</tr>
<tr>
<td></td>
<td>Focal positive</td>
<td>0</td>
<td>3 (33.4%)</td>
<td>2 (4.1%)</td>
</tr>
<tr>
<td></td>
<td>Diffuse positive</td>
<td>0</td>
<td>2 (22.2%)</td>
<td>20 (40.8%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>9</td>
<td>49</td>
</tr>
</tbody>
</table>

NB: * = Significant. ** = Highly significant.

- CK19 and HMWCK showed statistically significant higher diffuse positivity in malignant group in relation to atypical nodules & adenomas cases, p-value <0.05.

Benign and atypical cases didn’t show diffuse positivity for CK19 in relation to 47% diffuse positivity in malignant cases which was Statistically significant, p-value <0.05.

**Immunostaining of CK19 and HMWCK in malignant thyroid carcinomas variants were shown in Table (2):**

PTC cases showed negative CK19 expression in 6 out of 22 cases (27.3%), focal positivity in 7 out of 22 cases (31.6%) and diffuse CK19 positivity in 9 out of 22 cases examined (41%), FTC cases showed diffuse CK19 positivity in 14 out of 17 cases (82.4%), and focal positivity in 3 out of 17 cases (17.6%), AC cases showed focal positivity in 1 out of 5 cases (20%), MC cases showed negative CK expression in all examined 5 cases (100%).

PTC cases showed focal HMWCK positivity in 2 out of 22 cases (9%) and diffuse HMWCK positivity in 20 out of 22 cases examined (91%), FTC, AC and MC cases showed negative reactivity for HMWCK, Table (2).

A statistically significant higher CK19 diffuse positivity was found in follicular carcinoma cases compared to PTC while CK19 was not expressed in Medullary carcinomas or anaplastic carcinoma variants, p-value <0.05.

Conversely, only cases of PTC expressed HMWCK while cases of FTC, anaplastic carcinomas and Medullary carcinomas not expressed HMWCK.
which was statistically highly significant, $p$ value $<0.01$, Table (2).

Table (3) elicit CK19 and HMWCK diffuse positivity in PTC and FTC in relation to atypical nodules aiming to separate them from malignant cases: Diffuse CK19 positivity was seen in 14 out of 17 (82.4%) FTC cases, not seen in cases of atypical nodules and seen in 9 out of 22 (41%) cases of PTC which revealed statistically significant correlation, $p<0.05$, HMWCK showed diffuse positivity in 20 out of 22 (90%) of PTC cases, not seen in FTC and was present in 2 out of 9 (22%) of atypical nodules with statistically significant correlation, $p<0.05$, Table (3).

CK19 diffuse positivity not present in atypical nodules (0%), HMWCK diffuse positivity not seen in FTC cases, CK19 diffuse positivity can separate atypical nodules from PTC and FTC cases and could be considered more sensitive than HMWCK which showed diffuse positivity in PTC and atypical nodules. Similarly, HMWCK diffuse positivity can separate PTC from FTC cases (completely negative for HMWCK).

Table (2): Correlation between CK-19 and HMWCK expression in examined malignant thyroid carcinoma variants.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunostaining</th>
<th>PTC</th>
<th>FTC</th>
<th>AC</th>
<th>MC</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>Negative</td>
<td>6 (27.3%)</td>
<td>0</td>
<td>4 (80%)</td>
<td>5 (100%)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td></td>
<td>Focal positive</td>
<td>7 (31.6%)</td>
<td>3 (17.6%)</td>
<td>1 (20%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diffuse positive</td>
<td>9 (41%)</td>
<td>14 (82.4%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>HMWCK</td>
<td>Negative</td>
<td>0</td>
<td>17 (100%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td></td>
<td>Focal positive</td>
<td>2 (9%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diffuse positive</td>
<td>20 (91%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>70</td>
</tr>
</tbody>
</table>

NB: PTC = Papillary thyroid carcinoma. FTC = Follicular thyroid carcinoma. AC = Anaplastic carcinoma. MC = Medullary carcinoma.

Table (3): Immunoreactivity of CK-19 and HMWCK (34BE12) in PTC, FTC in relation to atypical nodules.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunostaining</th>
<th>PTC</th>
<th>Atypical nodule</th>
<th>FTC</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>Negative</td>
<td>6 (27.7%)</td>
<td>6 (66.7%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Focal positive</td>
<td>7 (31.6%)</td>
<td>3 (33.3%)</td>
<td>3 (17.6%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Diffuse positive</td>
<td>9 (41%)</td>
<td>0</td>
<td>14 (82.4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>9</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td>HMWCK</td>
<td>Negative</td>
<td>0</td>
<td>4 (44%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Focal positive</td>
<td>2 (9%)</td>
<td>3 (33.4%)</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Diffuse positive</td>
<td>20 (91%)</td>
<td>2 (22%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>9</td>
<td>17</td>
<td>31</td>
</tr>
</tbody>
</table>

NB: PTC = Papillary thyroid carcinoma. FTC = Follicular thyroid carcinoma. AN = Atypical nodules.
Fig. (1): A case of atypical nodule with questionable papillary thyroid carcinoma showing incomplete nuclear features (H & E, x200).

Fig. (2): Atypical thyroid nodule with incomplete nuclear features (clearing) showing diffuse Cytoplasmic immunostaining for HMWCK (arrow) (ABC, x200).

Fig. (3): PTC showing diffuse positive cytoplasmic staining for HMWCK (ABC, x100).

Fig. (4): Follicular thyroid carcinoma showing diffuse positive cytoplasmic (arrow) staining for CK19 (ABC, x400).

Fig. (5): Papillary thyroid carcinoma/follicular variant showing diffuse positive Cytoplasmic staining with membranous accentuation of tumor cells with HMWCK (ABC, x100).

Fig. (6): Follicular thyroid carcinoma showed diffuse positive Cytoplasmic (arrow) staining of CK-19 (ABC, x400).
Discussion

Human thyroid carcinoma is the most common cancer of endocrine system in United States Siegal et al., [9]. In western countries, thyroid cancer is more common in women than in men and is ranked seventh of all cancers affecting women [10,11].

According to Egyptian National Cancer Institute, malignant thyroid tumors constitute 65.3 1% of endocrine malignant tumors with highest incidence of PTC forming 67.59% [2].

Accurate diagnosis is critical for post-operative management of patients with thyroid nodules. Nowadays, most controversial issue in thyroid pathology is differential diagnosis of an encapsulated thyroid nodule with follicular architecture such as follicular adenoma, atypical nodule, follicular carcinoma, and follicular variant of papillary thyroid carcinoma [12].

Several immunohistochemical markers using different antibodies, alone or in panels have been proposed to overcome this challenge, including CK19, HMWCK (34 BE 12) and others, in the present work, adenomas showed negative CK19 expression in 11 out of 12 cases (91.6%), focal positivity in one out of 12 cases (8.4%) and diffuse CK19 positivity in no cases (0%). Similarly, atypical nodules showed diffuse CK19 positivity in no case (0%), and focal positivity in 3 out of 9 cases (33.3%). Conversely, malignant cases showed diffuse CK19 positivity in 23 out of 49 (47%) cases and focal positivity in 11 out of 49 (22.4%), these results coincided with results done by Choi et al., [13], Shelis [14] and Saleh et al., [12] who stated that diffuse positive CK19 immunostaining is not seen in follicular adenomas or in atypical encapsulated nodules but were evident in 86% of follicular carcinoma cases. Regarding variants of malignant cases, PTC cases showed negative CK19 expression in 6 out of 22 cases (27.3%), focal positivity in 7 out of 22 cases (31.6%) and diffuse CK19 positivity in 9 out of 22 cases examined (41%), while FTC cases showed diffuse CK19 positivity in 14 out of 17 cases (82.4%), and focal positivity in 3 out of 17 cases (17.6%), conversely, AC and MC cases did not show diffuse CK19 positivity in all examined 5 cases (100%) which was statistically highly significant ($p<0.01$), these results were supported by that done by many authors as Arora et al., [15], Kosem et al., [16] and Liu et al., [17] regarding CK19 immunoreactivity, in which malignant thyroid neoplasms originating from follicular epithelium can be separated from other malignant thyroid tumors as MC and AC, similarly, follicular thyroid carcinomas had higher diffuse CK19 positivity than PTC cases, this finding may provide evidence supporting CK19 as sensitive up to diagnostic marker for malignant thyroid tumors originating from follicular epithelium and successfully can separate them from follicular adenomas and atypical nodules (borderline) in which CK19 diffuse positivity is completely absent.

All examined adenomas showed negative HMWCK (100%), atypical nodules showed diffuse HMWCK positivity in 2 out of 9 (22%) cases examined and malignant cases showed diffuse HMWCK positivity in 20 out of 49 (40.8%) cases examined. So, HMWCK diffuse positivity is not seen in benign cases while was present in atypical and malignant cases, these results were coincide with that done by Scognamiglio et al., [18] and Nasr et al., [19] and Liu et al., [17] who stated that HMWCK is a marker of malignancy detection in thyroid tumors and can separate benign cases from atypical nodules and malignant thyroid tumors but can't separate atypical nodules from malignant cases. As regard HMWCK expression in malignant thyroid tumors variants, PTC cases showed negative HMWCK expression in no cases (0%), focal positivity in 2 out of 22 cases (9%) and diffuse HMWCK positivity in 20 out of 22 cases examined (91%), FTC, AC and MC cases showed diffuse HMWCK positivity in no cases (0%), these results were in agreement with that done by Nakamura et al., [20] and Liu et al., [17] concerning outcome of papillary thyroid carcinoma cases, in which diffuse HMWCK (34BE12) expression was statistically correlated with diagnosis of PTC regardless its variants ($p$-value<0.01), 100% of papillary thyroid carcinoma cases were positive HMWCK (34BE12). Similarly, Yang et al., [21] and Salajegheh et al., [22] recorded that HMWCK (34BE12) immunostaining was useful in papillary thyroid carcinoma diagnosis and can separate PTC/ follicular variant cases from FTC cases, these results were in accordance with the present work in which FTC cases showed diffuse positive staining in 14 out of 17 (82.4%) cases while showed diffuse HMWCK staining in no cases examined with statistically significant correlations, $p<0.05$. Accordingly, we can consider CK19 is a good diagnostic marker seperating FTC from Follicular variant of PTC. In the current work, HMWCK showed diffuse positivity in 2 out of 9 (22%) cases of atypical nodules, conversely, atypical nodules didn’t show diffuse positivity for CK19 in examined cases (0%), this statistically significant difference ($p$-value <0.05) can put CK19 superior to HMWCK in separating
atypical nodules from malignant thyroid lesions successfully. Regarding HMWCK diffuse positivity in atypical cases, it may be able to separate adenomas from atypical nodules with incomplete nuclear features not rising to threshold of classic papillary thyroid carcinoma, these results were coincide with that done by Kosem et al., [16], Prasad et al., [23], Scognamiglio et al., [18] and Arora et al., [15] who stated that lesions representing a biologic spectrum of papillary thyroid carcinoma still at early stage of malignant transformation with incomplete nuclear features that referred to them as atypical nodules can show immunoreactivity for HMWCK in this early stage. Moreover, HMWCK may be integrated in carcinogenic cascade for papillary thyroid carcinoma cases.

According to the present work, cases with atypical nodules showing incomplete nuclear features not rising to threshold of PTC can be separated from follicular variants of PTC using CK19 that showed completely negative expression pattern in adenomas as well as in atypical nodules in relation to diffuse positivity in 9 out of 22 (41%) of PTC cases examined. Results published by many authors as Kosem et al., [16], Prasad et al., [23], Scognamiglio et al., [18], Nasr et al., [19], Nakamura et al., [20] and Liu et al., [17] showed that HMWCK and CK19 immunoreactivity were very useful in discriminating follicular variant PTC from follicular adenomas and atypical nodules, at the same time all cases of follicular variant PTC showed positive HMWCK immunostaining, while it was absent in all cases of benign group.

In conclusion, HMWCK and CK19 can be considered as good diagnostic markers for evaluating benign versus malignant thyroid lesions, HMWCK may play an important role to separate adenomas from atypical nodules, CK19 can successfully separate atypical encapsulated nodules from early follicular thyroid carcinomas especially minimally invasive cases, and can separate atypical nodules exhibiting incomplete nuclear features supplied for PTC from cases with ordinary PTC. HMWCK can confirm diagnosis of follicular variant of PTC and successfully segregating them from follicular thyroid carcinomas, CK19 and HMWCK can separate dedifferentiated thyroid carcinoma with minimal follicular pattern from cases with Medullary and anaplastic carcinomas exhibiting follicular arrangement. Further researches may be mandatory to elicit role of HMWCK and CK19 in prognostic purposes regarding out come in patients with malignant thyroid tumors.


Effect of Mechanical Measures on Prevention of Deep Vein Thrombosis among General Surgical Patients

SHAWKEY S. GAD, M.D.* and AMAL A. EL-SHEIKH, D.N.Sc.**

The Departments of General Surgery, Faculty of Medicine* and Adult Health Nursing, Faculty of Nursing**, Menoufia University, Egypt

Abstract

Aim of the Study: To examine the effect of mechanical measures on prevention of deep vein thrombosis among general surgical patients.

Setting: The study was carried out in General Surgical Department of Menoufia University Hospital.

Subjects: A random sample of 120 general surgical patients. They were assigned randomly and divided alternatively into two equal groups, 60 patients for each groups.

Tools: 3 tools were used for data collection. I: An interviewing questionnaire of deep vein thrombosis II: Deep vein thrombosis risk factors assessment sheet (scale) III: Deep vein thrombosis clinical assessment sheet (physiological measurement).

Results: There was significant improvement in the knowledge about DVT after intervention among study group as compared to control group. The minority of the study and control group (13.3%) and (10%) respectively suffered from leg calf pain on admission, while after 3 weeks (6.7%) of the study group and (16.7%) of the control group suffered from leg calf pain. Regarding leg edema (13.3%) of the study group and (10%) of the control group suffered from leg edema on admission, while after 3 weeks the study group improved to become (6.7%) and control group (23.3%). Both study and control groups had a negative sign of homan’s test and Doppler result on admission, while after three weeks the study group still had negative sign of homan’s test and Doppler result compared to control group (6.31%) had positive sign of homan’s test and Doppler result.

Conclusions: General surgical patients who exposed to mechanical measures were not exposed to deep vein thrombosis than those who exposed to routine hospital care only from general surgical department.

Recommendations: A booklet about DVT prevention should be available and distributed for all general surgical patients in every ward.

Key Words: Mechanical measures – Deep vein thrombosis – General surgical patients.

Introduction

VENOUS thromboembolism (VTE) is the term that describes two clinical conditions: Deep vein thrombosis (DVT) and pulmonary embolism (PE). Deep vein thrombosis occurs when a blood clot or thrombus forms in a deep vein, usually restricting blood flow. Pulmonary embolism occurs if the thrombus is dislodges and travels to the lungs. VTE is considered to be the most common preventable cause of hospital related death [1].

The House of Commons Health Committee reported in 2005 that an estimated 25,000 people die from preventable hospital-acquired venous thromboembolism every year in the United States. This includes patients admitted to hospital for medical care and surgical interventions [2].

Risk for developing DVT increase with age, individual or family history of DVT, smoking, dehydration, cancer, varicose veins, surgery or other hospitalization, certain heart or respiratory disease, obesity and pregnancy. The hormones found in birth control pills or hormone replacement therapy, especially estrogen, is assumed to increase the risk of clot formation by 3 to 4 times [3].

Deep venous thrombosis results from three factors: Stasis of venous blood flow, damage to the endothelial lining of the vein wall and changes in the coagulation mechanism of the blood. These factors are still believed to be of primary importance in thrombus formation, and they contribute to the major predisposing risk factors of venous thromboembolism [4].

Warning signs for DVT can evolve over several days or develop rapidly over a few hours. These may include warmth, tenderness, redness or discoloration in the affected area usually in one calf.
The calf may feel tight, heavy or pulled. Sometimes, only slight discomfort or severe pain increasing upon standing or walking is experienced. Also it may have no signs or symptoms [8].

The morbidity associated with DVT is often under recognized and includes serious long term complications such as pulmonary embolism, chronic venous insufficiency, chronic edema, chronic pain and recurrent venous ulceration, collectively known as the post-thrombotic syndrome [6].

Prevention of venous thromboembolism (VTE), a combination of DVT and PE, is more effective than treatment and is an important aspect of patient care before, during, and after surgery. Identification of risk factors should be used as a basis to determine if pharmacological and/or mechanical thromboprophylaxis should be initiated [7].

Pharmacological measures when used early in combination with mechanical measures are effective in preventing DVT. Pharmacological measures include anticoagulant drugs as low dose heparin. Low molecular weight heparin, warfarin and dextran. It is important to start anticoagulant drugs quickly in patients at risk because thrombi can propagate in a matter of hours. Mechanical measures for DVT prevention include exercises, early ambulation, stockings, pneumatic compression devices, adequate hydration and diet. Each improve venous return and reduces venous stasis in the leg veins [8].

Perioperative nurses should be knowledgeable about venous stasis and should participate in multidisciplinary teams to develop policies, procedures, and protocols to reduce the risk of venous stasis. Preoperative nurses play an important role in assessment, prevention, and early recognition of deep vein thrombosis. Preoperative teaching is an important component of the nursing role, and can relieve patient anxiety, help prevent complications, and improve outcomes [9].

Aim of the study:

The aim of this study is to examine the effect of mechanical measures on prevention of deep vein thrombosis among general surgical patients.

Operational definition:

A mechanical measure for DVT prevention includes turning and positioning at least every 1 to 2 hours, exercises (foot and ankle exercises and deep breathing exercise), early ambulation, stockings and adequate hydration.

Research hypothesis:

The following research hypothesis is formulated in an attempt to achieve the aim of the study:

• There will be a decrease in the incidence of DVT in patient’s who receive DVT prophylaxis (mechanical measures) study group as compared to patient’s who did not receive control group.

Subjects and Methods

Research design:

An experimental design was utilized to achieve the aim of this study.

Setting:

The study was carried out in General Surgical Department of Menoufia University Hospital. Data were collected from 9/2012 to 1/2013.

Subjects:

A random sample of 120 general surgical patients. They were assigned randomly and divided alternatively into two equal groups, 60 patients for each groups:

A- Study group (I): Exposed to mechanical measure (turning and positioning at least every 1 to 2 hours, exercises (foot and ankle exercises and deep breathing exercise), early ambulation, stockings, and adequate hydration) along with routine hospital care.

B- Control group (II): Exposed only to routine hospital care.

The patients had been selected according to the following criteria:

• Age range from 21 to 60 years old.
• Both sexes.
• Conscious and willing to participate in the study.

Tools:

Three tools were utilized for data collection.

These tools are:

Tool I: An interviewing questionnaire of deep vein thrombosis.

It was developed and used by the researcher based on review of the related literature, to assess patient’s knowledge about deep vein thrombosis. It comprised of three parts:

Part one: Sociodemographic data.

It included information about patient’s age, sex, martial status, level of education and occupation.
Part two: Clinical data.
   It was composed of question about causes of hospital admission; take any medication, previous hospitalization and previous surgery.

Part three: Patient’s knowledge.
   It was comprised of questions related to patient’s knowledge regarding deep vein thrombosis causes, risk factors, signs and symptoms, method for prevention, treatment and complication.

Tool II: Deep vein thrombosis risk factors assessment sheet (scale).
   It was developed by Autar, (1996) [10] and utilized by the researcher to identify patient at risk and classified them into groups according to predisposing factors. The scale consisting of seven distinct categories identified as age, mobility, body mass index, special risk, trauma risk, surgical intervention and high-risk diseases. A score of ≤ 10 indicate low risk, while a score rang from 11-14 had indicated moderate risk and a score of ≥ 15 indicate high risk.

Tool III: Deep vein thrombosis clinical assessment sheet (physiological measurement):
   It was developed and used by the researcher based on review of the related literature except part one it was developed by Hirsh and Lee, (2002) [11]. The objective of this tool was to evaluate the patient’s sign and symptoms of DVT. It was comprised of 3 parts:

Part one: Clinical assessment.
   It was comprised of items to examine the patient’s signs and symptoms of DVT as presented by:
   • Pain in the calf.
   • Leg edema or swelling.
   • Erythema or cyanosis, warmth, dilated superficial vein.
   • Localized redness, pallor and a loss of the dorsalis pedis pulse.
   • Tenderness along the distribution of the affected deep leg veins.

Part two: Homan’s test:
   It is an active and subjective test in which the patient is asked to dorsiflex his or her foot. If pain in the calf, it was indicative of positive result and presence of deep vein thrombosis, negative human’s test does not exclude DVT.

Part three: Duplex ultrasound:
   Presence of clot it was indicative of positive result and presence of deep vein thrombosis, while absence of clot it was indicative of negative result and absence of deep vein thrombosis.

Methods:
   - An official permission: To carry out the study was obtained from the hospital director of Menoufia University Hospital prior to data collection and after explaining the significance of the study and its purpose.
   - Tools development: Tool I was developed by the researcher while tool II was developed by Autar, (1996) [10] and tool III was developed by the researcher except part one was developed by Hirsh and Lee, (2002) [11]. All tools were submitted to jury of 3 experts in nursing field, Faculty of Nursing, Menoufia University and 2 experts in surgical field, Faculty of Medicine, Menoufia University to obtain its content validity. Modifications were done accordingly.
   - Reliability: A test retest method was used to test tool reliability. This comparison is expressed through correlation coefficient alpha for tool I it was 0.93, for tools II it was 0.92 and for tool III it was 0.92.
   - Verbal consent: The researcher obtained a verbal consent for participation in the study from all participants, explaining the purpose of the study, and assuring that the confidentiality would be maintained throughout the study.
   - Pilot study: A pilot study was carried out before starting data collection on 10 patients to evaluate the tentative developed tools for clarity and applicability and to estimate the time needed to collect data then necessary modification were carried out before actual study. Data obtained from pilot study were excluded from the study.
   - Data collection:
     • The subjects who fulfill the inclusion criteria were selected randomly and divided alternatively into two equal groups, study group (I) and control group (II) 60 patients for each group.
     • All participants of both group were interviewed individually on admission at general surgical department to collect data about sociodemographic and clinical characteristics using tool I, knowledge assessment by using tool (I) part three, risk factors for DVT were assessed by using tool (II) and clinical assessment of DVT by using tool (III).
     • Patients of the control group (II) were exposed to routine hospital care.
     • Patients of the study group (I) were exposed to routine hospital care and to intervention as the following:
**Preoperative care:**

Health instruction was given to each patient in the study group individually before surgery by the researcher. Teaching was done in 3 sessions (2 session for teaching exercise program and early ambulation and one session about elastic stockings, and adequate hydration) using demonstration and redemonstration until the patient master the skills.

1. **The exercise that was followed involved the following:**
   - Turning and positioning at least every 1 to 2 hours and performing foot and ankle exercises. The exercises are flexion, extension, and rotation of the ankle and foot. Demonstrate all exercises to the patient and have the patient perform a return demonstration to acknowledge understanding.
   - Deep breathing exercise is beneficial because they produce increased negative pressure in the thorax, which ass in emptying the large vein.
   - E elevate the lower limb above the level of the heart periodically.
   - Early ambulation.

2. **Also health instruction was given to patients of the study group about:**
   - Correct application of elastic stockings.
   - Adequate hydration at the prescribed rate.

A booklet was distributed among patients of the study group for reinforcement.

**Intraoperative care:**

- The researcher assured that elastic stockings were not twisted or turned during the procedure, and keep the patient’s extremities at, but never below, the level of the operating room table.
- Remind operating room team members not to place pressure on the anesthetized patient to avoid circulatory compromise. Use extra padding on pressure points to prevent tissue damage and circulatory impairment.
- Confirm that straps to secure the patient on the table aren’t too tight.
- Instruct the surgical assistant or scrub nurse to avoid extreme degrees of flexion and internal rotation of hip and knee.
- Be aware that unnecessarily high tourniquet pressures and prolonged periods of inflation should be avoided, if possible, when a tourniquet is used.

Immediate postoperative care: Immediately after surgery the researcher carry out the following:
- Be sure that elastic stockings aren’t twisted or turned.
- Elevating the foot of the bed (unless contraindicated) to promotes venous return.
- Avoid placing pillows under the patient’s knees to prevent compressing the popliteal veins.
- Increase fluid intake according physician prescription.
- Encourage the patient to do all health instruction that was given before surgery.

After three weeks of surgery (after intervention) all participants of both group were interviewed individually for knowledge assessment by using tool (I) part three, and clinical assessment of DVT by using tool (III).

**Statistical analysis:**

Data was collected, tabulated and statistically analyzed using SPSS version II statistical program. Descriptive statistics were first applied (e.g., frequency, percentage, mean and standard deviation). Chi square test was used for comparison between 2 groups with qualitative data. While McNamara test was used for comparison between the same group before and after. p-values, which were less than 0.05, were considered as statistically significant.

**Results**

Table (1) revealed that, the mean age for study group was 42.03 ± 13.58 and for the control group was 41.76 ± 13.72 years. More than half of both groups (53.3%) were male. The majority of study group (83.3%) and control group (80%) were married. About half of study group (46.7%) and control group (50%) had secondary education.

Table (2) the findings revealed that about (56.7%) of study group and (46.7%) of control group admitted to hospital for abdominal surgery. The majority of the study group (83.3%) and control group (80%) did not take any medication. As regards the previous surgery, about (16.7%) of study group and (20%) of control group had cesarean section. There were no statistical significant differences between control and study groups regarding clinical data.

Table (3) showed that about two third of both groups (63.3%) of the study group and (66.7%) of control groups were at moderate risk for developing DVT. There were no statistical significant differences between control and study groups regarding risk factor of DVT.
Figures (1,2) it is clear from these figures that there was significant improvement in the knowledge of DVT after intervention among study group as compared to control group.

Table (4) the findings revealed that, the minority of the study and control group (13.3%) and (10%) respectively suffered from leg calf pain on admission, while after 3 weeks (6.7%) of the study group and (16.7%) of the control group suffered from leg calf pain. Regarding leg edema (13.3%) of the study group and (10%) of the control group suffered from leg edema on admission, while after 3 weeks the study group improved to become (6.7%) and control group (23.3%). As regards cyanosis in the leg (100%) of the study and control did not suffered from cyanosis in leg on admission, while after 3 weeks the control group become (10%) suffered from cyanosis in leg. Regarding to tenderness in leg the minority of the study and control group (10%) suffered from tenderness in leg on admission, while after 3 weeks the study group improved and (20%) of the control group suffered from tenderness in leg.

Figure (3) illustrated that all of both study and control groups had a negative sign of homan’s test and Doppler result on admission, while after three weeks the study group still had negative sign of homan’s test and Doppler result while (6.3 1%) of control group had positive sign of homan’s test and Doppler result.

Table (1): Distributions of patients of the both groups according to their socio-demographic data.

<table>
<thead>
<tr>
<th>Socio-demographic data</th>
<th>Study group No=60</th>
<th>Control group No=60</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>t-test</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>42.03±13.58</td>
<td>41.76±13.72</td>
<td>0.10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 53.3</td>
<td>32 53.3</td>
<td>0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>28 46.7</td>
<td>28 46.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>24 40</td>
<td>18 30</td>
<td>1.72</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Secondary</td>
<td>28 46.7</td>
<td>30 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>8 13.3</td>
<td>12 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>10 16.7</td>
<td>12 20</td>
<td>0.22</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Married</td>
<td>50 83.3</td>
<td>48 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional</td>
<td>8 13.3</td>
<td>18 30</td>
<td>6.36</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Manual work</td>
<td>36 60</td>
<td>24 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>16 26.7</td>
<td>18 30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Distributions of patients of the both groups according to their clinical data.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Study group No=60</th>
<th>Control group No=60</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causes of hospital admission:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal surgery</td>
<td>34 56.7</td>
<td>28 46.7</td>
<td>3.88</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>20 33.3</td>
<td>18 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hernia</td>
<td>6 10</td>
<td>14 23.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Take any medication:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 16.7</td>
<td>14 23.3</td>
<td>0.83</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>No</td>
<td>50 83.3</td>
<td>46 76.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Causes of previous hospitalization:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>36 60</td>
<td>24 40</td>
<td>3.36</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Surgery</td>
<td>22 36.7</td>
<td>30 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical problem</td>
<td>2 3.3</td>
<td>6 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past previous surgery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>10 16.7</td>
<td>12 20</td>
<td>2.40</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Tonsillectomy</td>
<td>6 10</td>
<td>10 16.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast surgery</td>
<td>6 10</td>
<td>8 13.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No previous surgery</td>
<td>38 63.3</td>
<td>30 50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (3): Distributions of risk factors of DVT for both study and control groups.

<table>
<thead>
<tr>
<th>Risk factors assessment of DVT:</th>
<th>Study group</th>
<th>Control group</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate risk 11 - 14</td>
<td>38</td>
<td>40</td>
<td>0.22</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>High risk &gt; 15</td>
<td>22</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Comparisons between patients of both groups regarding to clinical manifestation of DVT at two different intervals (on admission and after 3 weeks of surgery).

<table>
<thead>
<tr>
<th>Clinical manifestation of DVT</th>
<th>On admission</th>
<th>X2</th>
<th>P value</th>
<th>After three weeks of surgery (after intervention)</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>No.</td>
<td>%</td>
<td>Control group</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Calf pain:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>8</td>
<td>13.3</td>
<td></td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>52</td>
<td>86.7</td>
<td>54</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg edema:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>8</td>
<td>13.3</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>52</td>
<td>86.7</td>
<td>54</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanosis in the leg:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>60</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm leg:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Not present</td>
<td>54</td>
<td>90</td>
<td>54</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness in the leg:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>60</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can’t feel leg pulse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>60</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenderness in the leg:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Not present</td>
<td>54</td>
<td>90</td>
<td>54</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.B.: (100%) of both groups did not suffer from dilated superficial vein on admission and after 3 weeks of surgery.

Fig. (1): Patient’s knowledge about DVT of the study group at two different intervals pre and post intervention assessment.
Discussion

Deep vein thrombosis (DVT) is the primary cause of fatal and nonfatal pulmonary embolism. It is imperative for preoperative nurse to identify the risks factors for DVT and/or PE in their nursing assessments. Assessment of risk factors begins with the initial patient assessment. It continues throughout the patient’s surgical/interventional procedure and postoperative recovery until he or she is discharged from care. The process of assessing patients for DVT or PE begins with the identification of risk factors [9].

Sociodemographic characteristic of the studied sample:

As regard to age, the results of the present study revealed that the mean age for study group was 42.03 ± 13.58 and for the control group was 41.76 ± 13.72 years. This result was in line with Hitos et al., [12] who stated that deep vein thrombosis usually affect individuals older than 40 year and they added that any patient over the age of 40 years should be considered to be at significant high risk of surgical developing thromboembolism.

Concerning sex, the results of the present study revealed that more than half of both groups were male. This result was consistent with Abd El-Salam (2009) [13] who reported that more than two thirds of the sample were males while one third were females in both study and control groups.

Risk factors assessment for deep vein thrombosis:

Concerning risk factors assessment for deep vein thrombosis, it was noticed from the present study that about two thirds of both study and control group had moderate risk for deep vein thrombosis. The assessment allow the researcher to rate the patient’s risk as low, moderate or high. The researcher should focus on prevention by the early recognition and adequate prophylaxis of those at
increased risk. This result was in line with Abd El-Salam [13] who stated that more than three fourth of the study and control groups were at high risk for developing DVT.

Patient’s knowledge about deep vein thrombosis:

It was noticed from the present study that there was significant improvement in the knowledge of DVT after intervention among study group as compared to control group. This finding was in congruence with Abd El-Salam [13] who stated that after intervention there was a significant improved in total knowledge score of the study group as compared to control group. Bader [14] found that patients of the study group had a significant difference in their knowledge from preoperative to the discharge time as compared to the control group. Also Bonner [15] stated that patients need information about DVT, anticoagulation, compression stockings, and the possible complications of these. They also need to be aware of how and when to access help. The diagnosis of DVT may also mean a change in lifestyle and this information should be given verbally and reinforced with written information to increase concordance with treatment.

Regarding clinical assessment of DVT:

As regard to the clinical assessment it was noticed that after intervention the patients of study group had an improvement of clinical assessment as evident by absence of warm leg and tenderness in the leg than patients of control group who follow the routine care only. This may be due to improvement of knowledge and applying the instruction of mechanical measures. This finding of the study was in line with El-Sheikh [16] who stated that the patient who received the protocol of care on congestive heart failure would have an improvement of physical responses as evident by changes in vital signs, dyspnea, edema, fatigue, skin condition, laboratory finding than patient who did not receive the protocol of care.

According to the current study findings, the study group who received intervention did not have DVT compared of the control group who follow the routine care only, the risk for DVT is prevented this due to giving instruction to the patient about mechanical measures by the researcher. This was supported by Abd El-Salam [13] who stated that the study group who received intervention about lower limb exercises and diet had lower percentage of DVT occurrence than control group. Roderick et al., [17] stated that mechanical compression methods reduced the risk of DVT by about two-thirds when used as monotherapy and by about half when added to a pharmacological method. Also Geerts et al., [9] stated that without prophylaxis, the incidence of objectively confirmed, hospital-acquired DVT is approximately 10% to 40% among medical or general surgical patients and 40% to 60% following major orthopedic surgery.

The present study was also consistent with Amaragiri and Lees [18] who conducted a systematic literature review of randomized controlled trials evaluating the effectiveness of graduated compression stockings for preventing DVT in various groups of hospitalized patients. The analysis demonstrated a statistically significant reduction in DVT incidence with graduated compression stockings compared with control both among the nine trials in which stockings were used alone and among the seven trials in which stockings were used in addition to another method of thromboprophylaxis.

Conclusion:

General surgical patients who exposed to mechanical measures were not exposed to deep vein thrombosis compared with those who exposed to routine hospital care only from general surgical department.

Recommendations:

- A booklet should be available and distributed for all general surgical patients in every ward about DVT prevention
- Replication of the study with large probability sampling for generalizing the results.

References


14- BADR M.E.S.: Rehabilitation of the post cardiac surgery patient. Review Article. Faculty of Nursing, Cairo University, p. 25, 2002.


Effect of Vitamin D Supplementation and/or Physical Training on Cigarette Smoke Induced COPD in Rats

NASHWA ELTABLAWY, M.D.; SAMAH ELATTAR, M.D. and ZIENAB ABDEL WAHAB, M.D.
The Department of Physiology, Faculty of Medicine, Cairo University

Abstract

Background: Chronic obstructive pulmonary disease (COPD) is a major health problem with increasing morbidity and mortality. Vitamin D deficiency has been established as exceedingly prevalent in many of chronic lung disease populations and exercise training in COPD patients results in positive effects in dyspnea and exercise tolerance.

Aim of Work: The purpose of the present study was to investigate the effect of vitamin D supplementation and/or physical training on pulmonary functions, lung inflammation, antimicrobial production and matrix degradation in a rat model of COPD.

Methodology: Forty male Albino rats were used in this study and divided into 5 groups, 8 rats each: Group 1: Control group, Group 2: (COPD group): COPD rats maintained untreated for the experimental period, Group 3: (Vit. D+COPD): COPD rats were treated with vitamin D injection 1, 25 (OH) D3 was administered intraperitoneally (i.p.) at dose 0.5 µg/kg of body weight (BW), 3 times a week for 8 weeks, Group 4: (COPD+ Exercise): COPD rats performed daily exercise program and group 5: (COPD+Vit D+exercise) COPD rats treated with vitamin D injection (i.p.) at a dose of 0.5 µg/kg, 3 times a week for 8 weeks and performed daily exercise program. After 8 weeks of treatment, pulmonary functions were tested and blood samples were withdrawn for measuring vitamin D and Ca2+ levels and the lung tissues were excised to measure interleukin 12 (IL12), tumor necrosis factor alpha (TNF alpha), metalloproteinase-9 (MMP-9) and cathelicidin.

Results: Peak expiratory flow (PEF), forced vital capacity (FVC), vitamin D and Ca2+ were significantly reduced in COPD rats after 12 weeks of exposure to cigarette smoke. Vitamin D supplementation and swimming training for 8 weeks improved PEF, FVC, vitamin D and Ca2+ significantly as compared to untreated COPD. Combined vitamin D treatment and physical training significantly improved FVC level as compared with each treatment separately. The improvement was associated with significant reduction in inflammatory markers and MMP-9 as compared to COPD untreated rats. The antimicrobial cathelicidin was significantly increased in COPD rats and was further increased on vitamin D treatment but not with exercise training.

Conclusion: Our results showed that COPD is an inflammatory disease and it is associated with vitamin D deficiency. Vitamin D supplement or rehabilitation by physical training each separately improved the pulmonary functions, reduced inflammation, and attenuate lung parenchymal degradation. Vitamin D in addition induced an antimicrobial protection, however vitamin D supplement had a slightly better effects as compared with exercise training. Combination of both vitamin D supplementation and exercise training had a synergistic effect and produced a significant improvement as compared to each therapy separately. We can conclude that vitamin D supplement has a beneficial effects as a therapy in cases of COPD and it is better added to rehabilitation training programs for better results.

Key Words: COPD – Vitamin D – Physical training – Cathelicidin.

Introduction

CHRONIC obstructive pulmonary disease (COPD) is a major health problem with increasing morbidity and mortality; in 2020, COPD will be the 3rd leading cause of mortality worldwide and the 5th leading source in terms of burden of disease [1]. COPD is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients [2-4].

Exposure to cigarette smoke is known to significantly increase the risk for the development of COPD [5,6]. The exact pathogenesis has yet to be discovered; however, numerous cellular elements have demonstrated involvement in the pathophysiology of COPD. These include macrophages, neutrophils and cytokines such as interleukin (IL)-4, IL-5 and IL-13, along with interferon-gamma [6-8].

The alveolar wall destruction and loss of elastic recoil that occur in COPD are believed to be the
result of chronic inflammation and imbalance of antioxidants [7,9]. This oxidative stress causes a protease/antiprotease imbalance and is believed to be a contributing factor toward the pathogenesis of COPD [9].

Vitamin D is a steroid hormone that is synthesized in the epidermal keratinocytes under influence of UV-B light (290-315nm) or acquired in the diet. Dietary sources include supplemented dairy products, fish oil, fish liver and eggs. It is estimated that approximately 3 percent of the human genome is regulated directly or indirectly by the vitamin D endocrine system [10,11].

Recent research has revealed new sites of action that may force the re-examination of vitamin D and its role in human physiology. Vitamin D receptors (VDRs) have been found in organs not typically believed to be involved with bone metabolism, including the pancreas, gonads, liver, heart, brain and breast, as well as the hematopoietic and immune systems [10].

Vitamin D deficiency has been established as exceedingly prevalent in many of chronic lung disease populations [12]. COPD patients without any glucocorticoid use had significantly decreased 25-hydroxyvitamin D levels when compared with age-matched controls [13]. Children with a diagnosis of wheezy bronchitis had more than two-and-a-half times the incidence of rickets than the age-matched controls. Also it was noted a 10 times higher incidence of wheezy bronchitis when severe rickets was present [14]. Limited studies [15,16] in patients with chronic lung disease suggest that bone mineral density is correlated with lung function, whereas another study [17] was unable to confirm this.

Recent studies have shown that vitamin D has pleiotropic protective effects [18,19]. 1,25 (OH)2D3 (1,25-dihydroxyvitamin D3), an active metabolite of vitamin D is also a potent regulator of the immune response in Th1 cell-directed diseases [20,21]. Sunder and colleagues [22] have recently shown that VDR deficiency invokes lung inflammation and alterations in lung function. Hence, understanding the molecular mechanisms of dietary vitamin D for the treatment of lung disease and their exacerbations are an emerging area of research [23].

Skeletal muscle dysfunction is common in patients with advanced COPD and it contributes importantly to limiting their functional capacity and quality of life [24]. The role of exercise training in pulmonary rehabilitation of patients with severe COPD has been studied [25]. Exercise training in COPD patients results in positive effects in dyspnea and exercise tolerance [25,26], however, the mechanisms by which exercise affect the pulmonary functions in COPD have not been determined.

Aim of work: Accordingly, the purpose of the present study was to investigate the effect of vitamin D supplementation and/or physical training on pulmonary functions (peak expiratory flow rates (PEF) and forced vital capacity (FVC)) in a rat model of COPD induced by 3 months exposure to cigarette smoke. The effect of vitamin D supplementation and physical training on vitamin D level and calcaemic state, lung inflammation as determined by lung expression of TNF-α and IL-12 was measured. The effect of vitamin D and physical training on the antimicrobial protein cathelicidin and tissue breakdown as indicated by measuring lung metalloproteinase 9 (MMP-9) was also investigated.

Material and Methods

Experimental animals and groups:

Forty male Albino rats, weighing 100-120g, were used in this study. This work was conducted in the Physiology department, Cairo University from May 2013 – October 2013. The rats were kept under standard conditions. Placed in cages, at 20±5°C, average humidity, and normal light/dark cycles. Standard chow and water were available ad libitum.

Rats were divided into 5 groups:

Group 1: Control group (8 rats): These are normal rats serving as control rats for the different values measured in the other groups.

Remaining rats were exposed to passive cigarette smoke for 3 months in order to develop COPD and then tested by pulmonary function tests to examine the development of COPD.

32 male rats were daily exposed to the smoke resulted from burning of 12-15 cigarettes. COPD was induced by cigarette smoking exposure technique: Cigarette smoke (CS) exposure was achieved by same procedure which had been used in a previous study [27] with some modification in periods of exposure and number of cigarettes. Popular Egyptian filter-tipped cigarette were used containing (25mg) tar and (1.8mg) nicotine.

The exposure to the CS was initial progressive concerning the burned material mass and the exposure time in order to permit the biological adaptation of the animals and to avoid accidents such as smoke intoxication that could determine death of rats.
The cigarette smoke exposure lasts for 3 months. The rats were randomly divided into 4 groups after developing COPD:

Group 2: (COPD group): Eight rats were included in this group and maintained untreated for the experiment period.

Group 3: (Vit.D+COPD): Eight COPD rats were treated with vitamin D injection. 25(OH)D$_3$ was administered intraperitoneally (i.p.) at dose 0.5 g/kg BW, 3 times a week for 8 weeks [28].

Group 4: (COPD+Exercise): Eight COPD rats performed daily exercise program.

The exercise protocol consisted of swimming exercise (1hr/day, 5 days/week) [29] for 8 weeks in a swimming tank filled with water at a temperature of 36°C. Daily swimming period was divided into 2 sessions each formed of 30 minutes separated by 1 hour rest. At the completion of each period of swimming exercise, the rats were removed from the water, carefully dried and returned to their cages. The exercised rats underwent a swimming programme consisting of gradually increasing periods of swimming in the first 4 days the duration of exercise was gradually increased from an initial period of 15min to the maximum permissible period of 30min.

Group 5: (COPD+Vit.D+Exercise) eight COPD rats treated with vitamin D injection (i.p.) at dose 0.5 g/kg, 3 times a week for 8 weeks and performed daily exercise program similar to the protocol tried by group 4.

After 8 weeks, the animals were transferred to National Research Centre, Cairo, Egypt where pulmonary functions were assessed and blood samples were withdrawn by capillary tubes and left to clot to get the serum for measurement of vitamin D and Ca2+. The animals were then sacrificed and their chests were opened and the lungs were excised for measurement of IL1 2, TNF alpha, cathelicidin and MMP-9.

**Estimation of vitamin D and calcium:**
Blood samples were withdrawn and left to clot for 20min then centrifuged at 12,000rpm for 10 min then the separated serum was kept frozen at -80°C till analysis. Serum samples were examined for 25(OH) D levels by Enzyme-linked immuno- sorbent assay (ELISA) by kit supplied by (Immundiagnostic USA) briefly, monoclonal antibody identify 25, OH vitamin D was used in the assay. The samples were incubated with the detection antibody after the extraction step. Then Peroxidase-conjugated antibody was then added into microplate well, forming a complex of 25-hydroxy vitamin D-detection antibody-peroxidase conjugate. Tetramethylbenzidine (TMB) was used as a substrate, the colour density developed is proportional to vitamin d concentration. Finally, to terminate the reaction stop solution was added and the microplate were read by elisa reader at 520nm [31]. Serum calcium concentrations were measured by standard laboratory methods.

**Measurement of TNF-α and IL-12:**

TNF-α and IL-12 in lung tissues were measured by using ELISA (quantikine R&D system USA) according to the manufacturer’s instructions [32,33].

**Detection of MMP-9 & cathelcidin gene expression using real time:**

PCR (RT-PCR):

**RNA extraction:**

Total RNA was isolated from lung tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer’s instruction. The purity (A260/A280 ratio) and the concentration of RNA were obtained using spectrophotometry (GeneQuant 1300, Uppsala, Sweden). RNA quality was confirmed by gel electrophoresis.

**cDNA synthesis:**

First-strand cDNA was synthesized from 4 μg of total RNA using an Oligo (dT) 12-18 primer and SuperscriptTM II RNase Reverse Transcriptase, This mixture was incubated at 42°C for 1h, the kit was supplied by Super Script Choice System (Life Technologies, Breda, the Netherlands).

**Real-time quantitative polymerase chain reaction (PCR):**

Real-time PCR (RT-PCR) amplification was carried out using 10 μL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA USA), equiv-
Abstract

Background: Chronic obstructive pulmonary disease (COPD) is a major health problem with increasing morbidity and mortality. Vitamin D deficiency has been established as exceedingly prevalent in many of chronic lung disease populations and exercise training in COPD patients results in positive effects in dyspnea and exercise tolerance.

Aim of Work: The purpose of the present study was to investigate the effect of vitamin D supplementation and/or physical training on pulmonary functions, lung inflammation, antimicrobial production and matrix degradation in a rat model of COPD.

Methodology: Forty male Albino rats were used in this study and divided into 5 groups, 8 rats each: Group 1: Control group, Group 2: (COPD group): COPD rats maintained untreated for the experimental period, Group 3: (Vit. D+COPD): COPD rats were treated with vitamin D injection 1, 25 (OH) D3 was administered intraperitoneally (i.p.) at dose 0.5 µg/kg of body weight (BW), 3 times a week for 8 weeks, Group 4: (COPD+ Exercise): COPD rats performed daily exercise program and group 5: (COPD+Vit D+exercise) COPD rats treated with vitamin D injection (i.p.) at a dose of 0.5 µg/kg 3 times a week for 8 weeks and performed daily exercise program. After 8 weeks of treatment, pulmonary functions were tested and blood samples were withdrawn for measuring vitamin D and Ca2+ levels and the lung tissues were excised to measure interleukin 12 (IL12), tumor necrosis factor alpha (TNF alpha), metalloproteinase-9 (MMP-9) and cathelicidin.

Results: Peak expiratory flow (PEF), forced vital capacity (FVC), vitamin D and Ca2+ were significantly reduced in COPD rats after 12 weeks of exposure to cigarette smoke. Vitamin D supplementation and swimming training for 8 weeks improved PEF, FVC, vitamin D and Ca2+ significantly as compared to untreated COPD. Combined vitamin D treatment and physical training significantly improved FVC level as compared with each treatment separately. The improvement was associated with significant reduction in inflammatory markers and MMP-9 as compared to COPD untreated rats. The antimicrobial cathelicidin was significantly increased in COPD rats and was further increased on vitamin D treatment but not with exercise training.

Conclusion: Our results showed that COPD is an inflammatory disease and it is associated with vitamin D deficiency. Vitamin D supplement or rehabilitation by physical training each separately improved the pulmonary functions, reduced inflammation, and attenuate lung parenchymal degradation. Vitamin D in addition induced an antimicrobial protection, however vitamin D supplement had a slightly better effects as compared with exercise training. Combination of both vitamin D supplementation and exercise training had a synergistic effect and produced a significant improvement as compared to each therapy separately. We can conclude that

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9</td>
<td>Forward: 5’- CATTCGGTGAGAAGGATGTAACCTGCATGGTC -3’ according to gene bank accession number NM-013599</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’- ACCCTGGTTCACTCATGGTC -3’</td>
</tr>
<tr>
<td>Cathelicidin</td>
<td>Forward: 5’- AGGATTGTGACTTCAAGAAGGACG -3’ according to gene bank accession number XM_005253678.1</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5´ GTTTATTTCTCAGAGCCAGAGC -3´</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward 5´ TGCTGAGCTGATGCATGCAGC -3´ according to gene bank accession NM_017008</td>
</tr>
<tr>
<td></td>
<td>Reverse 5´ TTGAGAGCAATGCCAGCC 3´</td>
</tr>
</tbody>
</table>

Table (1): Changes in pulmonary functions (PEF and FVC) in untreated COPD rats and in COPD rats after 8 weeks of either vitamin D supplementation, exercise training or both.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>COPD</th>
<th>COPD+ Vit D</th>
<th>COPD+ exercise</th>
<th>COPD+ vit D+exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF (mL/min)</td>
<td>13.65±1.04a</td>
<td>6.78±1.15b</td>
<td>10.36±0.87c</td>
<td>8.80±0.89d</td>
<td>10.45±1.02c</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>9.24±0.78a</td>
<td>4.81±0.85b</td>
<td>7.90±0.54c</td>
<td>6.21±0.56e</td>
<td>8.43±0.63a</td>
</tr>
</tbody>
</table>

- (n=8).
- Values are Mean±SD.
- Values with different letters in the same row are significantly different from each other (p<0.05).
- Values with the same letters in the same row are insignificantly different from each other (p>0.05).
Effect of COPD, vitamin D supplementation, and exercise training on vitamin D levels and serum Ca2+ level in rats:

As expected from previous researches the present results showed deficiency of vitamin D and a hypocalcaemic state in rats with COPD and the levels of vitamin D and Ca2+ were significantly reduced in COPD rats as compared to control rats. It can be seen from Table (2) and Figs. (3,4) that vitamin D supplementation and/or exercise corrected the deficiency and the values of these two parameters were back to normal control values.

Changes in inflammatory markers TNF alpha and IL12 in lung tissues in untreated COPD rats and in COPD rats after 8 weeks of either vitamin D supplementation, exercise training or both:

The results of the present work confirmed that COPD is an inflammatory disease. As shown in...
Effect of Vitamin D Supplementation and/or Physical Training on COPD patients

Inflammatory effect and significantly reduced the level of TNF alpha and IL12 as compared to the untreated COPD rats but the reduction was significantly less than that produced by vitamin D treatment. Combined treatment with vitamin D and physical training induced a further significant decline in the levels of inflammatory markers as compared to each treatment alone.

Table (3): Effects of vitamin D supplement and physical training for 8 weeks on lung inflammatory markers (TNF alpha and IL12), matrix degradation (MMP-9) and antimicrobial (cathelicidin) in COPD rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>COPD</th>
<th>COPD+ Vit D</th>
<th>COPD+ exercise</th>
<th>COPD+ vit D+ exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12 (pg/ml) Mean±S.D.</td>
<td>30.56±4.05a</td>
<td>113.66±19.75b</td>
<td>56.93±7.03c</td>
<td>86.86±5.09d</td>
<td>44.99±4.60e</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml) Mean±S.D.</td>
<td>46.60±9.53a</td>
<td>266.56±59.59b</td>
<td>133.24±8.96c</td>
<td>152.63±17.78d</td>
<td>102.46±9.18e</td>
</tr>
<tr>
<td>MMP-9 Mean±S.D.</td>
<td>1.35±0.32a</td>
<td>9.51±1.85b</td>
<td>5.42±0.70c</td>
<td>6.62±0.35d</td>
<td>4.68±0.56</td>
</tr>
<tr>
<td>Cathelicidin Mean±S.D.</td>
<td>0.14±0.03a</td>
<td>0.38±0.06b</td>
<td>0.86±0.12c</td>
<td>0.39±0.13b</td>
<td>0.84±0.10b</td>
</tr>
</tbody>
</table>

- (n=8).
- Values are Mean±SD.
- Values with different letters in the same row are significantly different from each other (p<0.05).
- Values with the same letters in the same row are insignificantly different from each other (p>0.05).

Fig. (5): Effects of vitamin D supplement and physical training for 8 weeks on lung inflammatory marker (IL12) in COPD rats (Mean±SD).

Fig. (6): Effects of vitamin D supplement and physical training for 8 weeks on lung inflammatory marker (TNFalpha) in COPD rats (Mean±SD).

Effect of COPD, vitamin D supplementation, and exercise training on mRNA of lung matrix (MMP-9):

Table (3) and Fig. (7) show that in rats developed COPD there is enhanced degradation of lung parenchyma as indicated by elevated level of MMP-9 in lung tissues. Treatment of COPD rats with vitamin D for 8 weeks or exercise significantly reduced destruction of lung tissue by attenuation of MMP-9 production as compared to the untreated rats. It can be observed that vitamin D supplementation caused a more significant reduction in MMP-9 production than did exercise training. Combining both therapy together induced a better improvement and a significant reduction in MMP-9 in lung tissues as compared to each therapy alone.

Effect of COPD, vitamin D supplementation, and exercise training on mRNA of antimicrobial cathelicidin in lung tissue:

Table (2) and Fig. (8) showed that COPD was associated with a significant increase in the level of antimicrobial cathelicidin. Vitamin D supplementation for eight weeks alone or in combination with exercise training induced a further significant increase in its value. Exercise alone had no effect on the lung level of cathelicidin as compared to COPD untreated group.
Discussion

Chronic obstructive pulmonary disease (COPD) has been defined as a preventable and treatable pathologic condition characterized by partially reversible airflow limitation [35]. It is well known that cigarette smoke is one of the most important risk factors for COPD, and it can accelerate the development of COPD in humans [36]. In this study, we found that exposure to cigarette smoke caused COPD after 12 weeks in male albino rats; it produced a significant reduction in pulmonary function as detected by a significant decrease in mean values of PEF and FVC as compared to control group.

Short term exposure to noxious gases as cigarette smoke (days) [37] resulted in a pulmonary inflammatory infiltrate, increased mucus production, and pulmonary edema. Long term induction protocols (weeks or months) [38] produced, in addition to the inflammatory infiltrate, emphysema and pulmonary remodeling characterized by fibrosis, and thickened bronchiole and arterial walls.

In the present study, we found that vitamin D was deficient in rats with COPD and that the dose of vitamin D supplemented in our study was enough to raise the blood vitamin D level to be comparable with the control value. Exercise protocol performed in COPD rats also was able to significantly increase vitamin D level as compared to COPD untreated rats. In rats with COPD treated with both vitamin D and exercise, there was a significant increase in vitamin D levels as compared with the COPD untreated rats but the increase was insignificant when compared with vitamin D or exercise treated rats.

There are several factors that could account for vitamin D deficiency in COPD patients: Poor diet, a reduced capacity of aging skin for vitamin D synthesis, reduced outdoor activity and therefore sun exposure, an increased catabolism by glucocorticoids, impaired activation because of renal dysfunction, and a lower storage capacity in muscles or fat due to wasting [38]. Many steps of the vitamin D pathway (intake, synthesis, storage, metabolism) can potentially be disturbed in COPD patients.

In agreement with our results, Forli et al., [12] found vitamin D deficiency (in their study defined as below 20ng/ml) in more than 50% of a cohort waiting for lung transplantation. In an outpatient study on patients with COPD in Denmark, 68% of the participants had osteoporosis or osteopenia [40]. A recent study showed that vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D binding gene [41].

Moderate endurance exercise increases serum 1,25(OH)2D3 level [42,43]. Also, Sato et al., [44], reported that immobilization, in contrast to endurance exercise, 1,25-(OH)2D3 are suppressed. However, Maimoun et al., [45], reported that in exercise trained rats, serum 1,25-(OH)2D3 concentration was not affected.

In this study vitamin D supplementation, exercise training or both significantly improved pulmonary functions compared with COPD untreated group.

In agreement with our results, a strong relationship between serum levels of vitamin D and lung function (FEV1 and FVC) was found [46,47]. In another study, 25(OH)D was correlated with FEV1 [48]. Ferrari et al., [49] also demonstrated that the maximal exercise capacity and carbon monoxide transfer in the single breath method were both positively correlated with serum 25(OH)D concentrations.
A cross-sectional study found that higher plasma levels of vitamin D are associated with increased bone mineral density and exercise capacity in people with COPD [50]. Evidence also showed that high dose vitamin D supplementation improved respiratory muscle strength and exercise capacity in people with COPD [51]. Epidemiological studies revealed a dose-dependent association between serum 25(OH)D levels and pulmonary function so that adequate vitamin D supplementation may extend beyond its protection against osteoporotic fractures [52].

However Shaheen et al., [53] reported that total vitamin D intake was positively associated with forced expiratory volume in 1 s (FEV1); they did not confirm a positive association between blood 25(OH)D concentrations and adult lung function. They suggested that the apparent relationships with dietary vitamin D are likely to be explained by other highly correlated nutrients in the diet.

Also, Bjerk et al., [54], reported that short-term vitamin D supplementation in patients with COPD had no discernible effect on a simple measure of physical performance [55].

As regard the effects of physical exercise on pulmonary functions, there was a controversy. Maltais et al., [56] reported improvement of pulmonary functions FEV 1 and demonstrated physiologic gain following 12 weeks of exercise in persons with severe COPD. Similar improvement in physiologic parameters have been confirmed by several additional studies [57,58].

On the other hand, Flo et al., [59], reported worsening of pulmonary emphysema induced by exercise training. Their hypothesis is that the increase in mechanical forces on the connective tissue may contribute to the worsening of pulmonary function. One alternative explanation for the worsening of emphysema induced by exercise was the presence of exercise-induced oxidative stress. It has been demonstrated that strenuous aerobic exercise is associated with oxidative stress and tissue damage [60,61]. However, moderate exercise training is associated with adaptive responses in at least some antioxidant capacities [62].

The beneficial effect of vitamin D supplementation and or exercise on pulmonary functions could depend on the calcemic effects of vitamin D.

In the present study it was observed that the calcium levels in the blood of COPD rats were significantly lower than normal control. Treatment of the COPD rats for 8 weeks with vitamin D improved the calcemic state of the rats to the normal control values. Also in the exercise trained group or combined vitamin D and exercise group calcium levels were increased to be insignificantly changed as compared to the control rats.

The vital capacity and total lung capacity were found to decline with an increasing number of thoracic vertebral fractures as a direct consequence of vitamin D deficiency and hypocalcemia [63]. Nuti et al., observed 3030 ambulatory COPD patients and found a strong association between COPD severity and fractures [64]. Kyphosis related to osteoporosis caused limitation in rib mobility and inspiratory muscle function and correlated with a reduction in FEV 1 and FVC [65]. The altered properties of the thoracic skeleton could result in failure of the respiratory muscles contributing to the pathophysiology of COPD.

It was seen from the results of this study that exercise also improved pulmonary functions in COPD rats and we can find that also exercise improved the calcemic state in COPD rats. Blood Ca2+ levels were increased but not significantly in exercise trained groups as compared with untreated COPD rats.

In agreement with our results, Yeh et al., reported that in exercise trained rats plasma ionized calcium slightly increased [42]. Previous studies suggest that moderate endurance exercise increases serum 1,25(OH)2D3 level, decreases urinary calcium excretion [43]. By using a flat-bed treadmill exercise, Yeh and co-workers found that the endurance exercise trained female Sprague-Dawley rats had higher duodenal active, but not passive, calcium absorption than the control [42]. Although exercise-enhanced intestinal calcium absorption is likely mediated by an increase in serum 1,25-(OH)2D3 level, exercise may also stimulate calcium absorption by changing intestinal motility and epithelial permeability [66,67].

Acute exacerbations of COPD are an important cause of hospitalization and lead to a faster decline in FEV 1 [68]. Exacerbations are triggered by viruses, bacteria, atypical strains, or a combination of these [69,70].

In the present study it was found that vitamin D treatment enhances the innate immune system by inducing the production of antimicrobial cathelicidin and was associated with a significant increase in its lung production.

The occurrence of exacerbations, which are very often caused by bacterial or viral infections, increases the severity of COPD and causes a higher
death rate in humans [71]. 1,25(OH)_{2}D_{3} is a direct regulator of antimicrobial peptides, such as cathelicidin (camp) and defensin ß2 (defß2) genes that are driven by vitamin D response elements (VDRE)-containing promoters, revealing the potential therapeutic role of vitamin D3 analogs against opportunistic infections, including the infections in the respiratory tracts which occurs in patients with COPD susceptible to exacerbations [72].

In agreement with our results, Vaziri et al. [73] reported a beneficial effect of 1,25(OH)_{2}D on the innate immunity and they also found an elevation of the antimicrobial cathelicidin in cases treated with vitamin D.

Several interventional studies examining the effect of vitamin D supplementation on the risk of influenza [74,75] and in patients with active tuberculosis showed a significantly reduced risk for influenza an improved immunity against mycobacteria [76,77]. There was also a higher rate of tuberculosis symptom improvement in children [78].

Several mechanisms could explain vitamin D’s potentially beneficial effects on infectious diseases. In addition to its antimicrobial effect, vitamin D can affect the inflammatory response. In the present study, the increase of lung production of inflammatory mediators in untreated COPD rats relative to healthy controls are in agreement with those previously reported in patients with stable COPD of similar severity and body mass index [79]. Studies have also reported increased levels of circulating cytokines (interleukin (IL)-6 and -8 and tumour necrosis factor- ß (TNF-ß)) and acute phase reactant protein (C-reactive protein (CRP), both of which reflect systemic inflammation, in the peripheral circulation of stable COPD patients [80,81].

In agreement with our results, it was reported that 1,25D (1,25-dihydroxyvitamin D) decreases TNF-alpha [82]. Other studies showed that vitamin D can modulate the activity of various immune cells and inhibit inflammatory responses [83,84]. It was reported that vitamin D-induced inhibition of IL-12 release by dendritic cells has a profound effect on T lymphocyte differentiation [85]. Vitamin D-binding protein has immunomodulatory functions pertinent to the lung, and is associated with activation of macrophages and neutrophil chemotaxis [19].

In agreement with our results, regular exercise was noted to protect against diseases associated with chronic inflammation [86]. On the other hand, American Thoracic Society reported that exercise induced oxidative stress and could, inversely, induce abnormal exercise-induced inflammation in COPD. Plasma inflammatory mediators TNF alpha and IL-6 were not significantly modified by training. Pulmonary rehabilitation can induce peripheral muscle adaptations without decreasing the levels of systemic or local muscle inflammation [87].

In our study it can be observed that matrix metalloproteinasis-9 (MMP-9) is significantly elevated in lung tissues of COPD rats and a causative role has been suggested in the development of COPD [88]. The effect of vitamin D on extracellular matrix homeostasis not only in bone tissue, but also within the lung may have a role in COPD development. In the present study, vitamin D supplementation significantly reduced MMP-9 production in lung tissues of treated COPD rats as compared with untreated controls.

In agreement with our results, Boyan and Schwartz [89] found vitamin D to be an autocrine regulator of extracellular matrix turnover and growth factor release via matrix metalloproteinases. Also Bahar-Shany et al., [90] reported that vitamin D attenuates MMP-9 production in keratinocytes and they suggested that vitamin D deficiency may lead to a reduced attenuation of MMP-9 activity resulting in enhanced degradation of lung parenchyma.

Our results showed that exercise training reduced lung production of MMP-9, suggesting that exercise training may be important not only in pulmonary rehabilitation in COPD but also as an adjuvant in the prevention and progression of lung destruction due to cigarette smoking.

Few studies investigated the effect of physical training on lung production of MMP-9 in COPD. Toledo et al., [91] found a decrease in TIMP 1 in mice exposed to cigarette smoke that was reversed by aerobic exercise.

Conclusion:

We can see from the results of this work that, COPD is an inflammatory disease and it is associated with vitamin D deficiency. Vitamin D supplement or rehabilitation by physical training each separately improved the pulmonary functions, reduced inflammation, and attenuated lung parenchymal degradation. Vitamin D in addition induced an antimicrobial protection, however vitamin D supplement had a slightly better effects as compared with exercise training. Combination of both vitamin D supplementation and exercise training had a
synergistic effect and produced a significant improvement as compared to each therapy separately. We can conclude that vitamin D supplement has beneficial effects as a therapy in cases of COPD and it is better added to rehabilitation training programs for better results.

References


27- YOUNGEUNG HONG, SANG-RAE LEE, KYU-TAE CHANG and YONGGEUN HONG: Prophylactic effects of swimming


Association of Serum Fetuin-A with Insulin Resistance in Type 2 Diabetic Patients

MOHSEN KHALID, M.D.*; GHADA HUSSEIN, M.D.**; AYAT I. GHANEM, M.D.** and GHADA A. OMAR, M.D.**
The Departments of Internal Medicine* and Clinical & Chemical Pathology**, National Institute of Diabetes and Endocrinology, (NIDE), Cairo, Egypt

Abstract

Background: Recently, epidemiological studies showed that serum fetuin-A was associated with insulin resistance and its co-morbidities, such as metabolic syndrome and type 2 diabetes.

Aim of Work: This study was designed to evaluate the association between fetuin-A and insulin resistance among Egyptian diabetic patients.

Subjects and Methods: The study included 100 Egyptian patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type-2 diabetes (62 females, 38 males) attending the outpatient clinics of NIDE and 50 normal controls. Blood pressure, body mass index, fasting and 2hr plasma glucose, HBA1c, lipid profile and serum fetuin-A were measured. Fundus examination, chest X-ray and ECG were performed for all subjects and echocardiography was done when indicated.

Results: When we analyzed the results there was no significant difference as regard serum fetuin-A between males and females included in the study. There was a significant statistical difference as regard fetuin-A among studied subjects being higher in type 2 diabetic patients (m=335.31 ± 62.63), than patients having impaired fasting glucose, impaired glucose tolerance or both (m=294.86 ± 60.23), than normal controls (m=277.59 ± 67.22) (p-value<0.05). Serum fetuin-A levels were significantly correlated with BMI (r=+0.49; p<0.01), triglycerides level (r=+0.41; p<0.01), fasting insulin level (r=+0.45; p<0.01) and HOMA (r=+0.39; p<0.01).

Conclusions: The present study demonstrates the impact of fetuin-A on insulin resistance as there was a significant association between serum fetuin-A and insulin resistance in Egyptian diabetic patients. Fetuin-A could be a modulator of insulin resistance in humans.

Key Words: Fetuin-A – Insulin resistance – Type-2 diabetes.

Introduction

Fetuin-A (α2-Heremans Schmid glycoprotein) is a circulating glycoprotein which is exclusively secreted from hepatocytes in human serum [1]. It is a carrier for growth factors, binds to and inactivates transforming growth factor (TGF)-β and bone morphogenic protein, and is a major component of mineralized bone [2]. For a long time, fetuin-A has been considered to play a crucial role in the protection from vascular calcification by solubilizing calcium and phosphorus in serum [3]. It was also reported that fetuin-A can inhibit insulin receptor autophosphorylation and subsequent downstream signaling in vitro [4]. Recently, it has been reported the associations of serum fetuin-A levels with insulin resistance and vascular complications in patients with type 2 diabetes [5]. Also, the study by Ramadan et al., (2011) [6] demonstrated that fetuin-A levels seem to be associated with the development and progression of nephropathy in type 2 diabetic patients. These data indicate that fetuin-A might be a negative regulator of insulin signaling [7]. However, the physiological significance of fetuin-A in insulin resistance in type 2 diabetes remains unclear. Therefore our aim was to investigate the association of serum fetuin-A with insulin resistance in Egyptian type 2 diabetic patients.

Subjects and Methods

The study was conducted on 100 patients with IFG, IGT and type-2 diabetes (62 females, 38 males) with age ranging from 42-63 years old and 50 normal controls. Patients and controls were divided into three groups:

Group A: 50 patients having type 2 diabetes mellitus.

Group B: 50 patients having impaired fasting glucose, impaired glucose tolerance or both (15 patients having IFG, 15 patients having IGT and 20 patients having both.

Group C: 50 normal controls.
All patients were selected from the outpatient clinics of National Institute of Diabetes and Endocrinology (NIDE), between October 2012 and September 2013. IFG, IGT and type-2 DM was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [8]. The normal control subjects, with matched age and sex, were clinically free from any recognizable diseases, not receiving any medications and represented the control group.

**Inclusion criteria:**
- Type 2 DM.
- Age between 30-65 years.

**Exclusion criteria:**
- Type 1 DM.
- Other chronic diseases.

Demographic data was recorded for each subject using self-made questionnaire. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients and normal control subjects that described the aim of the study and the procedures that would be required from them. Samples were analyzed at Clinical and Chemical Pathology Department, National Institute of Diabetes and Endocrinology.

Blood pressure (mm Hg) was measured once after 10min rest. Weight (Kg) and height (m) were measured with the subjects wearing light clothes and without shoes. The waist circumference (cm) was measured at the level of the umbilicus. BMI was calculated as weight/hight^2 (Kg/m^2) [9].

Fundus examination, chest X-ray and ECG were performed for all subjects and echocardiography was done when indicated.

**Laboratory investigations:**
Ten ml of venous blood were withdrawn from each patient in dry sterile vacutainers after overnight fasting. First part of collected blood was left to clot. Serum was rapidly separated by centrifugation. It was tested for: Fasting Bl. Glucose, Lipid profile (Total cholesterol, Triglycerides, HDL and LDL), Creatinine and Urac acid levels by using ARCHI TECT 8000 chemistry analyzer (USA, supplied by Abbott, Al kamal company Cairo, Egypt). Serum fetuin-A and Fasting Insulin levels were measured using Enzyme Linked Immunosorbent Assay (ELISA) technique according to the manufacturer’s instructions. Second part of collated blood was taken on EDTA tubes for determination of HbA 1 c level by HPLC technique according to manufacturer’s instructions. Another Serum samples were collected for 2 Hours post prandial blood glucose levels. HOMA was calculated using the formula (fasting insulin [ µIU/ml] x fasting glucose [mmol/l])/22.5.

**Statistical analysis:**
Data was expressed as the mean±S.D. Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 10.0, 1999, Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare the variables between groups.

**Results**
The baseline characteristics of subjects included in the study are shown in Table (1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42</td>
<td>63</td>
<td>43.47</td>
<td>15.26</td>
</tr>
<tr>
<td>BMI (Kg/m^2)</td>
<td>21.73</td>
<td>32.31</td>
<td>26.18</td>
<td>9.12</td>
</tr>
<tr>
<td>Systolic BP (Sitting) (mmHg)</td>
<td>120</td>
<td>230</td>
<td>148.89</td>
<td>32.41</td>
</tr>
<tr>
<td>Diastolic BP (Sitting) (mmHg)</td>
<td>60</td>
<td>125</td>
<td>91.47</td>
<td>13.95</td>
</tr>
<tr>
<td>Fasting Bl. Glucose (mg/d)</td>
<td>91</td>
<td>225</td>
<td>126.18</td>
<td>24.51</td>
</tr>
<tr>
<td>2hr Bl. Glucose (mg/dl)</td>
<td>119</td>
<td>342</td>
<td>213.43</td>
<td>72.11</td>
</tr>
<tr>
<td>HbA 1 c (%)</td>
<td>6.1</td>
<td>13.7</td>
<td>9.14</td>
<td>3.22</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>195</td>
<td>311</td>
<td>207.16</td>
<td>27.39</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>111</td>
<td>358</td>
<td>243.55</td>
<td>74.61</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>31</td>
<td>54</td>
<td>39.14</td>
<td>9.3</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>82</td>
<td>191</td>
<td>151.73</td>
<td>35.15</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8</td>
<td>2.9</td>
<td>1.21</td>
<td>0.93</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>4.8</td>
<td>13.2</td>
<td>8.94</td>
<td>4.37</td>
</tr>
<tr>
<td>Fasting Insulin (µIU/ml)</td>
<td>3</td>
<td>95</td>
<td>31.62</td>
<td>19.23</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7</td>
<td>19</td>
<td>6.93</td>
<td>2.31</td>
</tr>
<tr>
<td>Serum Fetuin-A (mg/l)</td>
<td>212.9</td>
<td>391.3</td>
<td>323.8</td>
<td>31.5</td>
</tr>
</tbody>
</table>

- The mean age of patients studied was 43.47±15.26.
- Serum fetuin-A concentrations were not significantly different between male and female (326.92 vs. 321.53mg/l, p=0.24).
Comparison between the three groups regarding other parameters:

As regards body mass index, there was a highly significant statistical difference among the three studied groups being higher in group A (m=33.45 ± 4.6), than group B (m=28.61 ± 3.3) than group C (m=24.7 ± 2.5) (p-value >0.001) as shown in Table (2).

As regards systolic blood pressure, there was a highly significant statistical difference among the three studied groups being higher in group A (m=151.71 ± 24.1), than group B (m=144.52 ± 20.8) than group C (m=135.46 ± 19.8) (p-value >0.001) as shown in Table (2).

Regarding diastolic blood pressure, there was also a highly significant statistical difference among the three studied groups being higher in group A (m=93.34 ± 13.2), than group B (m=83.65 ± 11.5) than group C (m=72.11 ± 10.7) (p-value >0.001) as shown in Table (2).

As regards fasting plasma glucose, there was a highly significant statistical difference among the three studied groups being higher in group A (m=131.63 ± 31.8), than group B (m=115.34 ± 22.7) than group C (m=92.38 ± 17.6) (p-value >0.001) as shown in Table (2).

Regarding 2hr plasma glucose, there was also a highly significant statistical difference among the three studied groups being higher in group A (m=282.52 ± 42.5), than group B (m=161.12 ± 38.4) than group C (m=115.72 ± 35.2) (p-value >0.001) as shown in Table (2).

Regarding HBA1c, there was also a highly significant statistical difference among the three studied groups being higher in group A (m=11.9 ± 2.6), than group B (m=6.2 ± 1.1) than group C (m=4.8 ± 0.76) (p-value >0.001) as shown in Table (2).

On comparing the three studied groups regarding triglycerides level; there was a high statistical difference among the studied groups being higher in group A (m=261.12 ± 29.2), than group B (m=199.81 ± 24.4) than group C (m=142.72 ± 17.5) (p-value >0.001) as shown in Table (2).

On comparing the three studied groups regarding LDL-C; there was a high statistical difference among the studied groups being higher in group A (m=171.36 ± 54.3), than group B (m=134.82 ± 25.4) than group C (m=91.23 ± 19.2) (p-value >0.001) as shown in Table (2).

As regards HDL-C, there was a highly significant statistical difference among the three studied groups being lower in group A (m=31.46 ± 5.7), than group B (m=37.43 ± 6.9) than group C (m=49.15 ± 8.6) (p-value >0.001) as shown in Table (2). As regards fasting insulin level, there was a highly significant statistical difference among the three studied groups being higher in group A (m=38.42 ± 15.23), than group B (m=12.23 ± 7.68) than group C (m=5.2 ± 0.49) (p-value >0.001) as shown in Table (2).

Regarding HOMA-IR, there was also a significant statistical difference among the three studied groups being higher in group A (m=8.91 ± 2.94), than group B (m=2.92 ± 0.87) than group C (m=1.2 ± 0.21) (p-value >0.05) as shown in Table (2).

Table (2): Comparison between the three groups included in the study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type 2 D.M. (Group A)</th>
<th>Impaired glucose regulations (Group B)</th>
<th>Normal glucose tolerance (Group C) (control)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>44.22±15.17</td>
<td>40.21±12.56</td>
<td>37.87±11.79</td>
<td>35.54±14.36</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>33.45±4.6</td>
<td>25.7±2.9</td>
<td>27.7±3.1</td>
<td>30.7±3.6</td>
</tr>
<tr>
<td>Systolic BP (Sitting) (mmHg)</td>
<td>151.71±24.1</td>
<td>141.62±20.3</td>
<td>143.97±21.1</td>
<td>148.12±22.7</td>
</tr>
<tr>
<td>Diastolic BP (Sitting) (mmHg)</td>
<td>93.34±13.2</td>
<td>81.79±11.1</td>
<td>82.27±11.4</td>
<td>84.89±12.5</td>
</tr>
<tr>
<td>Fasting Bl. Glucose (mg/dl)</td>
<td>131.63±31.8</td>
<td>112.92±21.3</td>
<td>94.26±18.6</td>
<td>110.37±23.7</td>
</tr>
<tr>
<td>2hr Bl. Glucose (mg/dl)</td>
<td>282.52±42.5</td>
<td>123.74±16.9</td>
<td>158.23±18.1</td>
<td>169.37±19.3</td>
</tr>
<tr>
<td>HbA 1c (%)</td>
<td>11.9±2.6</td>
<td>6.03±1.3</td>
<td>6.1±1.4</td>
<td>6.3 ± 1.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>244.49±74.5</td>
<td>201.39±32.6</td>
<td>212.57±38.3</td>
<td>121.46±44.2</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>261.12±29.2</td>
<td>181.65±21.8</td>
<td>198.62±23.5</td>
<td>212.53±24.3</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>31.46±5.7</td>
<td>39.28±7.2</td>
<td>37.45±6.3</td>
<td>36.34±5.2</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>171.36±54.3</td>
<td>126.52±21.1</td>
<td>132.36±23.4</td>
<td>143.19±34.7</td>
</tr>
<tr>
<td>Fasting Insulin (µi/ml)</td>
<td>38.42±15.23</td>
<td>7.92±2.39</td>
<td>10.62±6.83</td>
<td>16.27±9.25</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>8.91±2.94</td>
<td>1.92±0.43</td>
<td>1.81±0.39</td>
<td>2.72±0.86</td>
</tr>
<tr>
<td>Serum Fetuin-A (mg/l)</td>
<td>335.31±62.63</td>
<td>291.84±71.95</td>
<td>293.62±77.31</td>
<td>296.84±52.48</td>
</tr>
</tbody>
</table>
As regards serum Fetuin-A, there was a significant statistical difference among the three studied groups; being higher in group A \((m=335.31 \pm 62.63)\), than group B \((m=294.86 \pm 60.23)\), than group C \((m=277.59 \pm 67.22)\) \((p\text{-value} >0.05)\) as shown in Table (2) and Fig. (1).

![Fig. (1): Comparison between the studied groups regarding Serum Fetuin-A.](image)

As regards correlations between serum Fetuin-A level and other parameters; there was a high statistically significant positive correlation between serum Fetuin-A level and BMI \((r=+0.49; p<0.01)\), triglycerides level \((r=+0.41; p<0.01)\), fasting insulin level \((r=+0.45; p<0.01)\) and HOMA \((r=+0.39; p<0.01)\) as shown in Table (3).

Table (3): Correlations between serum Fetuin-A level and other parameters.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum Fetuin-A (mg/l)</th>
<th>(r)</th>
<th>(p\text{-value})</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg/m(^2))</td>
<td></td>
<td>+0.49</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td></td>
<td>+0.41</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>Fasting insulin (µu/ml)</td>
<td></td>
<td>+0.45</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td>+0.39</td>
<td>&lt;0.01 **</td>
</tr>
</tbody>
</table>

** Highly significant difference.

Discussion

Fetuin/\(\alpha_2\)-HS glycoprotein (\(\alpha_2\)-HSG) homologs have been identified in several species including rat, sheep, pig, rabbit, guinea pig, cattle, mouse and human. Multiple physiological roles for these homologs have been suggested, including ability to bind to hydroxypatite crystals and to specifically inhibit the tyrosine kinase (TK) activity of the insulin receptor [4]. In our study, we found that fetuin-A was positively correlated with fasting serum insulin and HOMA-IR, the indicators of insulin resistance in type 2 diabetes. It was well known, that insulin resistance is the underlying mechanism of type 2 diabetes [10]. Previous studies demonstrated that fetuin-A is a natural inhibitor of the insulin receptor tyrosine kinase in vitro and in rodents [11]. The rate of autophosphorylation of insulin receptor tyrosine kinase and insulin receptor substrate-1 is decreased in rat liver and skeletal muscle by injection of human recombinant fetuin-A [12]. Fetuin-A knockout mice have improved insulin sensitivity and are resistant to weight gain on a high-fat diet [13]. In humans, high fetuin-A concentrations are associated with insulin sensitivity determined during the euglycemic-hyperinsulinemic clamp or HOMA-IR index in nondiabetic subjects [14]. We added the evidence that serum fetuin-A was associated with insulin resistance indicated as elevated HOMA-IR index and fasting serum insulin levels. We could assume that insulin resistance was an essential link between fetuin-A and type 2 diabetes. We also found that serum fetuin-A was associated with insulin resistance in the diabetics. It was consistent with what was found in a study conducted on 300 Japanese men [15]. In conclusion, we found that higher fetuin-A concentrations were associated with insulin resistance, IFG, IGT and type 2 diabetes in Egyptian patients. More studies are needed to uncover the mechanisms between elevated fetuin-A and insulin resistance and type 2 diabetes.

References

7- MATHEWS S.T., SINGH G.P., RANALLETTA M., CINTRON V.J., QIANG X., GOUSTIN A.S., JEN K.L.,


The Possible Protective Effect of Ginger Against Intestinal Damage Induced by Methotrexate in Rats

OMAIMA M. ABD-ALLAH, M.D.* and ABEER A.I. SHARAF EL-DIN, M.D.**
The Departments of Pharmacology & Therapeutics* and Forensic Medicine & Toxicology**, Faculty of Medicine, Benha University

Abstract

Methotrexate (MTX) is widely used in treatment of malignant tumors and autoimmune diseases. Gastrointestinal toxicity is an important factor limiting its use. This study was performed to assess the possible protective effect of ginger on MTX-induced intestinal damage and the mechanisms involved. Forty adult rats were randomly assigned into 4 Groups: Control group, Ginger group (200mg/kg/d, orally), MTX group (single dose, 20mg/kg/i.p.) and MTX+ ginger group (pretreated with ginger 3 days before and after MTX administration). At the end of the treatment period, rats were sacrificed, and jejunal tissue samples were taken for biochemical, histological and immunohistochemical assessment. Data showed that ginger produced significant decrease in lipid peroxidation product malondialdehyde (MDA) with significant increase in glutathione content (GSH), and antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) levels compared to MTX group. Also, pretreatment with ginger resulted in significant decrease in intestinal levels of myeloperoxidase (MPO) and pro-inflammatory cytokines (TNF-α and IL-1β) levels. The total microscopic damage score of MTX+ ginger-treated rats was found to be significantly reduced compared to the MTX group. Additionally, a significant decrease in enterocyte apoptosis in the jejunum of MTX+ ginger rats was accompanied by decreased Bax protein and increased Bcl-2 protein expression. The results of this study demonstrated that administration of ginger powder protects the jejunal mucosa from damage caused by MTX. Ginger was shown to reduce oxidative stress and lessen inflammation and apoptosis in the jejunal tissues of rats with MTX-induced mucositis.

Key Words: Ginger – MTX – Mucositis – Intestinal damage – Oxidative stress – Apoptosis.

Introduction

INTESTINAL mucositis is a dose limiting side-effect of cancer chemotherapy, which leads to decreased absorption of nutrients, increased epithelial permeability, recurrent diarrhea, and weight loss [1]. Methotrexate (MTX), a folate antagonist agent, is mainly used in the treatment of malignant tumors; it has also been found to have a major therapeutic role in non-neoplastic diseases as an anti-inflammatory and immunosuppressive agent. MTX is a well-known cause of intestinal mucositis, which impairs rapidly dividing cells, such as epithelial stem cells within intestinal crypts, thereby causing diminished enterocyte replacement [2]. It has previously demonstrated that MTX administration in rats causes villus atrophy with consequent reduction of the overall mucosal absorptive surface area [3].

MTX induced small intestinal damage is characterized by marked inflammation and increased production of reactive oxygen species and therefore increased oxidative stress [4]. Huang et al., [5] demonstrated that MTX-induced apoptosis in the small intestine which is reactive oxygen species (ROS)- dependent and occurs along a mitochondria-mediated pathway. Based on this result, numerous studies aiming to prevent MTX-induced damage by using antioxidant agents such as N-Acetylcyisteine [6], lactoferrin [7], melatonin [8], prostaglandin E1 [9] and garlic extract [10] revealed beneficial effects.

Plant derived products have been used for medicinal purposes for centuries and also being used in our daily food intake. Focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems [11].

Ginger (Zingiber officinale Rosc.) has been cultivated for thousands of years as a spice and for medicinal purposes [12]. It has been used as an ingredient of Chinese traditional medicine for
thousands of years [13]. The major pungent constituents of ginger, 6-gingerol and 6-shogaol, have been shown to have many interesting pharmacological effects, such as antithrombotic [14], antioxidant, antitumor promoting and anti-inflammatory effects [15,16]. Ginger has staring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer. Also, it has antimicrobial potential as well which can help in treating infectious diseases [17-19]. Furthermore, it has been used to treat a number of medical conditions, affecting the digestive tract such as dyspepsia, flatulence, nausea and abdominal pain [20]. Aromatic, spasmyloytic, carminative and absorbent properties of ginger are probably responsible for the therapeutic applications in digestive tract ailments [21]. Ginger is generally considered a safe herbal medicine with only few and insignificant adverse/side effects [22].

Taking into consideration the potential clinical use of MTX and the numerous health benefits of ginger, the present work was planned to explore whether ginger has a possible protective effect on MTX-induced intestinal injury.

Material and Methods

Animals:
Adult male Sprague-Dawley rats weighing 200-250g were purchased from Helwan farm (VAC-SERA), Egypt. The animals were housed (4 per cage) in the animal facility of the Pharmacology Department, Faculty of Medicine, Benha University, Egypt, during June 2013 and left for one week before beginning the experiment for acclimatization. Rats were kept under the standard laboratory conditions (12h light/dark cycles at 25°C ± 2°C) with free access to standard balanced diet and freshwater supply.

Drugs:
Methotrexate (Ebewe Pharma, Austria), 50mg in 5ml was dissolved in normal saline. Ginger pure powder (Sigma), was suspended in 0.5% carboxymethylcellulose (CMC) in distilled water (vehicle) so that 1ml of the vehicle contained the desired dose. All drugs were freshly prepared immediately just before administration.

Experimental design:
A total of 40 rats were randomly assigned into four groups, 10 rats in each. The total duration of the experiment was 6 days. MTX was injected intraperitoneally (i.p.) at a single dose on day 3 of the experiment. Ginger was administered for 6 days to the second and forth groups, 3 days before and after MTX injection.

Control group: Received 1ml of saline (the vehicle of MTX) intraperitoneally once and 1ml of 0.5% CMC in water (the vehicle of ginger) by gavage for 6 days.

Ginger group: Received ginger powder (200 mg/kg, orally) [23], suspended in 1ml of 0.5% CMC in water for 6 days. Rats also received single dose of saline (1ml, i.p.) on day 3 of the experiment.

MTX group: Received a single dose of MTX (20mg/kg, i.p.). Dosage and route of administration of MTX were determined according to a model previously described [24]. Rats also received 1ml of 0.5% CMC in water orally for 6 days.

MTX+ ginger group: Received a single dose of MTX (20mg/kg, i.p.), and also received ginger (200mg/kg, orally) 3 days before and after MTX administration.

At the end of the experiment, all rats were anesthetized using urethane (0.6ml/100g body weight of 25% solution i.p.), and sacrificed by decapitation. The tissue samples of jejunum were taken from each animal for biochemical, histopathological and immunohistochemical studies.

Determination of body weight:
Body weight of all animals in each group was recorded using an electronic balance which is considered the initial body weight. At the end of the experiment, body weight was again recorded for all animals in each group.

Biochemical study:
The first portion of jejunum was washed two times with cold saline solution and homogenized using a tissue homogenizer in phosphate buffer saline (10% w/v). The homogenate was centrifuged for 10 minutes at 4°C to remove the cell debris. The clear supernatants were separated and used for determination of malondialdehyde (MDA, a biomarker of oxidative damage) according to the method of Uchiyama and Mihara [25], and reduced glutathione (GSH, a biomarker of protective oxidative injury) was also determined [26].

In addition, superoxide dismutase (SOD) was determined according to the method of Sun et al., [27] and catalase (CAT) activity was assayed using the method of Cohen et al., [28]. Tissue-associated
myeloperoxidase activity (MPO, an index of the degree of neutrophil accumulation and inflammation) was measured using a procedure similar to that documented by Hillegass et al., [29]. Evaluation of proinflammatory cytokines, tumor necrosis factor- alpha (TNF-α) and interleukin-1 beta (IL-1β) in rat small intestine cells homogenate were evaluated by ELISA assay according to manufacturer's instructions and as previously described [30,31].

**Histopathological study, including apoptotic scoring:**

The tissue samples of jejunum were fixed in 10% neutral buffered formalin and were embedded in paraffin. Sections of tissue were cut at 5-6 µm mounted on slides, stained with hematoxylin and eosin (H & E) [32]. An overall score of intestinal damage severity was assessed in stained tissue sections as follows by scoring each of the following histological observations: A- Villus shortening and fusion. B- Epithelial atrophy. C- Crypt loss. D- Inflammatory infiltrate in the lamina propria and E- Goblet cell loss as 0, none; 1, mild; 2, moderate; 3, severe. Thus, the maximum total score was 15. [6]. A total of 10 fields of section were examined per animal.

In hematoxylin and eosin-stained sections, apoptotic cells were identified under light microscopy based on its morphology including nuclear fragmentation (karyorrhexis) and cell shrinkage with condensed nuclei (pyknosis) [33]. The apoptotic cells were counted as the mean of cells in 3 visual fields of one section.

**Immunohistochemical study:**

The jejunum sections were fixed in neutral buffered formalin and processed for preparation of 5 µm paraffin section slides. Immunohistochemical technique for Bcl2 and Bax expression were performed using labeled streptavidin biotin technique according to the manufacturer’s guidelines. Bax and Bcl2 staining was cytoplasmic and shown as brown granules. Monoclonal Bcl-2 (Biovision, USA) and monoclonal bax (Santa Cruz Biotechnology, USA) antibodies were employed in immunohistochemical staining. The positive cells were counted in 100 adjacent epithelial cells and repeated in three high power fields, and the total number of positive cells was expressed as a percentage of 300. The staining was scored as 0, negative; 1, 1-5%; 2, 6-15%; 3, 16-100% [34].

**Statistical analysis:**

All data were expressed as Mean±Standard error of mean (S.E.M.). The statistical analysis of data was done by using SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 16. One-way analysis of variance (ANOVA) was used to determine statistically significant differences among the groups, and means of every two different groups were compared with Student's t-test. *p*<0.05 was considered statistically significant.

**Results**

**Body weight changes:**

The means of initial and final body weight in all groups are summarized in Table (1). The results showed that there was no statistical difference in initial body weight between all groups (*p*>0.05). Comparison of the final body weight with the initial body weight in all groups revealed a significant (*p*<0.05) increase in control and Ginger groups and significant (*p*<0.05) decrease in MTX-treated rats while MTX+ ginger-treated rats showed a non-significant decrease in final body weight (*p*>0.05).

The data also revealed that the animals treated with MTX showed significant decrease in final body weight in comparison with control or ginger-treated rats (*p*<0.001). On the other hand, the final body weight of MTX+ ginger group was significantly increased (*p*<0.05) when compared to MTX-treated rats and significantly decreased (*p*<0.05) when compared to control group.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Control group</th>
<th>Ginger group</th>
<th>MTX group</th>
<th>MTX+ ginger group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>245.73±5.32</td>
<td>241.77±4.35</td>
<td>246.28±7.35</td>
<td>244.40±5.4</td>
</tr>
<tr>
<td>Final</td>
<td>266.76±8.4</td>
<td>261.24±6.78</td>
<td>224.20±6.2</td>
<td>243.63±5.9†*</td>
</tr>
</tbody>
</table>

*Values are expressed as Mean±SE, number 10 rats for each group.

* Significant compared to Control group.

† Significant compared to MTX group.
Biochemical results:

Tissue malondialdehyde (MDA) level:

The level of MDA was found to be significantly ($p<0.0001$) higher in the MTX-treated group when compared with control group. MTX+ ginger group showed a significant ($p<0.05$) decrease in MDA level when compared to MTX group and non-significant ($p>0.05$) difference when compared to control rats. Ginger treatment alone had no effect on MDA (Table 2).

Reduced glutathione content (GSH) and antioxidant enzymes level (superoxide dismutase (SOD) and catalase (CAT)):

As shown in Table (2), administration of MTX significantly ($p<0.05$) reduced the GSH level in the jejunum tissue as compared to control group. However, reduced GSH content was significantly ($p<0.05$) increased in the group pretreated with ginger as compared to MTX group. At the same time, MTX+ ginger group showed a non significant difference ($p>0.05$) when compared to control group.

On measuring the enzyme activity of SOD; the data recorded a significant ($p<0.05$) decrease in MTX-treated rats compared with control group. Also, there was a significant ($p<0.05$) improvement in MTX+ ginger group as compared to MTX group and non-statistically ($p>0.05$) difference when compared to control rats.

The current work revealed that CAT activity was significantly ($p<0.01$) attenuated in MTX-treated rats compared to control rats. However, a significant ($p<0.05$) increase in CAT activity was observed in MTX+ ginger-treated rats as compared to MTX group. At the same time, MTX+ ginger-treated rats showed non-significant difference ($p>0.05$) when compared to control group. Ginger alone did not cause any significant ($p>0.05$) alteration in GSH, SOD or CAT compared to control group.

Tissue myeloperoxidase (MPO) activity:

As depicted in Fig. (1), tissue MPO activity was found to be significantly ($p<0.0001$) increased in MTX-treated rats when compared to the control group. However, pretreatment with ginger to MTX-treated rats produced a significant ($p<0.001$) reduction in MPO activity as compared to MTX group but still significantly ($p<0.001$) higher than the control value.

Table (2): Effect of ginger pretreatment on malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in the jejunum segment of rats exposed to MTX-induced intestinal mucositis.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (nmol/mg)</th>
<th>GSH (nmol/mg)</th>
<th>SOD (U/mg)</th>
<th>CAT (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td>0.5±0.1</td>
<td>7.9±0.8</td>
<td>61.8±6.8</td>
<td>16.6±3.8</td>
</tr>
<tr>
<td>Ginger group</td>
<td></td>
<td>0.4±0.3</td>
<td>7.5±0.7</td>
<td>57.5±8.4</td>
<td>19.2±3.4</td>
</tr>
<tr>
<td>MTX group</td>
<td></td>
<td>1.71±0.2*</td>
<td>5.07±0.9*</td>
<td>43.6±4.4*</td>
<td>9.5±2.6*</td>
</tr>
<tr>
<td>MTX+ ginger group</td>
<td></td>
<td>0.8±0.3†</td>
<td>9.3±1.5†</td>
<td>80.5±8.12†</td>
<td>21.3±4.3†</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SE, number 10 rats for each group.

*Significant compared to Control group.
†Significant compared to MTX group.

Results are expressed as Mean±SE (n=10 rats/group).

*Significant compared to Control group.
†Significant compared to MTX group.

Fig. (1): Effect of Ginger pretreatment on myeloperoxidase in jejunal tissue of MTX-induced intestinal injury.
Tumor necrosis factor-alpha (TNF-α) concentration:

Fig. (2A) demonstrates that MTX- treated rats showed a significant ($p<0.001$) increase in intestinal TNF-α level compared to control group. Pretreatment of the MTX- treated rats with Ginger abolished this increase and resulted in a significant reduction ($p<0.001$) in the level of intestinal TNF-α as compared to those in MTX- treated rats only. There was a significant difference ($p<0.001$) between MTX+ ginger treated rats and control group.

Interlukin-1 beta (IL-1β) level:

The concentration of IL-1β was significantly ($p<0.0001$) increased after the administration of MTX compared to those seen in the control rats. At the same time, pretreatment with ginger to MTX- treated rats attenuated this increase significantly ($p<0.01$) when compared to MTX group with a significant ($p<0.0001$) difference when compared to control group (Fig. 2B).

Histopathological results:

As shown in Fig. (3A), jejunal sections of control group revealed no significant pathological changes, where the jejunal mucosa showed long slender, finger like villi. Each villus had a core of loose connective tissue (extending from the lamina propria) and a covering of tall columnar epithelium with goblet cells. The lamina propria, forming the villus core contained a small lacteal vessel and was separated from the epithelium by a thin well defined basement membrane. Intestinal crypts of Lieberkühn extended from the bases of villi into the lamina propria. The tall columnar epithelial cells (enterocytes) exhibited eosinophilic cytoplasm and basophilic basal oval nuclei. The luminal surface of the villi was covered by striated (brush) border. The crypt epithelium demonstrated paneth cells among the enterocytes, which appeared pyramidal with basal rounded nuclei and apical eosinophilic granules. Light microscopic examination of the jejunum in ginger group showed normal morphology similar to that of the control group (Fig. 3B).

In the jejunal specimens of the MTX- treated rats, shortening of villus or fusion of villi and atrophy were noticed. The surface epithelium of some villi was severely distorted and those of others was markedly detached and completely separated from the underlying lamina propria. Some areas of lamina propria showed cellular inflammatory infiltration, interstitial edema, crypt loss and marked absence of goblet cells (Figs. 4-6). Apoptotic cells were also detected (Fig. 7).

The pathological changes of the jejunum of MTX+ ginger group were markedly attenuated and showed more or less normal appearance when compared to MTX- treated rats. The surface epithelium of some villi was intact and continuous but others still showed slight epithelial detachment. Some cellular infiltration and small areas of hemorrhage were detected (Fig. 8). The total microscopic score of MTX+ ginger-treated rats was found to be significantly ($p<0.05$) reduced ($6.83 \pm 1.04$) when compared to the MTX group ($13.29 \pm 2.43$). Histological intestinal damage scores of all groups are summarized in Table (3).

Apoptosis assessment revealed that the count of apoptotic cells in control and Ginger groups...
was low. Compared to the control group, MTX-treated rats tended to have an elevated number of apoptotic cells with a significant difference \((p<0.0001)\). Pretreatment of rats with ginger resulted in a significant \((p<0.0001)\) decrease in apoptotic cells when compared to MTX group and a significant \((p<0.0001)\) increase when compared to control group (Table 3).

### Table (3): Scoring values of the jejunal damage in the histopathological examination.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Ginger group</th>
<th>MTX group</th>
<th>MTX+ ginger group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus damage</td>
<td>0.0±0.0</td>
<td>0.1±0.0</td>
<td>2.75±0.26*</td>
<td>1.10±0.18*†</td>
</tr>
<tr>
<td>Crypt damage</td>
<td>0.2±0.0</td>
<td>0.1±0.0</td>
<td>2.63±0.38*</td>
<td>1.15±0.22*†</td>
</tr>
<tr>
<td>Epithelial atrophy</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2.38±0.11*</td>
<td>1.7±0.15*†</td>
</tr>
<tr>
<td>Cellular infiltration</td>
<td>0.3±0.0</td>
<td>0.4±0.0</td>
<td>2.95±0.27*</td>
<td>1.25±0.16*†</td>
</tr>
<tr>
<td>Goblet cell depletion</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2.58±0.25*</td>
<td>1.63±0.26*†</td>
</tr>
<tr>
<td>Total score of damage</td>
<td>0.5±0.0</td>
<td>0.6±0.0</td>
<td>13.29±2.43*</td>
<td>6.83±1.04*†</td>
</tr>
<tr>
<td>Apoptotic cell count</td>
<td>41.97±1.1</td>
<td>40.15±0.6</td>
<td>82.76±2.6*</td>
<td>57.15±0.8*†</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SE, \((n=10/group)\).
* Significant compared to Control group.
† Significant compared to MTX group.

Fig. (3): Photomicrographs of section of jejunum of Control rats (A) and Ginger group (B) showing normal morphology with intact villi, normal crypts and goblet cells (H & E x200).

Fig. (4): Photomicrograph of section of jejunum of MTX-treated rats showing villus fusion, crypt loss and goblet cells depletion with inflammatory infiltration (H & E x100).

Fig. (5): Photomicrograph of section of jejunum of MTX-treated rats showing severe shortening of villi with blunting surface and goblet cell depletion (H & E x100).
Fig. (6): Photomicrograph of section of jejunum of MTX-treated rats showing mucosal sloughing, loss of normal villus structure and small areas of hemorrhage (H & E x200).

Fig. (7): Photomicrograph of section of jejunum of MTX-treated rats showing apoptotic cells (arrows) and inflammatory infiltrate in lamina propria (H & E x400).

Fig. (8): Photomicrograph of section of jejunum of MTX+ Ginger treated rats showing preservation of normal height of the villi with small areas of hemorrhage in lamina propria (H & E x200).

**Immunohistochemical results:**

Immunohistochemistry was used to examine distribution of Bcl-2 family members in the jejunum of experimental rats. The Bax and Bcl-2 positive staining was located in the cytoplasm of intestinal epithelial cells. Goblet cells did not stain positively for any protein investigated. Bcl-2 staining was observed in the cytoplasm of villus epithelial cells and in the lamina propria but was almost negligible in the crypts. Bax staining was observed in the cytoplasm of villus and crypts. Sections of jejunal tissues of control and Ginger groups stained immunohistochemically for Bax and Bcl-2 delineated the normal distribution of Bax and Bcl-2 (Figs. 9A, 10A).

As shown in Table (4), Bax expression was up-regulated in jejunum \((p<0.001)\) of MTX- treated rats compared to control animals. Pretreatment of MTX- treated rats with ginger attenuated the pro-apoptotic effects of MTX. MTX+ ginger rats showed a significant \((p<0.001)\) decrease in Bax expression in the jejunum compared to MTX group with a significant \((p<0.05)\) increase when compared to control rats (Figs. 9B-D). On the other hand, treatment with MTX resulted in a significant \((p<0.001)\) down-regulation of Bcl-2 in jejunum compared to control group. MTX+ ginger rats showed a significant \((p<0.05)\) increase in Bcl-2 expression in jejunum compared to MTX- animals with a significant \((p<0.001)\) decrease when compared to control rats (Fig. 10B-D).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>Ginger group</th>
<th>MTX group</th>
<th>MTX+ ginger group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bax</td>
<td>0.43±0.5</td>
<td>0.48±0.7</td>
<td>2.75±0.19*</td>
<td>1.85±0.11 †</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>1.93±0.23</td>
<td>1.86±0.17</td>
<td>0.47±0.20*</td>
<td>0.95±0.11 †</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SE, \((n=10/group)\).

* Significant compared to Control group.
† Significant compared to MTX group.
The Possible Protective Effect of Ginger Against Intestinal

Fig. (9): Immunohistochemical staining of Bax in jejunum of the experimental rats from (A) Control group and (B) Ginger group (C) MTX group showing a significant increase in immunoreactivity in the cytoplasm of the cells when compared to control or ginger group; (D) MTX+ ginger group demonstrating a significant reduction in Bax immunostaining when compared to MTX group. Brown color indicates Bax positivity (Immunostaining x400).

Fig. (10): Immunohistochemical staining of Bcl2 in jejunum of the experimental rats from (A) Control group and (B) Ginger group; (C) MTX group showing a significant decrease in immunoreactivity in the cytoplasm of the cells when compared to control or ginger- treated rats; (D) MTX+ Ginger group demonstrating a significant increase in Bcl-2 immunostaining when compared to MTX group. Brown color indicates Bcl-2 positivity (Immunostaining x400).
Discussion

Methotrexate (MTX) is widely used as a chemotherapeutic agent. It is currently the most common anti-rheumatic drug prescribed for the treatment of rheumatoid arthritis and other rheumatic disorders. However, one of the major toxic effects of MTX is intestinal injury and enterocolitis \[35\]. The small intestinal damage induced by MTX treatment results in malabsorption and diarrhea \[36\].

In the present study MTX treatment in rats resulted in significant weight loss which implies the presence of mucosal injury and malabsorption. In accordance with this, the morphological appearance of the intestinal mucosa revealed marked villous shortening and fusion, epithelial atrophy, crypt loss, inflammatory infiltrate, and goblet cell depletion. Our findings were similar to other studies where MTX has been reported to cause severe damage in the small intestine \[37,38\]. The inflamed intestine demonstrates the presence of oxidative stress, as evidenced by enhanced lipid peroxidation (as indicated by increase in MDA content and reduction in reduced glutathione). It is well established that depletion of reduced glutathione in tissues promotes oxidative stress and tissue injury \[39\]. Thus significant decrease in reduced glutathione level promoted by MTX, leads to a reduction of effectiveness of the antioxidant enzyme defense system, thereby sensitizing the cells to reactive oxygen species (ROS) \[40\]. Many studies have shown a decrease in the level of reduced glutathione in the small intestine following MTX administration \[8,41,42\]. With respect to the activities of other antioxidant enzymes, superoxide dismutase and catalase, a significant decrease in the activities of both enzymes was observed in the present study. Many studies also support the view that oxidative stress plays a role in MTX-induced small intestinal damage \[6,42\]. Reactive oxygen species trigger the accumulation of leukocytes in the tissues, and thus aggravate tissue injury indirectly through activated neutrophils. The activated neutrophils secrete myeloperoxidase (MPO) and other proteases \[43\]. In turn, MPO plays important role in oxidant production by neutrophils. In the current study a marked elevation in MPO activity was observed after MTX treatment of rats, indicating that neutrophil accumulation contributes to MTX-induced small intestinal damage.

In this study, the administration of Ginger powder significantly enhanced intestinal recovery following MTX-induced damage. This is evident from the significant decrease in MTX-induced body weight loss indicated that ginger reserves the mucosal function. Histologically, ginger-treated rats showed more preserved architecture as well as the presence of newly formed crypts and regeneration. In addition, ginger significantly increased the levels of reduced glutathione and the antioxidant enzymes (i.e., catalase and superoxide dismutase) and decreased MDA content and MPO activity. These results were in accordance with other published studies \[44-46\] in which ginger was demonstrated to be a strong antioxidant. Its antioxidant activity has been attributed to its major active phenolic ingredients (e.g., zingerone, gingerdiol, zingibrene, gingerols and shogaols). In addition, the administration of ginger has been shown to improve oxidative stress by decreasing lipid peroxidation and protein oxidation as free radical-generating sources and elevating the levels of enzymes implicated in the antioxidant defense system. A study by Ahmed et al., \[47\] showed that Ginger has an equal antioxidant effect to that of ascorbic acid. Animal modeling showed that ginger significantly lowered induced lipid peroxidation and raised the levels of antioxidant enzymes, together with serum glutathione. In one study, ethanol extract of zingeriber officinale alone and in combination with vitamin E partially ameliorated cisplatin-induced nephrotoxicity. This protection is mediated by renal antioxidant defense system \[48\]. In the other study, the protective effect of the ginger extract was examined on carbon tetrachloride (CCl₄) and acetaminophen-induced liver damage and indicated that ginger could be useful in preventing acute liver injury \[49\].

Cell loss in the small intestine MTX-induced mucositis is mainly regulated by programmed cell death. In addition, oxidative stress is known to induce apoptosis by damaging DNA, oxidizing membrane lipids, and/or directly activating the expression of the genes/proteins responsible for apoptosis \[50,51\]. Our results showed that the intrinsic pathway, with its regulation by the Bcl-2 family of proteins, was altered by MTX consistent with changes in cell apoptosis: The expression of the pro-apoptotic protein Bax increased, while those of the anti-apoptotic Bcl-2 protein decreased. These changes correlate with the enhanced enterocyte apoptosis during MTX-induced mucositis. In consistent with our results, Verburg et al., \[52\] found that MTX induced apoptosis at days 1 and 2 after MTX treatment in the proliferative region of small intestinal crypts, as judged by morphological criteria and TUNEL staining.
In this study, results showed that rats treated with Ginger powder had a decrease in apoptosis in the injured intestinal tissues, as the expression of protein levels of the pro-apoptotic Bax decreased, while those of the antiapoptotic Bcl-2 protein levels increased suggesting increased enterocyte survival. Therefore, the inhibitory effect of ginger on MTX-induced ROS production may be the underlying mechanism for its protective effect against apoptosis, which plays a role in the pathogenesis of MTX-induced mucositis.

In accordance with our results, Lee et al. [53] reported that (6)-gingerol protected against β-amyloid-induced cytotoxicity and apoptotic cell death, such as DNA fragmentation, disruption of mitochondrial membrane potential, elevated Bax/Bcl-2 ratio, and activation of caspase-3, by inhibition of intracellular accumulation of reactive oxygen species and/or reactive nitrogen species and subsequent oxidative and/or nitrosative damages. Recently, Kim and Kwon [54], suggested that following oxidative stress, (6)-shogaol protects astrocytes from oxidative damage and apoptosis by attenuating the impairment of mitochondrial function proteins such as Bcl-2 and Bcl-xL. Additionally, (6)-shogaol inhibits the expression of the apoptotic proteins Bax and caspase-3 in hydrogen peroxide-treated astrocytes.

In the current study the intestinal inflammatory response appeared higher in MTX-treated rats than in control, since we observed an increase in the inflammatory cellular infiltration histologically. Furthermore, the levels of TNF-α and IL-1β in the jejunal mucosa were significantly increased. It has been suggested that proinflammatory cytokines, e.g., TNF-α and IL-1β, may be involved in the amplification phase of intestinal mucositis [58] but that inflammation may be the functional consequence of the weakened barrier function, i.e., weakened epithelial integrity (atrophy and altered protein metabolism) and altered mucus protection. Alamir et al., [56] found that intramuscular concentration of proinflammatory cytokines, interleukin-1β and cytokine-induced neutrophil chemoattractant, was markedly increased in methotrexate-treated rats.

Our results revealed that Ginger decreased the level of TNF-α and IL-1β significantly in the jejunal mucosa. The effect on inhibition of TNF-α production by the ginger extract was earlier reported in synoviocytes by Frondoza et al., [57]. Extensive studies in recent years have displayed that ginger and its pungent ingredients exhibit anti-inflammatory responses. Gingerol, shogaol, and other structurally-related substances in Ginger can inhibit synthesis of pro-inflammatory cytokines such as IL-1, TNF-α, and IL-8 [58]. In another investigation, Pan et al., [59] showed that in macrophages, (6)-shogaol can down-regulate inflammatory inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) gene expression. Jung, et al., [60] indicated that rhizome hexane fraction of Z. officinale inhibited the excessive production of NO, PGE2, TNF-α, and IL-1β. In addition, Habib et al., [61] showed that Ginger extract can reduce the elevated expression of NF κB and TNF-α in rats with liver cancer. Ko and Leung, [45] reported that ginger extract mainly inhibits the expression of the chemokines and to some extent TNF-α. It significantly reduces the gastric ulcer area in a dose-dependent manner, with concomitant attenuation of the elevated activities of xanthine oxidase and myeloperoxidase, as well as malondialdehyde level in the ulcerated mucosa. Studies by Nonn et al., [62] have shown that (6)-gingerol inhibited the TNF-α, and IL-1β-induced increase in the p38-dependent NF-κB activation and expression of pro-inflammatory genes of IL-6 and IL-8 in normal prostatic epithelial cells. Our results are in accordance with these studies and suggest an effect of ginger on inflammatory processes at cell level.

In conclusion:

Our findings provide evidence that Ginger may be used to protect against severe intestinal injury induced by MTX. The possible mechanisms of ginger effects in attenuating tissue injury and apoptosis in the intestine involves the anti-inflammatory and anti-oxidant actions of ginger, including reduced proinflammatory cytokines (TNF-α and IL-1β) and increased antioxidant enzyme activities, decreased oxidative stress and lipid peroxidation, additionally, lessened neutrophil activation. Therefore, further studies are required to establish its clinical application.

Acknowledgement:

Deepest thanks to Dr. Ghada A. Abd-Elfattah, Lecturer of Pathology, Faculty of Medicine, Benha University for her participation in performing the histopathological part of this study.

References


Abstract

Diabetes is frequently found in patients with chronic HCV infection even before the development of advanced liver disease. The underlying mechanism responsible for derangements in glucose tolerance is poorly understood.

Aim of Study: To detect the presence of anti-islet cell antibodies in hepatitis C virus infected patients and also measure insulin resistance in them, which are highly predictive for the development of type 2 DM and which may occur years before the development of manifest diabetes.

Patients and Methods: This study was conducted on 80 chronic hepatitis C patients, excluding patients co-infected with hepatitis B and patients with type 1 diabetes. All patients subjected to full clinical examination, routine chemical labs, abdominal ultrasound, child classification, islet antibodies testing, fasting insulin and HOMA-R calculation.

Results: This study showed very low prevalence of islet cell antibodies in HCV infected patients (3.74%, 3 out of 80), with no correlation between these antibodies and diabetes or insulin resistance. On the contrary insulin resistance had a very high prevalence among the patients (HOMA-R >2.5, in 80%). Insulin resistance was significantly correlated to advanced age, male sex, and presence of liver cirrhosis. A significant negative correlation was also detected between mean value of insulin resistance and serum creatinine values.

Conclusions: Islet cell antibodies does not appear to play a significant role in the pathophysiology of diabetes in HCV infected patients.

Key Words: Hepatitis C – Diabetes – Islet cell antibodies – Insulin resistance.

Introduction

Type 2 diabetes is a common complication of all liver diseases, independently of the etiology, especially at the advanced stage. However, clinical and experimental data suggest a direct role of HCV in the perturbation of glucose metabolism. The prevalence of type 2 diabetes in patients with HCV is much higher than that observed in the general population and in patients with other chronic liver diseases [1].

It is now clear that hepatitis C conveys a risk to develop diabetes mellitus, in particular type 2 [2]. Other factors, such as obesity, which is characterized by a high body mass index (BMI); advanced age and family history of diabetes, are also associated with the higher incidence of diabetes in the HCV-infected population [3].

However, the pathogenetic basis for the association between HCV infection and diabetes has not been clearly understood. A direct involvement of the virus in the development of insulin resistance has been proposed, and a direct cytopathic effect of HCV at the islet cell level leading to β-cell dysfunction in HCV-positive patients has been observed [4,5].

Aim of study:

To detect the presence of anti-islet cell antibodies in hepatitis C virus infected patients and also measure insulin resistance in them, which are highly predictive for the development of type 2 DM and which may occur years before the development of manifest diabetes.

Patients and Methods

This study was conducted on 80 patients with chronic HCV infection: 57 males and 23 females, mean age 50.93 (±8.15) years, ranging from 27-74, without diabetes being an inclusion criteria.

All the patients were inpatients in either Kasr El Aini Medical School or new Kasr El Aini teaching Hospital from January till December 2007.
Inclusion criteria:
- Patients who were previously diagnosed as chronic hepatitis C patients.
- Patients who presented with complications of chronic liver disease and were proved through hepatitis markers performed during our study to have chronic hepatitis C.

Exclusion criteria:
- Patients co-infected with hepatitis B.
- Patients with type 1 diabetes.

All patients were subjected to the following:
- Thorough history taking.
- Clinical examination.
- Complete blood count.
- Liver enzymes and liver function tests (albumin-prothrombin time and concentration & INR-total & direct bilirubin).
- Kidney function tests (blood urea & serum creatinine) and urine analysis.
- Fasting and post prandial blood sugar.
- Lipid profile (total cholesterol, LDL, HDL, & triglycerides).
- Testing for islet cell antibodies.
- Fasting insulin level.
- Calculation of insulin resistance.
- Abdominal ultrasound examination.

Islet cell antibodies:
A search for islet cell antibodies was conducted using ICA FLUOR kit, manufactured by DiaSorins.r.l. [Strada per Crescentino-Saluggia (Vercelli)], which is an indirect fluorescent antibody test system utilizing cryostat tissue sections of monkey pancreas as a substrate for detection of circulating anti-islet cell antibodies (ICA) in human serum.

Fasting insulin level:
Quantitative determination of fasting insulin level was performed by the DRG® Insulin Enzyme Immunoassay Kit.

Calculation of insulin resistance:
The HOMA-R model was used to calculate an index for insulin resistance.

Insulin resistance = Fasting plasma insulin (microunits per milliliter) X plasma glucose (millimoles per liter) / 22.5.

The HOMA cut off point of >2.5 is valid for adults.

Results

Table (1): Ultrasound, islet cell antibodies status, fasting insulin level and insulin resistance (HOMA-R) of studied patients.

<table>
<thead>
<tr>
<th>Number</th>
<th>Abdominal U/S</th>
<th>Islet cell antibodies</th>
<th>Fasting insulin (IU/ml)</th>
<th>Insulin resistance (HOMA-R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bilateral pathological kidneys, pleural effusion, liver cirrhosis</td>
<td>Negative</td>
<td>9.9</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>Liver cirrhosis, splenomegaly</td>
<td>Negative</td>
<td>5.64</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>Liver cirrhosis, splenomegaly, calculor cholecystitis</td>
<td>Negative</td>
<td>45.5</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>56</td>
<td>26.8</td>
</tr>
<tr>
<td>5</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>83.7</td>
<td>21.5</td>
</tr>
<tr>
<td>6</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>84.7</td>
<td>14.7</td>
</tr>
<tr>
<td>7</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>13.7</td>
<td>4.6</td>
</tr>
<tr>
<td>8</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>63</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>Bilharzial liver, focal splenic lesions, pathological kidneys</td>
<td>Negative</td>
<td>11.55</td>
<td>3.4</td>
</tr>
<tr>
<td>10</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>54.6</td>
<td>7.9</td>
</tr>
<tr>
<td>11</td>
<td>Liver cirrhosis, splenomegaly, ascites, portal hypertension</td>
<td>Negative</td>
<td>11.13</td>
<td>4.9</td>
</tr>
<tr>
<td>12</td>
<td>Liver cirrhosis, splenomegaly, ascites, thickened portal tracts</td>
<td>Negative</td>
<td>45.3</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>11.55</td>
<td>3.4</td>
</tr>
<tr>
<td>14</td>
<td>Liver cirrhosis, splenomegaly, ascites, pathological kidneys</td>
<td>Negative</td>
<td>14.96</td>
<td>3.1</td>
</tr>
<tr>
<td>15</td>
<td>Liver cirrhosis, splenomegaly, ascites, pathological kidneys</td>
<td>Negative</td>
<td>26</td>
<td>4.6</td>
</tr>
<tr>
<td>16</td>
<td>Fine thickened periportal tracts, calculor gall bladder</td>
<td>Negative</td>
<td>28.77</td>
<td>7.4</td>
</tr>
<tr>
<td>17</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>52.59</td>
<td>13.3</td>
</tr>
<tr>
<td>18</td>
<td>Liver cirrhosis, splenomegaly, colonic gaseous distension</td>
<td>Negative</td>
<td>83</td>
<td>21</td>
</tr>
<tr>
<td>19</td>
<td>Liver cirrhosis, hepatic focal lesions, splenomegaly, ascites</td>
<td>Negative</td>
<td>78</td>
<td>17.5</td>
</tr>
<tr>
<td>20</td>
<td>Liver cirrhosis, splenectomy, ascites, gaseous distension</td>
<td>Negative</td>
<td>11.55</td>
<td>2.9</td>
</tr>
<tr>
<td>21</td>
<td>Liver cirrhosis, splenectomy</td>
<td>Negative</td>
<td>79</td>
<td>18</td>
</tr>
<tr>
<td>22</td>
<td>Hepatomegaly with diffuse pathology, gaseous distension</td>
<td>Negative</td>
<td>14.81</td>
<td>2.6</td>
</tr>
<tr>
<td>23</td>
<td>Liver cirrhosis, splenomegaly, ascites, portal hypertension</td>
<td>Negative</td>
<td>168</td>
<td>28.3</td>
</tr>
</tbody>
</table>
Cont. Table (1).

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis &amp; Abnormalities</th>
<th>Result</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Liver cirrhosis, splenomegaly, ascites, calcular gall bladder</td>
<td>Negative</td>
<td>45.2</td>
<td>11.2</td>
</tr>
<tr>
<td>25</td>
<td>Hepatomegaly, 2 focal lesions</td>
<td>Negative</td>
<td>57.79</td>
<td>18</td>
</tr>
<tr>
<td>26</td>
<td>Hepatomegaly with diffuse pathology, calcular gall bladder</td>
<td>Negative</td>
<td>32.71</td>
<td>14.6</td>
</tr>
<tr>
<td>27</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>30.3</td>
<td>13.6</td>
</tr>
<tr>
<td>28</td>
<td>Liver cirrhosis, splenomegaly, ascites, portal hypertension</td>
<td>Negative</td>
<td>5.94</td>
<td>1.4</td>
</tr>
<tr>
<td>29</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>13</td>
<td>11.4</td>
</tr>
<tr>
<td>30</td>
<td>Liver cirrhosis, splenomegaly</td>
<td>Negative</td>
<td>10</td>
<td>2.3</td>
</tr>
<tr>
<td>31</td>
<td>Hepatomegaly, thrombosed portal vein, thickened omentum</td>
<td>Negative</td>
<td>6.09</td>
<td>1.5</td>
</tr>
<tr>
<td>32</td>
<td>Periportal fibrosis, splenomegaly, calcular gall bladder</td>
<td>Negative</td>
<td>8.23</td>
<td>2.8</td>
</tr>
<tr>
<td>33</td>
<td>Liver cirrhosis, left lobe hepatoma, splenomegaly, ascites</td>
<td>Negative</td>
<td>24.2</td>
<td>9.3</td>
</tr>
<tr>
<td>34</td>
<td>Liver cirrhosis, splenomegaly, calcular gall bladder</td>
<td>Negative</td>
<td>99.8</td>
<td>32.4</td>
</tr>
<tr>
<td>35</td>
<td>Liver cirrhosis, splenomegaly, portal hypertension</td>
<td>Negative</td>
<td>7.3</td>
<td>1.4</td>
</tr>
<tr>
<td>36</td>
<td>Liver cirrhosis, right lobe focal lesion, ascites</td>
<td>Negative</td>
<td>45.3</td>
<td>14.5</td>
</tr>
<tr>
<td>37</td>
<td>Fatty liver, gall bladder stone</td>
<td>Negative</td>
<td>12.5</td>
<td>2.8</td>
</tr>
<tr>
<td>38</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Positive</td>
<td>60.2</td>
<td>18.2</td>
</tr>
<tr>
<td>39</td>
<td>Left hydronephrosis</td>
<td>Positive</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td>40</td>
<td>Normal</td>
<td>Negative</td>
<td>32.8</td>
<td>7.3</td>
</tr>
<tr>
<td>41</td>
<td>Liver cirrhosis, focal lesions, splenomegaly, ascites</td>
<td>Negative</td>
<td>49</td>
<td>20</td>
</tr>
<tr>
<td>42</td>
<td>Average sized uniform bright liver</td>
<td>Negative</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>43</td>
<td>Liver cirrhosis, right lobe mass, splenomegaly, ascites</td>
<td>Negative</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>44</td>
<td>Liver cirrhosis, splenomegaly, ascites, portal hypertension</td>
<td>Negative</td>
<td>36</td>
<td>11</td>
</tr>
<tr>
<td>45</td>
<td>Liver cirrhosis, splenomegaly, ascites, calcular gall bladder</td>
<td>Negative</td>
<td>12.7</td>
<td>9</td>
</tr>
<tr>
<td>46</td>
<td>Normal</td>
<td>Negative</td>
<td>10.2</td>
<td>2.3</td>
</tr>
<tr>
<td>47</td>
<td>Liver cirrhosis, splenomegaly, ascites, calcular gall bladder</td>
<td>Negative</td>
<td>20.4</td>
<td>4.7</td>
</tr>
<tr>
<td>48</td>
<td>Liver cirrhosis, splenomegaly, ascites, calcular cholecystitis</td>
<td>Negative</td>
<td>82.6</td>
<td>26.8</td>
</tr>
<tr>
<td>49</td>
<td>Hepatosplenomegaly, hepatic focal lesion</td>
<td>Negative</td>
<td>21.8</td>
<td>5.1</td>
</tr>
<tr>
<td>50</td>
<td>Liver cirrhosis, calcular cholecystitis, left minimal hydronephrosis</td>
<td>Negative</td>
<td>36.1</td>
<td>14.8</td>
</tr>
<tr>
<td>51</td>
<td>Hepatosplenomegaly</td>
<td>Negative</td>
<td>11.13</td>
<td>5</td>
</tr>
<tr>
<td>52</td>
<td>Liver cirrhosis, ascites</td>
<td>Negative</td>
<td>13</td>
<td>13.7</td>
</tr>
<tr>
<td>53</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>5.5</td>
<td>1.2</td>
</tr>
<tr>
<td>54</td>
<td>End-stage kidney disease, splenomegaly, ascites</td>
<td>Negative</td>
<td>7</td>
<td>1.3</td>
</tr>
<tr>
<td>55</td>
<td>Liver cirrhosis, splenomegaly</td>
<td>Negative</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>56</td>
<td>Bright hepatomegaly, end-stage kidneys</td>
<td>Negative</td>
<td>6</td>
<td>1.4</td>
</tr>
<tr>
<td>57</td>
<td>End-stage kidneys</td>
<td>Negative</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>58</td>
<td>Coarse liver, splenomegaly, ascites, bilateral echogenic kidneys grade I, suggestive of chronic parenchymal renal disease</td>
<td>Negative</td>
<td>11.5</td>
<td>2.9</td>
</tr>
<tr>
<td>59</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>49</td>
<td>20</td>
</tr>
<tr>
<td>60</td>
<td>Liver cirrhosis, colonic gaseous distension</td>
<td>Negative</td>
<td>28.7</td>
<td>7.4</td>
</tr>
<tr>
<td>61</td>
<td>Bilateral pathological kidneys, bilateral pleural effusion, liver cirrhosis</td>
<td>Negative</td>
<td>9.8</td>
<td>2.3</td>
</tr>
<tr>
<td>62</td>
<td>Liver cirrhosis, splenomegaly</td>
<td>Positive</td>
<td>5.4</td>
<td>0.75</td>
</tr>
<tr>
<td>63</td>
<td>Liver cirrhosis, splenomegaly,cholecystitis</td>
<td>Negative</td>
<td>45.2</td>
<td>13</td>
</tr>
<tr>
<td>64</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>56</td>
<td>26.8</td>
</tr>
<tr>
<td>65</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>63.7</td>
<td>21.5</td>
</tr>
<tr>
<td>66</td>
<td>Liver cirrhosis, splenomegaly, ascites, dilated gall bladder</td>
<td>Negative</td>
<td>40</td>
<td>11.7</td>
</tr>
<tr>
<td>67</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>13</td>
<td>4.3</td>
</tr>
<tr>
<td>68</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>60</td>
<td>17</td>
</tr>
<tr>
<td>69</td>
<td>Liver cirrhosis, splenomegaly, end-stage kidneys</td>
<td>Negative</td>
<td>11</td>
<td>3.2</td>
</tr>
<tr>
<td>70</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>40</td>
<td>7.8</td>
</tr>
<tr>
<td>71</td>
<td>Splenomegaly, thickened periportal tracts</td>
<td>Negative</td>
<td>26.7</td>
<td>7</td>
</tr>
<tr>
<td>72</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>73</td>
<td>Liver cirrhosis, splenomegaly, gaseous distension</td>
<td>Negative</td>
<td>80</td>
<td>21</td>
</tr>
<tr>
<td>74</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>75</td>
<td>16.6</td>
</tr>
<tr>
<td>75</td>
<td>Liver cirrhosis, splenomegaly, ascites, gaseous distension</td>
<td>Negative</td>
<td>10</td>
<td>2.6</td>
</tr>
<tr>
<td>76</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>6</td>
<td>1.4</td>
</tr>
<tr>
<td>77</td>
<td>Splenomegaly, diffuse periportal fibrosis</td>
<td>Negative</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>78</td>
<td>Liver cirrhosis, splenomegaly, ascites</td>
<td>Negative</td>
<td>25</td>
<td>9.5</td>
</tr>
<tr>
<td>79</td>
<td>Liver cirrhosis, splenomegaly, calcular gall bladder</td>
<td>Negative</td>
<td>98</td>
<td>30</td>
</tr>
<tr>
<td>80</td>
<td>Liver cirrhosis, splenomegaly, portal hypertension</td>
<td>Negative</td>
<td>7.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>
Table (2): Frequency and percent distribution of different categories of glucose tolerance among the studied patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (N=80)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>IFG and/or IGT</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Diabetes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously diagnosed</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>Discovered in our study</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Table (3): Frequency and percent distribution of extrahepatic manifestations of HCV infection in the studied patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (N=80)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid disease (hyperthyroidism)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Joint affection (knee effusion, arthralgia)</td>
<td>3</td>
<td>3.75</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Porphyria cutanea tarda</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leukocytoclastic vasculitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Renal disease:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>7</td>
<td>8.75</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>5 (4 are diabetics, 1 is not)</td>
<td>6.25</td>
</tr>
<tr>
<td>Nephrotic syndrome, cryoglobulinemia</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td>Pruritus</td>
<td>26</td>
<td>32.5</td>
</tr>
<tr>
<td>Cryoglobulinemic vasculitis</td>
<td>3</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Table (4): Frequency and percent distribution of islet cell antibodies in the studied patients.

<table>
<thead>
<tr>
<th>Islet cell antibodies</th>
<th>Frequency (N=80)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>3.75</td>
</tr>
<tr>
<td>Negative</td>
<td>77</td>
<td>96.25</td>
</tr>
</tbody>
</table>

Table (5): Frequency and percent distribution of insulin resistance (HOMA-R > 2.5) among the studied patients.

<table>
<thead>
<tr>
<th>Insulin resistance</th>
<th>Frequency (N=80)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td>Absent</td>
<td>16</td>
<td>20</td>
</tr>
</tbody>
</table>

Table (6): HOMA-R values in the studied patients.

<table>
<thead>
<tr>
<th>N=(80)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-R</td>
<td>Mean deviation</td>
<td>Minimum</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>9.89</td>
<td>8.2</td>
<td>0.8</td>
<td>32.4</td>
<td></td>
</tr>
</tbody>
</table>

Table (7): Correlation of insulin resistance to age.

<table>
<thead>
<tr>
<th>Insulin resistance (HOMA-R) Pearson Correlation</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.343</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table (8): Correlation of insulin resistance to age values based on ROC analysis.

<table>
<thead>
<tr>
<th>Age</th>
<th>Insulin resistance</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤50</td>
<td>40</td>
<td>6.86</td>
<td>5.96</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>40</td>
<td>12.92</td>
<td>9.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Fig. (1): Correlation of insulin resistance to age.

Fig. (2): ROC analysis of age with insulin resistance.

Table (9): Correlation of insulin resistance to liver cirrhosis, Child class, and serum creatinine.

<table>
<thead>
<tr>
<th>Insulin resistance</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=59)</td>
<td>11.71</td>
<td>8.51</td>
<td></td>
</tr>
<tr>
<td>No (N=21)</td>
<td>4.77</td>
<td>4.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Child class:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (N=26)</td>
<td>6.78</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>B (N=35)</td>
<td>11.61</td>
<td>9.87</td>
<td></td>
</tr>
<tr>
<td>C (N=19)</td>
<td>10.97</td>
<td>5.92</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum creatinine:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.2mg/dl (N=19)</td>
<td>6</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>&lt;1.2mg/dl No (N=61)</td>
<td>11</td>
<td>8.69</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Discussion

HCV infection is able to trigger autoimmune mechanism(s) against certain antigenic and cellular components of the body. Autoantibodies are common in patients with chronic HCV infection [6]. Studies have shown that HCV may induce IR irrespective of the stage of advancement of the underlying liver disease, and that IR is less frequent in chronic hepatitis B than in matched chronic hepatitis C cases [7,8].

We thus postulated that the pathogenetic mechanisms of type 2 diabetes mellitus in HCV-infected patients may include the production of antibodies against the insulin producing pancreatic beta cells, and the development of insulin resistance.

The concern of our study was to detect whether insulin resistance or an auto-immune mechanism is more responsible for development of diabetes in HCV-infected population, accordingly we searched for islet cell antibodies and measured insulin resistance by the HOMA-R model in all of our patients to detect which of them is more prevalent.

Out of the 80 HCV infected patients included in our study; 34 patients were proved diabetic (42.5%), 30 of them were previously diagnosed and 4 were discovered to be diabetic in our study. Another 16 patients were discovered to have IFG and/or IGT (20%) which are two categories of increased risk for diabetes known as prediabetes, and only 30 patients were normoglycemics (37.5%).

These results go in concordance with several clinic-based studies and the general population-based NANNES-III study that have previously came to similar conclusions, which reinforce the hypothesis of a causal association between HCV infection and type 2 diabetes [9].

In our study, mean age of diabetic patients was 53.7 years, mean age of normoglycemic patients was 48.3, which is not a statistically significant difference. However, advanced age was proved to be a predisposing factor for the development of diabetes in studies assessing the incidence of type 2 diabetes among HCV-positive persons [10,11].

Our study failed to demonstrate a correlation between male sex and development of diabetes in HCV infected patients; a relation that was again found in studies assessing predisposing factors for the development of diabetes in HCV infected persons following liver transplantation [12,13].

At least 80% of patients with cirrhosis have glucose intolerance, and prevalence of diabetes in cirrhosis is 12.3-57% [14].

In our study prevalence of diabetes in cirrhotic patients was 45.8%, matching with the previously determined prevalence, but unfortunately, the study could not detect a positive correlation between diabetes and liver cirrhosis. Child class B represented the majority of diabetic patients (47%), while Child classes A and C shared equal portions (26.5%) each. The increased prevalence of diabetes in Child B patients may be interpreted by the fact that advanced liver disease is associated with derangements of normal glucose homeostasis, while similar values for Child classes A and C could be attributed to poor hepatic glycogen reserve in late stages of liver cirrhosis, reducing the hyperglycemia that develops in the intermediate class B. Further studies for relation between Child class and diabetes are warranted.

Up to our knowledge, no previous studies have searched for islet cell antibodies in HCV infected patients. Our study has detected ICA with a prevalence of 3.75% in the studied group. Neither a statistically significant difference could be detected in ICA distribution among diabetics and non-diabetics, nor a relation could be found between ICA and presence of insulin resistance. We had 3 patients of the studied group who had positive cryoglobulins as an extrahepatic manifestation of HCV infection, again ICA were present in none of these 3 patients. According to our study, an autoimmune mechanism does not seem to be responsible for the development of diabetes in HCV infected patients.

A direct involvement of the virus in the development of insulin resistance has been proposed [4]. HCV may induce IR irrespective of the stage of advancement of the underlying liver disease, an effect that seemed to be genotype specific [7]. IR was associated with genotypes 1 and 4 and high serum HCV RNA levels [8]. A correlation between HCV RNA levels and HOMA score has been reported also by other studies, especially in genotype 1 [15]. Genotype 3 patients may have significantly lower HOMA scores than other genotypes [7].

Our results agree with the previously mentioned studies, as we could detect insulin resistance (measured by HOMA-R model with a cutoff value of >2.5) in 80% of our patients, with a mean value of 9.89 (±8.2). As the study was conducted in Egypt that is known for an extraordinary high prevalence of genotype 4, we conclude with the
previous studies that IR may be associated with genotype 4.

These results are not, however, confirmed by all investigators. In one study, lean patients with non-3 genotypes had HOMA scores comparable to those of the controls [16]. Negative results have also been reported from Japan, where studies failed to identify HCV infection as independent predictor of IR [17].

Furthermore, it is impossible to determine whether HCV replication is responsible for increased IR or whether HCV replication is favored by hyperinsulinemia, as suggested by some in vitro data, and/or by the increased serum levels of free fatty acids typically observed in IR and type 2 diabetes [18]. However, data supporting the role of HCV in increasing the level of IR and the development of glucose metabolism disturbances, was concluded in one study which showed that eradication of HCV improved the HOMA score [19]. Similar results have been reported in a cohort of 181 genotype 4 patients from Egypt [20].

A fair positive correlation could be demonstrated in our study between age and insulin resistance. Being a prediabetic condition, so our results agree with [3], who stated that advanced age is associated with the higher incidence of diabetes in the HCV-infected population.

A significantly positive correlation could also be found between male sex and IR in agreement with [12,13] who stated male sex as a risk factor for development of diabetes.

Insulin resistance was present in 94% of diabetic patients with a mean value of 13.66 (±8.52) and in 30% of non-diabetic patients with a mean value of 7.1 (6.85), demonstrating that at least 30% of non-diabetics at the time of our study may develop diabetes in later life. It has to be stated clearly, however, that it is not clear whether IR associated with HCV infection invariably evolves towards type 2 diabetes in all infected persons, especially those without other risk factors for type 2 diabetes. There is a clear need of longitudinal studies that may clarify this issue [21].

The pathogenesis of the proposed insulin resistance is not known, although a receptor or postreceptor abnormality is postulated [22]. This postulation was reinforced by results of our study, where a strongly positive correlation could be detected between insulin resistance and presence of cirrhosis.

Up to our knowledge, no previous studies have searched the relation between insulin resistance and serum creatinine values in HCV infected patients. Our study has found a negative correlation between mean values of insulin resistance and those of serum creatinine. Further work is needed to confirm this relation and to search for possible underlying mechanisms.

Conclusion:

Our study showed that HCV is strongly associated with the development of insulin resistance. Important predisposing factors favoring the development of IR in our patients were:

- Advanced age.
- Male gender.
- Presence of liver cirrhosis.

References

5- MATILDE MASINI, DANIELA CAMPANI, UGO BOGGI, MICHELE MENICAGLI, NICOLA FUNEL, MARIA POLLERA, ROBERTO LUPI, SILVIA DEL GUERRA, MARCO BUGLIANI, SCIlla TORRI, STEFANO DEL PRATO, FRANCO MOSCA, FRANCO FILIPPONI and PIERO MARCHETTI: Hepatitis C virus infection and human pancreatic beta-cell dysfunction. Diabetes Care, Volume 28, Number 4, April, 2005.


Synovial Sarcoma of Extremities: Evaluation of Prognostic Factors and Clinical Outcomes

SEHAM E. ABDELKHALEK, M.D. and RASHA HAMDY, M.D.
The Department of Clinical Oncology & Nuclear Medicine, Mansoura University Hospital

Abstract

Purpose: As synovial sarcoma has a poor prognosis. In this retrospective study we tried to evaluate its clinical outcome and to identify which prognostic factors influence its clinical outcomes.

Patients and Methods: This is a retrospective study of patients with synovial sarcoma of extremities between January 2000 and January 2010 at Clinical Oncology & Nuclear Medicine, Mansoura University Hospital.

Results: Of 74 patients, 44 males (59.5%) and 30 females (40.5%) with a median age of 38 (range;1-69 years). Thirty two (43.2%) had metastasis at the first diagnosis and 42 (56.7%) had only a localized tumor. The 5-year overall survival of all the patients was 37.83%; 66.7% in patients with localized disease and 0% in patients with metastasis at first diagnosis. Forty one patients (55.4%) died of the disease at a median duration of 12 months (range 4-48 months). Metastasis at first diagnosis influenced overall survival for patients with synovial sarcoma ($p<0.001$). According to a univariate analysis, the significant adverse factors were biphasic histological subtype and an inadequate surgical margin of the definitive surgery ($p<0.05$).

Conclusion: Synovial sarcoma is still a disease with a poor prognosis. Distant metastasis at initial diagnosis is a significant adverse prognostic factor for overall survival. A biphasic histological subtype and an inadequate surgical margin are significant adverse prognostic factors in localized synovial sarcoma.

Key Words: Synovial sarcoma – Prognostic factors – Extremities – Clinical outcomes.

Introduction

SYNOVIAL sarcoma accounts for approximately 8% of all soft tissue sarcomas and is the fourth most common type of sarcoma. Synovial sarcoma can occur anywhere in the body, but it most often occurs in the paraarticular areas of the lower extremities and they have a poor prognosis [2]. They occur predominantly in the young and the middle aged and in both genders (median age 35 years) [3,4]. Synovial sarcoma usually occurs in the extremities but any location may be affected and, despite its name, synovial sarcomas do not arise from synovial tissue. The histological pattern, however, resembles a developing synovium, 5 and is classified into three subtypes: Monophasic, biphasic and poorly differentiated [6]. It is aggressive and the development of distant metastasis is therefore common. The most common sites of metastasis are the lung and lymph nodes [3].

Overall survival in patients with metastasis at first diagnosis is quite low while the 5-year survival of patients with localized disease at first presentation range from 57 to 88.2% [2,4,7-10]. Surgery is the main treatment modality, with the aim to achieve adequate surgical margins, while adjuvant chemotherapy and radiotherapy are controversial [4,9,11-13]. It is well known from the literature that the key prognostic factors at diagnosis of synovial sarcoma are tumor stage [2,14], tumor size [8,15], older age [4,8,9,11], primary tumor site [9,12,16], initial surgical treatment with adequate surgical margins [8,10,14] And adjuvant radiotherapy [17]. However, there is no consensus on which is the most useful [28-10]. This is a retrospective study was performed to evaluate clinical outcomes and identify which prognostic factors influenced outcome of synovial sarcoma of extremities.

Patients and Methods

This is a retrospective study of patients with synovial sarcoma of extremities treated at Clinical Oncology & Nuclear Medicine Department, Mansoura University Hospital during the period from 1st January 2000 till 1st January 2010.

A total 79 patients were diagnosed with synovial sarcoma while five were excluded from this study five because of incomplete data. All patients had chest X-ray, chest computed tomography, bone
scan and magnetic resonance imaging of the extremity involved. The patients were divided into two groups: Those with localized tumor at the time of diagnosis, and those with metastatic disease.

Patient data were recorded including: age, sex, primary tumor site (upper extremity at or distal to the shoulder joint, lower extremity) and size (maximum diameter < 5 cm or ≥5 cm), previous treatment, histological subtype (monophasic, biphasic, poorly differentiated), type of surgical procedure (closed, wide excision or amputation), microscopic surgical margins (an inadequate margin was defined as an area with close (tumor present ≤ 2 mm or less from the inked margins) or positive margins or a tumor at the inked margin), chemotherapy, radiotherapy and status of the patients after treatment.

Statistical analysis:

Disease-free survival time was calculated from the time of admission to the occurrence of a local recurrence, distant metastasis or death. Overall survival time was calculated from the time of admission to death or last follow-up visit. The survival analysis was calculated using the Kaplan-Meier method.

Patients with localized disease, the following parameters were analyzed by the log-rank test for prognostic value: Age, sex, primary tumor site and size, previous treatment, histological subtype, microscopic surgical margins, chemotherapy and radiotherapy. All statistical analyses were performed using SPSS 15 statistical software (SPSS, Chicago, IL, USA).

Results

Patients’ characteristics:

Table (1) showed patients characteristics; of 74 patients, 44 males (59.5%) and 30 females (40.5%) with a median age of 38 (range, 1-69 years), 32 (43.2%) had metastasis at the first diagnosis and 42 (56.7%) had only a localized tumor. The most common location of tumors was: Thigh (43 patients, 58.1%), leg and foot (15 patients; 20.3%), arm (10 patients; 13.5%), forearm and hand (six; 8.1%). The tumor size ranged between 0.6 and 21 cm (median, 6 cm). Fifteen patients (20.3%) had tumors < 5 cm while 59 patients (79.7%) had tumors ≥ 5 cm. There were 48 monophasic (64.8%) and 26 biphasic (35.1%) synovial sarcomas.

In all, 58 patients underwent surgical treatment, twenty two (29.7%) with a wide excision, fifteen (20.2%) amputations and twenty one (28.3%) with marginal excisions. Sixteen patients (21.6%) refused all surgical procedures.

Postoperative external beam irradiation was administered to 32 patients (43%); 60-76 Gy over 6-7.5 weeks according to margins. While ten patients (14%) received preoperative radiotherapy to shrink tumor size by external beam irradiation 50 Gy over 5 weeks and boost after surgery 10-26 Gy over 1-2.5 weeks according to margins. Palliative radiotherapy was given to relieve symptoms for fourteen patients (19%).

Regards to chemotherapy 59 patients (79.7%) received chemotherapy while fifteen (20.3%) did not receive (nine refused and six patients had comorbidities and cannot tolerate chemotherapy). Multiple drug regimens were used based on combinations of adriamycin, mesna and ifosfamide. In eight patients the regimen was changed after the administration of chemotherapy because the tumors were not responding. Four patients received cisplatin and ifosfamide. Four patient received dacarbazine, mesna, adriamycin and ifosfamide. There were no deaths related to chemotherapy toxicity.

<table>
<thead>
<tr>
<th>Sex:</th>
<th>Localized disease (n=42)</th>
<th>Metastases at first diagnosis (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22 (52)</td>
<td>13 (41)</td>
</tr>
<tr>
<td>Female</td>
<td>20 (48)</td>
<td>19 (59)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 (1-64)</td>
<td>46 (16-77)</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site:</th>
<th>Localized disease (n=42)</th>
<th>Metastases at first diagnosis (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper extremities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>5 (12)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Distal</td>
<td>4 (10)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Lower extremities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>25 (60)</td>
<td>18 (56)</td>
</tr>
<tr>
<td>Distal</td>
<td>8 (19)</td>
<td>7 (22)</td>
</tr>
<tr>
<td>Size:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 cm</td>
<td>9 (21)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>≥ 5 cm</td>
<td>33 (79)</td>
<td>26 (81)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histologic subtype:</th>
<th>Localized disease (n=42)</th>
<th>Metastases at first diagnosis (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monophasic</td>
<td>26 (62)</td>
<td>22 (69)</td>
</tr>
<tr>
<td>Biphasic</td>
<td>16 (38)</td>
<td>10 (31)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of definite surgery:</th>
<th>Localized disease (n=42)</th>
<th>Metastases at first diagnosis (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide excision</td>
<td>13 (31)</td>
<td>9 (28)</td>
</tr>
<tr>
<td>Radical (amputation)</td>
<td>9 (21)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Marginal excision</td>
<td>13 (31)</td>
<td>8 (25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemotherapy:</th>
<th>Localized disease (n=42)</th>
<th>Metastases at first diagnosis (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoadjuvant</td>
<td>17 (40)</td>
<td>13 (41)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>18 (43)</td>
<td>11 (34)</td>
</tr>
<tr>
<td>None</td>
<td>7 (17)</td>
<td>8 (25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radiation:</th>
<th>Localized disease (n=42)</th>
<th>Metastases at first diagnosis (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant</td>
<td>21 (50)</td>
<td>11 (34)</td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>10 (24)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Palliative</td>
<td>4 (10)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>No</td>
<td>7 (17)</td>
<td>11 (34)</td>
</tr>
</tbody>
</table>

Table (1): Baseline and tumor characteristics for the 74 patients with synovial sarcoma (n, %).
Table (2): Statistical analysis for prognostic factors by log-rank test.

<table>
<thead>
<tr>
<th></th>
<th>5-year overall survival (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>0.07</td>
</tr>
<tr>
<td>Female</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>98</td>
<td>0.76</td>
</tr>
<tr>
<td>&gt;35</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td><strong>Site:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>37</td>
<td>0.63</td>
</tr>
<tr>
<td>Distal</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td><strong>Size:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5cm.</td>
<td>100</td>
<td>0.07</td>
</tr>
<tr>
<td>&gt;5cm.</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td><strong>Histological subtype:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monophasic</td>
<td>83</td>
<td>0.01</td>
</tr>
<tr>
<td>Biphasic</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td><strong>Microscopic surgical margin:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate (wide excision/amputation)</td>
<td>98</td>
<td>0.001</td>
</tr>
<tr>
<td>Inadequate (marginal excision)</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td><strong>Chemotherapy:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td>0.21</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><strong>Radiation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77</td>
<td>0.07</td>
</tr>
<tr>
<td>No</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

**Clinical outcome:**

The median follow-up time was 22 months (range 4-113 months). None of the patients was lost to follow-up. The overall 5-year survival for the patients was 37.83%; it was 66.7% in patients with localized disease (Fig. 1) and 0% for patients with metastasis at diagnosis. A total of 41 patients (55.4%) died from the disease at a median duration of 12 months (range 3-47 months); none died from any unrelated causes. A total of 24 patients (32.4%) have remained alive and continuously free of disease at a median follow-up time of 52 months (range 6-110 months). Nine patients (12.1%) have remained alive with disease at a median follow-up time of 8 months (range 5-52 months).

Local recurrence after treatment in patients with localized disease occurred in eight patients (19%): The median being 12 months (range 2-62 months). Metastasis after treatment occurred in ten patients (24%): The median being 12 months (range 3-60 months). Seven patients had both lung and lymph node metastases; three had only lung metastasis and one had both lung and brain metastases. The 5-year disease-free survival was 58% (Fig. 2).

Metastasis at first diagnosis influenced the overall survival of synovial sarcoma patients ($p<0.001$) (Fig. 1). The prognostic factors of death in patients with localized disease are presented in Table (2). According to the univariate analysis, the significant adverse factors were biphasic histological subtype and inadequate surgical margins in definitive surgery ($p<0.05$).

**Discussion**

Synovial sarcoma is a high-grade aggressive soft tissue tumor with high rate of local recurrence and distant metastasis in spite of aggressive treatment (i.e., wide excision plus adjuvant radiotherapy and chemotherapy) [18]. During the last 40 years, there have been many studies to determine prognostic factors in this disease. Anatomic site [19], tumor size [20], patient age [21], microscopic margins [20], and histologic subtype (monophasic vs biphasic) [19,22] have all been reported to be significant determinants of outcome for patients with synovial sarcoma.
Regarding the histological subtypes of synovial sarcoma, there are mixed reports of a histological correlation with survival between monophasic and biphasic histologies [23-27]. Our study found that the monophasic type had a better overall survival than the biphasic type ($p=0.01$). A tumor size >5 cm had a negative influence on overall survival in many studies; [3,4,8,9] whereas in our study there was a non-statistically significant trend of tumor size affecting overall survival ($p=0.07$). This is comparable to tumor size described by Taweechok et al., [28].

The rate of metastasis at the first diagnosis ranges between 9 and 54% [8,14]. In the present study distant metastases at diagnosis were found in 43.2% of all patients. The 5-year overall survival rate in this series was quite low (37.8%) because the patients who had metastasis at first presentation were included. When patients with only localized disease were considered, the 5-year overall survival rate increased to 66.7%, which is comparable to other similar studies [2,7,8]. Metastases and local recurrences in initially localized tumors after treatment were 24 and 19% respectively. This is comparable to other studies [8,23,28].

Surgery is the mainstay of treatment for local control of tumors [12,23]. A wide excisions with a limb-sparing procedure is the method of choice except when neurovascular structures are involved [29]. Ten patients in the current study underwent an amputation because of locally recurrent disease and the tumor involved a neurovascular structure. A marginal margin (excision through the pseudocapsule or the reactive tissue surrounding the tumor) is insufficient for the management of a soft tissue sarcoma. In the entire marginal margin patients in the current series the tumors were very close to neurovascular bundles and postoperative radiation and chemotherapy were given. We found that the overall survival of an inadequate surgical margin was worse than a free surgical margin ($p<0.001$). A wide surgical margin (excision through normal tissue) affected overall survival significantly compared to a marginal margin as described by other studies [11,28].

The benefit of chemotherapy for synovial sarcoma is controversial [2,11,13]. At our institution chemotherapy was given as neoadjuvant or/adjuvant to patients who had tumors larger than 5cm and who could tolerate its side-effects. The usual regimen was ifosfamide-based, resembling other studies [11,13,30]. In the present study we found that chemotherapy did not improve the clinical outcome for patients with a localized synovial sarcoma; however, there were only 35 patients in the localized disease group so a larger study is needed.

Some studies have shown that radiation improved local control of soft tissue sarcoma with a combination of surgery and radiation [12,13]. Ferrari et al., studied postoperative external-beam radiotherapy combined with adjuvant chemotherapy for synovial sarcoma patients, which resulted in high rates of local control [29]. However, local control is not always certain if adequate surgical margins are not achieved [4]. In the present study we did not find an association between radiation and survival in patients with a localized synovial sarcoma ($p=0.42$), as other studies have done [11,25,28].

Conclusion:

Synovial sarcoma is a high-grade tumor that is associated with poor prognosis. Soft tissue sarcomas have many factors that can influence the overall survival of the patients. These factors are related to the patients, tumor and treatment. Distant metastasis at initial diagnosis is a significant adverse prognostic factor for overall survival. A biphasic histological subtype and an inadequate surgical margin are significant adverse prognostic factors in localized synovial sarcoma. To achieve the best possible result, the physician must take all of them in consideration and try to control the factors that have influence in the outcome and are possible to be handled to minimize the effects of the others which he can’t modify. As it was retrospective single institution study and the number of patients was small due to the rarity of the disease and this problem was faced by other studies [2,8,10,25,28]. However a multicenter study may be needed to achieve a larger population.

References


Stressors Facing Mothers of Children with Cerebral Palsy

MAGDA M. EL-SAYED YOUSSEF, D.N.Sc.; FATEN F. AHMED, D.N.Sc. and SHAIMAA M. MAHMOUD, M.Sc.
The Department of Pediatric Nursing, Faculty of Nursing, Alexandria, Suez Canal and Zagazig Universities

Abstract

Background: Cerebral palsy (CP) is a static, nonprogressive disorder caused by brain insult or injury in the prenatal, perinatal, and postnatal time period. It is a major developmental disability affecting function in children. The present study aimed to identify the stressors facing mothers of children with cerebral palsy. A descriptive design was used. The study was carried out on 100 mothers with cerebral palsy children at Mansoura University Children’s Hospital. The data were collected using a structured interview questionnaire sheet developed by the researcher. It included personal and social data about mothers and their children, mothers' knowledge about the disease and the stressors that faced mothers with their children. The result of this study revealed that the majority of mothers suffered from stressors related to management followed by psychological stressors, then physical stressors, and social stressors. Training and educational programs for nursing team to help mothers with cerebral palsy children for coping and reducing the stressors facing during treatment of their children were recommended.

Conclusion: Stressors related to management were the highest perceived stressors by the mothers of children with cerebral palsy, followed by psychological stressors. Physical and social stressors were the least perceived ones.

Key Words: Cerebral Palsy – Stressors – Mothers – Children.

Introduction

CEREBRAL palsy (CP) is a group of permanent and non-progressive disorder of movement and posture caused by a central nervous system lesion, damage or dysfunction originating early in life [1]. The prevalence of CP in Egypt ranges from 2 to 3 per 1000 live births, this rate increases to 40-100 per 1000 live births among babies born very early or with very low birth-weight [2].

The etiology of CP is not well understood, and brain lesions are thought to be associated with prenatal events of varying causes. Risk factors for CP have multifactor and can include birth asphyxia, bilirubin encephalopathy, post infectious brain damage [3], multiple gestation, intrauterine growth restriction, maternal thyroid abnormalities, prenatal strokes, maternal methyl mercury exposure, and maternal iodine deficiency [4,5].

There are various types of cerebral palsy, these include spastic cerebral palsy, athetoid cerebral palsy, ataxic cerebral palsy and mixed type [6]. Depending on the location of the brain injury, these children can experience sensory and communicative difficulties cognitive impairment, hearing and vision problems, sensory deficits and seizures [7]. The birth of a child with cerebral palsy places the family in a dilemma. Parents may experience periods of panic, anxiety and helplessness, as well as periods of indifference and anger, at which time they face nearly overwhelming depression, apathy and bitterness [8].

A stressor is any event, experience, or environmental stimulus that causes stress in an individual. These events or experiences are perceived as threats or challenges to the individual and can be either physical or psychological. Researchers have found that stressors can make individuals more prone to both physical and psychological problems, including heart disease and anxiety [9].

Pediatric nurse has important role in helping those mothers to overcome difficulties that faces them in caring for their children. In addition, she has responsibility in minimizing the stressors that facing those mothers and helping them to cope healthfully with their children's conditions. The pediatric nurse should provide those mothers with the needed information and guidance regarding their children's care and management [10]. Answering mothers' questions and respecting their beliefs, considering their level of education and understanding is also an important issue in helping those
mothers to overcome their stressors [10]. Therefore, this study aims to identify the stressors of mothers of children with cerebral palsy.

Aims of the study:
To identify the stressors facing mothers of children with cerebral palsy.

Subjects and Methods
A descriptive study design was used, with measurement of all study variables at the same point in time, carried out in the Out-patient and Rehabilitation Centers of Mansoura University Children's Hospital.

Sample:
A convenient sample of 100 mothers of children with cerebral palsy who were diagnosed as cerebral palsy since at least 6 months and free from other chronic disorder comprised the sample. Data were collected from at 1st of January to the 1st of March 2012.

Tool of data collection:
Stressors of Mothers of Children with Cerebral Palsy Structured Interview Questionnaire Sheet. This tool was developed by the researcher to identify the stressors facing mothers of CP children. It included two parts:

Part 1:
A- Demographic data about children and their families, such as, age, sex, mother's age, education and occupation.
B- Medical history, such as, type of cerebral palsy and duration of diagnosis.
C- Problems of children with CP and mothers actions toward these problems.

Part 2:
Stressors facing mothers of children with cerebral palsy. It included the following:
- Physical stressors, such as, exhaustion, fatigue due to bringing child to the clinic or hospital and helping child with his/her hygiene care.
- Psychological stressors, such as, feel unhappy for child's inability to play with his/her peers, frequent hospitalization, and mothers' dependence on others.
- Social stressors as financial problems, worrying about relatives, sibling and peers' interaction with her child.
- Stressors related to management, such as, rehabilitation and difficulty of swallowing.

Results
Table (1) shows the sociodemographic characteristics of mothers of children with cerebral palsy. It was found that almost half of the studied mothers (51.0%) were in the age group of 25 to less than 35 years (47.4% for mothers of male children and 55.8% for mothers of female children). While, 32.0% of mothers were in the age group 35 years and more (40.3% for mothers of male children compared to 20.9% for mothers of female one) with a mean age 30.58 ± 6.22 years.

Table (2) shows the sociodemographic characteristics of children with cerebral palsy. It is clear from the table that almost half of children of the studied mothers (48.0%) were in the age group of less than 4 years (49.1% for males and 46.5% for females), followed by 30.0% of children were in the age group 4 to less than 8 years (28.1% for males and 32.5% for females), with a mean age 4.79 ± 3.30 years.

Table (3) illustrates the mean percent scores of the perceived stressors by mothers of children with cerebral palsy. As shown from the table stressors related to management were the highest stressors that were perceived by mothers as its mean percent score was 62.00 (61.58 for mothers of male children and 62.56 for those of female children), followed by the psychological stressors where their mean percent score was 57.67 (58.77 and 56.20 for mothers of male and female children respectively). Social stressors were the least perceived stressors (mean score 48.20). Yet, the mean percent score of social stressors related to financial aspects was 60.28 (62.15 and 57.81 for mothers of male and female children respectively). Regarding management, physical, psychological and social stressors no statistical significant differences were found between mothers of male children and those who have female one.

Figure (1) shows mothers' knowledge about type of disease of their children with cerebral palsy. It is clear from figure that the most common type of disease was spastic cerebral palsy (49.3%), followed by ataxic cerebral palsy (42.7%), athetoid and mixed types represented the lowest ones.

Figure (2) illustrates treatment of children with cerebral palsy. It is revealed from figure that physiotherapy was the most common type of treatment (61%), followed by occupational therapy (29%), then speech therapy represented the least one (10%).

Figure (3) represents the problems facing mothers of children with cerebral palsy. It is clear from figure that urine incontinence represented the highest
problems (86.0%) followed by difficulty of speech (83.0%). Difficulty of walking and stool incontinence were reported by 82.0% and 81.0% of mothers respectively. Visual and hearing difficulties represented the least reported problems (20.0% and 14.0%) respectively.

Table (1): Sociodemographic characteristic of mothers of children with cerebral palsy.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=57</td>
<td>N=43</td>
<td>N=100</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age/years:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;25</td>
<td>7</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>25-</td>
<td>27</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td>35 &amp; More</td>
<td>23</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Mean±S.D</td>
<td>31.56±6.30</td>
<td>29.28±5.93</td>
<td>30.58±6.22</td>
</tr>
<tr>
<td><strong>Marital status:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>54</td>
<td>41</td>
<td>95</td>
</tr>
<tr>
<td>Divorced</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Widow</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>Educational level:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate &amp; read and write</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Primary/preparatory education</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Secondary education</td>
<td>32</td>
<td>26</td>
<td>58</td>
</tr>
<tr>
<td>Higher education</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><strong>Mothers work:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>54</td>
<td>40</td>
<td>94</td>
</tr>
<tr>
<td>Employee and worker</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><strong>Residence:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>23</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>Rural</td>
<td>34</td>
<td>28</td>
<td>62</td>
</tr>
</tbody>
</table>

Table (2): Sociodemographic characteristics of children with cerebral palsy.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=57</td>
<td>N=43</td>
<td>N=100</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age/years:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>28</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>4-</td>
<td>16</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td>8-</td>
<td>8</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>12 and More</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Mean±S.D</td>
<td>4.86±3.48</td>
<td>4.70±3.07</td>
<td>4.79±3.30</td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of sibling</td>
<td>57</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td>Only child</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>One sibling</td>
<td>17</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>Two sibling</td>
<td>23</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>Three and more</td>
<td>13</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td><strong>Birth order:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>17</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Second</td>
<td>14</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Third</td>
<td>19</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>Forth and more</td>
<td>7</td>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>
Table (3): Mean percent scores of perceived stressors by mothers of children with cerebral palsy.

<table>
<thead>
<tr>
<th>Stressors</th>
<th>Male Mean % score (n=57)</th>
<th>Female Mean % score (n=43)</th>
<th>Total Mean % score (n=100)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stressors related to management</td>
<td>61.58</td>
<td>62.56</td>
<td>62.00</td>
<td>0.309</td>
<td>0.758</td>
</tr>
<tr>
<td>Psychological stressors</td>
<td>58.77</td>
<td>56.20</td>
<td>57.67</td>
<td>0.702</td>
<td>0.484</td>
</tr>
<tr>
<td>Physical stressors</td>
<td>50.35</td>
<td>46.98</td>
<td>48.90</td>
<td>0.841</td>
<td>0.403</td>
</tr>
<tr>
<td>Social stressors:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A- Related to family</td>
<td>50.05</td>
<td>45.76</td>
<td>48.20</td>
<td>1.108</td>
<td>0.270</td>
</tr>
<tr>
<td>B- Related to financial stressors</td>
<td>62.15</td>
<td>57.81</td>
<td>60.28</td>
<td>1.109</td>
<td>0.270</td>
</tr>
</tbody>
</table>

Fig. (1): Mean scores of type of disease for studied children with cerebral palsy.

Fig. (2): Mean scores of type of treatment for studied children with cerebral palsy.

Fig. (3): Mean scores of problems facing mothers of children with cerebral palsy.

Discussion

Cerebral palsy (CP) is an abnormality of motor function and postural tone that is acquired at an early age, even before birth. Signs and symptoms of cerebral palsy usually show in the first year of life. This abnormality in the motor system is the result of brain lesions that are nonprogressive [11].

Chronic illness such as cerebral palsy have a substantial impact not only on the affected child but also on family as a whole. Family members, mainly the mothers, have many responsibilities toward their cerebral palsied children [12]. These supportive measures may decline across adolescence stage. Mothers of these children always report many stressors during their care for their cerebral palsied children [13].

This study was carried out to identify the stressors facing mothers of children with cerebral palsy. Regarding the treatment of children with cerebral palsy, it is revealed from the current study that physiotherapy was the most common type of treatment especially massage and scrub (Fig. 2). This finding was consistent with [14-17], who stated that physiotherapy is very important for children with cerebral palsy in which it will encourage those children to control their movements in order to sit, move around, play and interact with people and the environment. Physiotherapy aims at maintaining the range of movement in all the joints so the children's abilities are not limited by joint stiffness where various techniques, such as, exercises, mobility training, orthotics (braces, splints ... etc), and other equipment can be used.
In relation to the problems facing mothers of children with CP, it is clear from the current study that urine incontinence, difficulty of speech, and difficulty of walking represented the highest problems facing mothers having CP children (Fig. 3). This result may be explained in the light of the fact that these are the most common manifestations of CP. The main cause of urinary incontinence in children with cerebral palsy is lack of executive function, and inability to suppress the urge to void. The degree of urinary incontinence varies with cerebral palsy severity, partly due to the communication problems that exist for more severely affected patients.

The results of the present study revealed that stressors related to management was the highest stressors perceived by the studied mothers (Table 3). This finding was in same line with [19,20], who showed that most mothers with chronic ill children suffer from high level of stress due to daily giving medication, difficulty of giving medication and side effect of treatment such as nausea, vomiting, weight loss, and constipation where mothers suffered from anxiety, depression, frustration, sadness, worry about body image and think about future of their children.

The finding of the present study also revealed that psychological stressors were the second highly perceived stressors by the studied mothers (Table 3). This finding was consistent with [21], who stated that having a child with cerebral palsy double the risk of occurrence of depression. Similar finding was reported by [22], who stated that having a CP child may lead to maternal depression. The findings of this study could be attributed to the fact that mothers of children with cerebral palsy are always under a lot of pressures and they have the responsibility to adjust themselves and their children immediately with the problems associated with their chronic disease. In addition, these mothers are always afraid of losing their children who will never be healthy children as they are.

Physical stressors were the third stressors perceived by the studied mothers after the stressors related to management and psychological stressors (Table 3). This finding was supported by [23,24], who mentioned that mothers with chronically ill children suffer from physical stressors due to frequent follow-up, hospitalization, fatigue due to spending all their times with their children. The result of the current study could be explained by the fact that the majority of the studied mothers were housewives, so they devided their time and effort for caring of their sick children. In addition, small family size reduces the physical burden on mothers which in-turn reduces the physical stressors that could face them in caring for their children.

Social stressors were the least stressors perceived by the studied mothers as shown in the result of the present study (Table 3). This finding is congruent with [25], who stated that social stressors were the final stressors perceived by the studied mothers. This finding could be explained in the light of the fact that most of the mothers have a support from relatives as, husbands, grandparents and neighbors so that social stressors did not represent high level of stress to mothers.

Conclusion:

Based on the findings of the present study, it is concluded that stressors related to management were the highest perceived stressors by the mothers of children with cerebral palsy, followed by psychological stressors. Physical and social stressors were the least perceived ones. No statistical significant difference was found between mothers of male or female children regarding their perceived stressors.

Recommendations:

- Educational sessions should be provided for mothers of cerebral palsy children about care of their children, as well as, community resources from whom they may seek assistance and support to reduce their stressors.

- Nurses must alleviate stressors of mothers with cerebral palsy children that lead to better quality of life for children through providing the appropriate knowledge about the disease and community resources.

- The Ministry of Health should arrange for summer camp programs for cerebral palsy children and their mothers which can provide them with adequate information and skills regarding management of cerebral palsy which leads to stressors reduction.

References


Role of Inflammatory Markers on Left Ventricular Functions in Vitamin D Deficiency Rickets

WAFAA S. MOHAMMED, M.D.* and KOTB A. METWALLEY, M.D.**
The Departments of Clinical Pathology* and Paediatrics**, Faculty of Medicine, Assiut University, Egypt

Abstract

Background: Circulating 25-hydroxyvitamin D (25(OH)D), an accurate measure of vitamin D status, is markedly reduced in rachitic infants. Aside from the known relationship between vitamin D and bone, vitamin D has also been implicated in cardiovascular homeostasis, immune function and inflammation. Furthermore, a mass of evidence is accumulating that vitamin D deficiency could lead to cardiovascular complications and imbalance of cytokines profile. Our objective was to study the relationship between vitamin D status (as determined by serum 25(OH)D concentrations) and inflammatory markers and left ventricular function in rachitic infants. Also, to evaluate the effect of vitamin D supplementation on the above parameters.

Subjects and Methods: This study included two groups; vitamin D deficiency rickets (VDDR) group (25 infants) and an age matched control group (15 infants). After subsiding of the acute illness, the rachitic infants received vitamin D supplementation for 6 months. Blood samples were collected in the morning before the start of treatment and analyzed for serum 25(OH)D, intact parathyroid hormone (iPTH), Alkaline phosphatase (ALP), calcium (Ca), Phosphorus (Ph) and inflammatory markers [interleukin-6 (IL-6), and C-reactive protein (CRP). Electrocardiogram (ECG) and echocardiography measuring left ventricular functions were done. The biochemical variables, ECG and echocardiography were assessed at baseline and after 6 months of vitamin D supplementation.

Results: VDDR group had significant lower 25(OH)D, Ca, Ph and significant higher iPTH, ALP, IL-6 and CRP compared to the age matched control group at baseline. Echocardiographic finding revealed significant increase in LVEDD and LVESD and significant decrease in EF% and FS% in VDDR group compared to the age matched control group at the study entry. Also, ECG finding showed abnormality in some patients at baseline. The biochemical, echocardiographic and ECG variables improved significantly after 6 months of vitamin D supplementation and reached to those levels found in the age matched control group. Finally, we found negative correlations between 25(OH)D level and IL-6, CRP, LVEDD and LVESD. Also, positive correlations were found between 25(OH)D and EF% and FS%. These correlation were observed at baseline and after 6 months of vitamin D treatment.

Conclusion: VDDR is associated with increased inflammatory markers and impairment of left ventricular functions in rachitic infants. Vitamin D supplementation ameliorated these effects. Also, results gleaned from this investigation support the possible contributing role of the elevated inflammatory markers in the pathophysiology of left ventricular impairment in vitamin D deficiency rachitic infants. More studies are needed to fully characterize the relationship between Vitamin D induced inflammation and cardiac function in rachitic infants.

Key Words: Vitamin D deficiency rickets – Infants – Inflammatory markers– Left ventricle function.

Introduction

NUTRITIONAL rickets is a disease resulting from impaired bone mineralization due to insufficient calcium or phosphorus in growing children. It ranks as one of the five commonest diseases in children from developing countries and is still quite common in the Middle East [1]. It is thought to be secondary to vitamin D deficiency [2].

Several studies have shown that vitamin D may play a role in many biochemical mechanisms in addition to bone and calcium metabolism. Recently, vitamin D has sparked widespread interest because of its involvement in the homeostasis of the cardiovascular system [3]. There is growing evidence that vitamin D either directly or indirectly affects cardiac structure and function [4]. The vitamin D receptor knockout mouse model demonstrates marked cardiomyocyte hypertrophy and increased left ventricular weight [5], and 1,25(OH)2D3 attenuates cardiomyocyte proliferation [6] and hypertrophy [7] in vitro. In human, Vitamin D deficiency has been shown to be associated with an increased incidence of left ventricular hypertrophy and con-
gestive heart failure [8]. Although it has been reported that asymptomatic left ventricular dysfunction may develop in infants with vitamin D deficiency rickets (VDDR) and it improves with treatment, dilated cardiomyopathy and congestive heart failure are rare [9].

There is increasing evidences that low vitamin D status may lead to immune dysregulation. Studies have shown defective macrophage function, such as impaired chemotaxis, phagocytosis, and increased production of proinflammatory cytokines in vitamin D deficiency [10]. Vitamin D supplementation improved cytokines profiles in animals [11], patients with congestive heart failure [12] and human coronary arterial endothelial cells [13].

To date there is little evidence on the associations of 25(OH)D with indicators of inflammation and cardiac functions in rachitic infants. So, the aim of this work was to evaluate the effect of vitamin D deficiency on the inflammatory markers; interleukin-6 (IL-6) and C-reactive protein (CRP) and the left ventricular function in the rachitic infants. Also, we examined the effect of vitamin D supplementation on the above mentioned parameters. Moreover, we searched for potential correlations between 25(OH)D and IL-6, CRP and selected echocardiographic parameters.

Material and Methods

This study included 40 infants with age range from 6 months to 2 years. 25 (14 boy and 11 girls) infants with vitamin D deficiency rickets (VDDR). 15 (9 boys and 6 girls) apparently healthy, age matched infants were studied as a control. Both patients and controls were recruited from Paediatric Outpatients Clinics and Paediatric Emergency department in Assiut University Children Hospital, Egypt.

The diagnosis of VDDR was based on a combination of clinical, radiographic and biochemical features of VDDR [14]. Exclusion criteria were previous history of heart disease or any other condition that affect cardiac functions, history of prematurity or intrauterine growth retardation, renal, liver, intestinal or central nervous system disease, family history of hereditary forms of rickets, treatment with vitamin D, malnutrition and anemia. The work was approved by the Assiut University Ethics Scientific Committee and an informed consent from the parents of infants had been performed.

At the study entry, blood samples were taken from all patients (VDDR group) and then received intramuscular injection of vitamin D (cholecalciferol) (600 000 IU) once and oral calcium lactate for 2 weeks followed by oral maintenance dose of vitamin D 400 unit/day for 6 months.

A- Biochemical analysis:

Blood samples were drawn in the morning between 8 AM and 11 AM at baseline and at the end of the 6 months of treatment. After centrifugation at room temperature for 20 minutes, aliquots of the serum samples were frozen consecutively and stored at -20°C until analyzed.

The following biochemical parameters were measured using ELISA kits: IL-6 (AviBion Human IL-6 ELISA kit, Orgenium Laboratories, Finland), C-reactive protein (highly sensitive CRP ELISA Kit Monobind Inc., USA).

25-hydroxyvitamin D (25OHD) was measured using enzyme immunoassay (Immundiagnostic Systems Inc., Fountain Hills, AZ) and intact parathyroid hormone (i-PTH) was measured using immunoassay (Immulate 1000, Diagnostic Products Corporation). Alkaline phosphatase was determined using Abbot Aeroset Autoanalyzer by spectrophotometric method. Ca and Ph levels were measured using routine laboratory tests.

B- Electrocardiographic measurements:

Resting12-lead electrocardiograms (ECG) studies were performed for all rachitic cases and interpreted in accordance with the patient's age and sex, and the QT segment was corrected for heart rate (QTc) [15].

C- Echocardiography:

After improvement of acute illness of studied cases. For all patients and controls, left ventricle functions were evaluated by echocardiography using Vivid 3, Aloka machines with transducers of 3.5,7 MHz. We used different echocardiography Modes: 1) two dimensional (2D) to verify cardiac chambers structures and details of anatomy. 2) M mode study to estimate the other echocardiographic variables according to the criteria of the American Society of Echocardiography [16].

Statistical analysis:

Data are expressed as mean±standard deviation (SD) for all parameters. The data were analysed by using GraphPad Prism data analysis program (GraphPad Software, Inc., San Diego, CA, USA).
For the comparison of statistical significance between cases and control, Student Newman-Keuls t-test for unpaired and paired data was used. Linear correlations were performed by Spearman’s or Pearson’s test. A value of \( p \leq 0.05 \) was considered statistically significant.

**Results**

The biochemical and echocardiographic variables of the two groups at the baseline are summarized in Table (1). Patients with VDDR had significantly lower level of serum Ca, Ph and 25(OH) vitamin D (for all \( p<0.001 \)) and significantly higher level of alkaline phosphatase, parathyroid hormone, IL-6 and CRP (for all \( p<0.001 \)) in comparison with age matched control group. The echocardiographic parameters of VDDR group; LVEDD and LVESD, were significantly higher (for both \( p<0.001 \)) while EF\% and FS\% were significantly lower (for both \( p<0.001 \)) when compared to the control group. No significant difference in IVSWT, LVPWT, I/L, LVM, LVMI and E/A between VDDR group and the control group.

After 6 months of treatment (Table 2), the serum Ca, Ph and 25(OH) vitamin D of the VDDR group were significantly higher (for all \( p<0.0001 \)) compared to the levels found at the baseline. Alkaline phosphatase, parathyroid hormone, IL-6 and CRP levels of VDDR participants after 6 months of treatment were significantly lower (for all \( p<0.001 \)) compared to levels showed at baseline. All of these parameters return to normal levels and were not significantly different when compared to an age matched control group. The echocardiographic variables; LVEDD and LVESD, were significantly lower (for both \( p<0.001 \)) while EF\% and FS\% were significantly higher (for both \( p<0.001 \)) when compared to the baseline levels. These variables were not significantly different compared to the age matched control group.

ECG of VDDR group showed T wave abnormalities in 3 cases and prolonged QT interval in 5 cases at the baseline. These changes disappeared after 6 months of vitamin D supplementation (data not shown).

**Correlation analysis:**

Figures (1A,B & 2A,B) showed correlation coefficient between 25(OH) vitamin D level and IL-6, CRP, echocardiographic variables: LVEDD, LVESD, EF\% and FS\% among VDDR group at baseline and after 6 months of treatment. At baseline, serum 25(OH) vitamin D level had significant positive correlation with IL-6 (\( r=0.68 \) and \( p<0.001 \)), CRP (\( r=-0.59 \) and \( p<0.01 \)), LVEDD (\( r=-0.66 \) and \( p<0.001 \)), LVESD (\( r=0.79 \) and \( p<0.001 \)) and significant positive correlation with EF\% (\( r=0.71 \) and \( p<0.001 \)) and FS\% (\( r=0.69 \) and \( p<0.001 \)). After 6 months of treatment, serum 25(OH) vitamin D level had significant positive correlation with IL-6 (\( r=-0.94 \) and \( p<0.001 \)), CRP (\( r=-0.53 \) and \( p<0.01 \)), LVEDD (\( r=-0.69 \) and \( p<0.001 \)), LVESD (\( r=0.77 \) and \( p<0.001 \)) and positive correlation with EF\% (\( r=0.87 \) and \( p<0.001 \)) and FS\% (\( r=0.56 \) and \( p<0.01 \)).

<table>
<thead>
<tr>
<th>Variables</th>
<th>VDDR group</th>
<th>Control group</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.1±0.6</td>
<td>9.1±0.6</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>1.8±0.6</td>
<td>5.5±0.8</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>ALP (IU)</td>
<td>490±50.2</td>
<td>142±31.6</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>212.8±25.3</td>
<td>44±6.6</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>25(OH) vitamin D (ng/ml)</td>
<td>5.18±0.68</td>
<td>24.5±1.4</td>
<td>( p&lt;0.001 )</td>
</tr>
</tbody>
</table>

Quantitative variables are expressed as Mean ±SD, student t-test were used to compare between the two groups.

ALP : Alkaline phosphatase.

iPTH : Intact parathyroid hormone.

CRP : C-reactive protein.

IL-6 : Interleukin-6.

LVEDD : Left ventricular end diastolic diameter.

LVESD : Left ventricular end systolic diameter.

FS : Fractional shortening.

EF : Ejection fraction.

IVSWT : Interventricular septal wall thickness.

LVPWT : Left ventricular posterior wall thickness.

I/L : Interventricular posterior wall thickness/left ventricular posterior wall thickness.

LVM : Left ventricular mass.

LVMI : Left ventricular mass index.

E/A ratio : E wave/A wave ratio.
Table (2): Biochemical and echocardiographic variables of the study groups at baseline, after 6 months of treatment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>VDDR at base line</th>
<th>VDDR after 6 month of treatment</th>
<th>Control</th>
<th>p-value</th>
<th>At base line vs after 6 months of treatment</th>
<th>After 6 months vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.1±0.6</td>
<td>9.2±0.6</td>
<td>9.4±0.8</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>1.8±0.4</td>
<td>5.0±0.6</td>
<td>5.2±0.8</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALP (IU)</td>
<td>490±50.2</td>
<td>172±58.6</td>
<td>155±42.6</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>212.8±25.3</td>
<td>47.5±7.2</td>
<td>44.6±6.6</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>25(OH) vitamin D (mg/ml)</td>
<td>5.18±0.68</td>
<td>23.9±2.34</td>
<td>24.12±1.95</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>14.01±1.73</td>
<td>6.08±1.08</td>
<td>5.65±1.12</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (ng/l)</td>
<td>26.07±5.01</td>
<td>6.64±1.27</td>
<td>6.08±1.13</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>31.92±1.04</td>
<td>20.37±2.55</td>
<td>21.1±2.1</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>23.57±1.15</td>
<td>14.26±1.21</td>
<td>13.98±1.5</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EF (%)</td>
<td>57.41±3.43</td>
<td>64.06±6.1</td>
<td>66.5±8.6</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FS (%)</td>
<td>23.96±0.99</td>
<td>34.08±3.73</td>
<td>35.9±3.9</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LVWT(mm)</td>
<td>3.75±0.5</td>
<td>4.0±0.6</td>
<td>4.1±0.6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>I/L</td>
<td>1.06±0.06</td>
<td>1.07±0.07</td>
<td>1.05±0.04</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>67.42±11.5</td>
<td>65.43±11.7</td>
<td>64.76±12.4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.33±0.05</td>
<td>1.35±0.09</td>
<td>1.34±0.07</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Quantitative variables are expressed as Mean ±SD, student t-test were used to compare between the two groups.

ALP : Alkaline phosphatase.
iPTH : Intact parathyroid hormone.
CRP : C-reactive protein.
IL-6 : Interleukin-6.
LVEDD : Left ventricular end diastolic diameter.
LVESD : Left ventricular end systolic diameter.
FS : Fractional shortening.
EF : Ejection fraction.
IVSWT : Interventricular septal wall thickness.
LVPWT : Left ventricular posterior wall thickness.
I/L : Interventricular posterior wall thickness/left ventricular posterior wall thickness.
LVM : Left ventricular mass.
LVMI : Left ventricular mass index.
E/A ratio : E wave/A wave ratio.

Fig. (1): A and B correlation coefficients between Vitamin D and IL-6 and CRP in VDDR group at baseline (A) and after 6 months of treatment (B).
Discussion

Vitamin D has received worldwide attention not only for its importance for bone health in children and adults but also for reducing risk for many chronic diseases including autoimmune diseases, type 2 diabetes, heart disease, many cancers and infectious diseases [17].

Vitamin D has net effect of increasing serum levels of calcium and phosphorus levels and achieves this by increasing intestinal calcium and phosphorus absorption. Vitamin D deficiency results in reduced serum calcium, which triggers secretion of parathryoid hormone to release calcium and phosphorus from bone in an attempt to maintain normal serum calcium levels [18].

Regarding the cardiovascular system, investigators have found an association between vitamin D deficiency and cardiovascular diseases and risk factors [19,20].

Vitamin D reduces the expression of several genes which are upregulated in myocardial hypertrophy, e.g. by suppressing the cardiac rennin-angiotensin system and natriuretic peptides. Vitamin D has been shown to exert antihypertrophic effects on cardiomyocytes by increasing thrombomodulin and decreasing tissue factor [21]. Also, vitamin D exerts various effects on the growth and differentiation of cardiomyocytes, which are largely suggested to improve myocardial structure and function [21]. In addition, it has been shown that cardiac myocytes and fibroblasts express the enzymes 1α-hydroxylase [22]. Furthermore, the expression of myosin, a major contractile protein of the myocardium, is also regulated by vitamin D which may explain the associations of vitamin D status and myocardial contractility [23].

In the present study, serum Ca level was low in VDDR at baseline compared to the control group and reach to the normal level after 6 months of treatment with vitamin D. Within the heart, calcium
ions are essential for the initiation of excitation-contraction coupling via an influx through L-type calcium channels. Once it is released from the sarcoplasmic reticulum by ryanodine receptors, calcium determines contractility by mediating the tension developed between actin and myosin filaments via the troponin-tropomyosin complex. Decreased amounts of available calcium lead to diminished responses in both of these areas and decreased cardiac function [24].

In the present work, PTH levels were high in VDDR group at baseline and decreased after 6 months of vitamin D treatment. As 25(OH) vitamin D falls, intestinal absorption of calcium falls leading to decreased serum calcium. This causes a rise in the serum PTH, which stimulate conversion of 25(OH)(OH)2 D and thereby maintains absorption of calcium [25]. Thus optimal level of 25(OH) D is defined as level which causes maximal suppression of PTH and maximum Calcium absorption [26]. Elevated PTH was level reported to be a cardiovascular risk factor independent of calcium and phosphorus levels [27]. PTH is pro-atherosclerotic, stimulates systemic and vascular inflammation, augmenting atherogenesis [28]. Also, high PTH levels activates the renin-angiotensin system, causing increased blood pressure and left ventricular hypertrophy (with subsequent apoptosis and fibrosis) [19]. It is debated whether the beneficial effects of vitamin D on the cardiovascular system are direct or related to the physiological vitamin D-related lowering of PTH levels [29].

Results of the present study showed a decrease in phosphorus level at baseline of VDDR group which improved after treatment. Liu et al., [30] reported that hypophosphatemia caused left ventricular hypertrophy with upregulation of catecholamine and renin-angiotensin system components. Also, a previous study illustrated that the hypophosphatemia that resulted from vitamin D deficiency resulted in muscle weakness [12]. They suggested that the muscle weakness could result from central importance of phosphorus in muscle function involving large amounts of ATP and the high level of phosphorylation and dephosphorylation of proteins during contraction and relaxation.

In our study, alkaline phosphatase (ALP) level was high at the study entry of the VDDR group which was normalized after 6 months of vitamin D supplementation. ALP is an excellent marker of rickets activity because it participate in the mineralization of bone and growth plate cartilage. Serum ALP is elevated in hypocalcemic rickets [31]. Sahay and Sahay [26] suggested that ALP may be used for the screen of rickets.

In our study, serum IL-6 and CRP levels were elevated at the baseline and reached the normal levels after 6 months of vitamin D treatment. Alterations in the inflammatory markers with vitamin D deficiency were observed by many investigators [32,34]. Thota et al., [34] showed that vitamin D caused down regulation of IL-6 and upregulation of anti-inflammatory cytokines. Also, Beilfuss et al., [35] found that 1 year of vitamin D supplementation reduces the level of IL-6 in vitamin D deficient subjects. In addition, a study done on infants with congestive heart failure who have baseline 25-hydroxyvitamin D below the lower end of the reference range. 12 weeks of vitamin D supplementation resulted in improvement of LVEDD, LVESD, EF%, FS% and decreased IL-6 level [36]. He suggested that vitamin D is a potent anti-inflammatory agent that improved cytokine profile balance. Moreover, experimental evidences has been identified that vitamin D deficiency induced hypertrophy in cardiomyocytes with decreased expression of vitamin D receptor and suppressor of cytokine signaling (SOCS3) in cardiomyocyte which was also associated with increased inflammatory markers in epicardial adipose tissue [37]. Liss and Fisherman [38] proposed that increment of proinflammatory cytokines tumor necrosis-α (TNFα) and IL-6 are one of the pathophysiological mechanisms involved in heart disease with vitamin D deficiency.

In the present study, EF% and FS% were lower while LVEDD and LVESD were higher in VDDR group at the baseline and normalized after 6 months of treatment. EF% and FS% are the most commonly used parameters in the clinical evaluation of systolic functions of the left ventricle [39]. This indicated the presence of systolic dysfunction and poor left ventricular contraction at baseline that reach normal values after treatment. Also, increased LVEDD and LVESD signified the presence of dilated left ventricle among the studied subjects. The combination of dilated left ventricle and poor contractility of left ventricle implying dilated cardiomyopathy among VDDR subjects. Verma et al., [9] reported that VDDR caused asymptomatic left ventricular dysfunction that improves with treatment. They concluded that VDDR must the considered as an important curable cause for dilated cardiomyopathy among children especially in regions where nutritional rickets is still common.

Finally, our results demonstrated significant -ve correlations in VDDR group between Vitamin D and each of IL-6, CRP levels, LVESD and LVEDD at baseline and after 6 months of treatment. On the other hand, significant +ve correlations
were observed between vitamin D and FS% and EF%. These results are in line with Eleftheriadis et al., [40] who found inverse correlation between Vitamin D and IL-6 and CRP and Fall et al., [41] who observed higher circulating vitamin D concentrations to be associated with better left ventricular systolic function and smaller LVESD. This means that the increment of vitamin D concentration in VDDR is associated with improvement of cytokines profile and left ventricular function.

In Conclusion:
Vitamin D deficiency in rachitic infants is associated with increment of inflammatory markers and left ventricular impairment. Vitamin D supplementation in rickets reduce the cardiovascular complication and improve the associated systemic inflammation. Also, our results support the concept of a possible contributing role of the elevated inflammatory markers in the pathophysiology of impaired left ventricular function in vitamin D deficient rachitic infants.

References


32- JAMALI Z., ARABABADI M.K. and ASADIKARAM G.: Serum levels of IL-6, IL-10, IL-12, IL-17 and IFN-γ and their association with markers of bone metabolism in vitamin D-deficient female students. Inflammation; doi: 10.1007/s10753-012-9531-9, 2012.


Is there Still a Place for Laparotomy in the Management of Tubal Ectopic Pregnancy?

AHMED M.M.K. NOOH, M.D.
The Department of Obstetrics & Gynaecology, City Hospital, Birmingham, UK, Zagazig University Students’ Hospital, Zagazig, Egypt

Abstract

**Background:** Due to the perceived advantages of laparoscopy over laparotomy, the last two decades have witnessed an enormous increase in the number of tubal ectopic pregnancies which were managed successfully laparoscopically to the point that the role of laparotomy in the management of this condition might have become questionable.

**Objective:** To investigate whether there is still a role for laparotomy in the management of tubal ectopic pregnancy and, if any, to what extent?.

**Subjects and Methods:** Fifty women were treated surgically for tubal ectopic pregnancy over 14 months at a university-affiliated British hospital. Out of these cases, 24 (48%) had a laparotomy and these constituted the subjects of this study. Case notes were reviewed and patients’ data were collected. These were analysed mainly for the indications of laparotomy and capacity of operator.

**Results:** The most common indication for laparotomy was haemodynamic instability. Only one laparotomy was performed by a consultant, another one by a senior resident (year 4-5) and the remaining 22 by junior residents (year 1-3). The consultant, senior resident and only one junior resident have had adequate operative laparoscopic experience. Nineteen cases (38%) could have avoided laparotomy had they been managed by a surgeon with adequate operative laparoscopic experience. This leaves only 5 cases (10%) in which laparotomy was unavoidable.

**Conclusion:** A certain percentage (10% in this study) of all tubal ectopic pregnancy would eventually have a laparotomy. The main indication for unavoidable laparotomy is haemodynamic instability. Other reasons include extensive pelvic adhesions and history of multiple pelvic/abdominal surgeries.

**Key Words:** Tubal ectopic pregnancy – Laparotomy – Laparoscopy.

Introduction

ECTOPIC pregnancy is defined as a pregnancy in which the fertilized ovum implants outside the uterine cavity [1,2]. Between 95.5% and 98% of ectopic pregnancies implant in the Fallopian tube while rarely an ectopic pregnancy implants at an extra-tubal location such as the ovary (3.2%), abdomen (1.3%), cervix, liver, spleen or Caesarean section scar. The most common sites of tubal ectopic pregnancy are the ampullary region (73.3%), isthmus (12.5%) or fimbria (11.6%). Interstitial or cornual ectopics, where the pregnancy implants in the intra-myometrial portion of the tube, are less common (2.6%) but have a mortality twice that of any other type of tubal ectopic pregnancy [1,3].

Around 1-2% of all reported pregnancies in the developed world are ectopic [4,5]. The incidence is thought to be higher in the developing countries, but specific numbers are unknown. Although the incidence in the developed world has remained relatively static in recent years, between 1972 and 1992 there has been an estimated six-fold rise [6]. This increase was attributed to an increase in risk factors in women of reproductive age (e.g. pelvic inflammatory disease mainly due to Chlamydia infection, and smoking), increased use of assisted reproductive technology (ART) and increased awareness of the condition, facilitated by the development of specialised early pregnancy assessment units [7,8]. The incidence after oocyte retrieval/embryo transfer may be as high as 4.5%, although this may be due to already existing tubal pathology in these patients and not solely to ART intervention [9]. Nevertheless, more than half of diagnosed ectopic pregnancies are not associated with risk factors [10].

Surgery for tubal ectopic pregnancy may be radical (salpingectomy) or conservative (usually
salpingostomy), and it may be carried out through traditional open surgery (laparotomy) or laparoscopically depending on the surgeon’s skill, equipment availability and condition of the patient [11].

Due to the perceived advantages of laparoscopy over laparotomy which include reduced overall cost, shorter hospital stay and early return to work, the last two decades have witnessed an enormous increase in the number of tubal ectopic pregnancies which were managed successfully laparoscopically [12].

In view of the continuously escalating rate of laparoscopic procedures in the management of tubal ectopic pregnancy, the purpose of this study was to investigate whether there is still a place for laparotomy in the management of this condition and, if any, to what extent?.

**Subjects and Methods**

This study was carried out at City Hospital, Birmingham, UK. All cases of tubal ectopic pregnancy treated by laparotomy over the period from October 2001 to December 2002 were identified from the theatre register. Case notes were retrieved from the medical records and reviewed. Patients’ data were collected, using a purpose-designed proforma, and analysed mainly for the indications of laparotomy and capacity of operator.

**Ethical approval:** This study was approved by the hospital ethical committee. Women were counselled and a clear explanation about the intervention was given prior to surgery. An informed consent was then obtained from all participants.

**Inclusion criteria:** All cases of tubal ectopic pregnancy which ended up having a laparotomy were included in this study.

**Exclusion criteria:** All cases of tubal ectopic pregnancy which were managed totally laparoscopically were excluded from the study.

All cases were subjected to thorough history taking, general and local examinations. Serum $\beta$-hCG monitoring and abdominal and/or transvaginal ultrasound examinations were carried out to confirm the diagnosis of ectopic pregnancy.

Pre-operative preparation included saving two units of packed red blood cells for each patient. All operations were carried out under general anaesthesia with muscle relaxant. Pre-medication, induction, maintenance and reversal medications were the same for all cases.

Prior to surgery, all cases were given prophylactic antibiotics, and anti-thrombo-embolic measures were taken, if needed, according to the unit protocol.

Laparotomy was performed through a low transverse incision and salpingectomy was the surgical procedure of choice.

Patients were divided into three groups; group (A): Those who had immediate laparotomy, group (B): Those who had diagnostic laparotomy prior to laparotomy and group (C): Those who had conversion to laparotomy following attempted operative laparoscopy.

To detect persistent viable trophoblastic tissue after surgery, all patients were followed-up by serial serum $\beta$-hCG levels on day 4 and day 7 and then weekly until non-pregnant levels (<5 IU/L) were reached, with weekly clinical examination and trans-vaginal ultrasound scans, if needed.

**Results**

Fifty cases of tubal ectopic pregnancy were dealt with over the 14 months study period of which 26 cases (52%) were treated totally laparoscopically. These were excluded as they were beyond the scope of this study. The remaining 24 cases (48%) who ended up having a laparotomy constituted the subjects of this study. As shown in Table (1), 5 cases were in group (a), 18 cases in group (b) and only one case was in group (c).

The indications of laparotomy are shown in Table (2), while Table (3) shows the capacity of operator.

### Table (1): Patients groups.

<table>
<thead>
<tr>
<th>Patients groups</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>Group (B)</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>Group (C)</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>24</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table (2): Indications of laparotomy.

<table>
<thead>
<tr>
<th>Indications of laparotomy</th>
<th>Patients groups</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemodynamic instability</td>
<td>Group (A)</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>(circulatory shock)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of operative laparoscopic experience</td>
<td>Group (B)</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>Failed operative laparoscopy</td>
<td>Group (C)</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>24</td>
<td>100</td>
</tr>
</tbody>
</table>
Ahmed M.M.K. Nooh

Table (3): Capacity of operator.

<table>
<thead>
<tr>
<th>Capacity of operator</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consultant</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Senior Resident (year 4-5)</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Junior Resident (year 1-3)</td>
<td>22</td>
<td>91.6</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100</td>
</tr>
</tbody>
</table>

The surgeons involved had operative laparoscopic experience at different levels of competence. The consultant, senior resident and only one junior resident had adequate operative laparoscopic experience while the remaining 21 junior residents were at beginning of the learning curve.

All, except two, cases in this study went uneventful with no major complications. The two exceptions were as follows: One patient had to return to theatre the same day with internal haemorrhage. She was re-opened and the bleeding source was secured. The other case was re-admitted to hospital 3 days after discharge. She was found to have a severe wound infection. Treatment with I.V. antibiotics was started and the wound was laid open in theatre. Eventually, her wound healed completely.

Discussion

As a standard, it was stated that 90% of cases of ectopic pregnancy should have a laparoscopic approach for surgery [13,14]. Nevertheless, a success rate of laparoscopic management of tubal ectopic pregnancy of 84.3% with 7% unavoidable laparotomy rate was reported from a teaching hospital with a dedicated sub-specialised minimal access surgery unit [15], while two other studies from two different district general hospitals, showed a rate of 62% and 73.8% respectively, with a higher rate of laparotomy [16,17]. Neither of the latter two studies investigated the reasons of such a high rate of laparotomy.

Twenty two out of 24 cases (91.6%) which had a laparotomy in this study were managed out of hours by residents in different grades of training with limited or no operative laparoscopic experience when, unfortunately, no senior laparoscopic surgeon was available, or called late.

It is recommended to provide junior staff with formal structured laparoscopic training [18]. Instead, under ideal circumstances, inexperienced residents, supervised by senior laparoscopic surgeons willing to teach, could perform the laparoscopic procedures [19].

In this study, 19 cases (38%); 18 in group (b) and the single case in group (c), could have avoided laparotomy had they been managed by an operator with adequate operative laparoscopic experience.

However, not all cases of tubal ectopic pregnancy will be suitable for laparoscopic surgery, and haemodynamic instability remains an absolute contraindication of laparoscopy. For that reason, laparotomy was mandatory and unavoidable in the remaining 5 patients (10%) in this study. Other common indications of laparotomy are history of multiple abdominal/pelvic surgeries, extensive adhesions, persistent bleeding during attempted operative laparoscopy and extreme obesity [19].

Especially at district general hospitals where most of ectopic pregnancies are likely to be managed, laparotomy, however, remains the most common approach. This slow uptake of laparoscopic surgery is attributed to several reasons; among which are the need for further training, included capital cost of equipment and unpredictability of operating time [20,21].

As this is a retrospective study, there was no random assignment to the type of surgical approach, and selection bias could not be eliminated. The experience and skill of the operating surgeons are other confounding factors in any surgical study. As most of the operations in this study were carried out by surgeons learning both procedures (laparotomy and operative laparoscopy), this confounding effect should - to some extent - have been reduced. A prospective randomised study would be required to minimise the effects of biases in case selection and surgeon experience.

Conclusion:

It has been shown that of all tubal ectopic pregnancies, a certain percentage (10% in this study) would eventually have a laparotomy. The main reason for unavoidable laparotomy remains as haemodynamic instability. Other reasons include extensive pelvic adhesions and history of multiple pelvic/abdominal surgeries.

Acknowledgement:

The author would like to thank the nursing staff at City Hospital, Birmingham, England, UK for their contribution to collection of the data of this study.

References

1116 Is there Still a Place for Laparotomy in the Management


Abstract

Background: Newborns treated in a neonatal intensive care unit (NICU) are exposed to a variety of painful procedures. Unrelieved pain in newborns may lead to potential long term physiological and behavioral consequences. Nurses in NICU have a professional and ethical accountability to have knowledge about assessment and treatment of pain in newborns.

The Aim of this Study: Was to investigate nurses' knowledge about physiological and behavioral pain indicators of newborn. The present study was a descriptive study. The study included all nurses working in the intensive care units for newborns in Port Said Hospitals (N=70). The data were collected using a Nurses’ Pain Knowledge Structured Questionnaire Sheet. The results of this study indicated that; the majority of the nurses had lack of knowledge about physiological and behavioral indicators of pain in newborn. Moreover; no statistical significant differences were found between nurses’ knowledge scores about pain in newborns and their age, their level of education or their experience in neonatal intensive units. It is recommended that nurses who cares for newborns should be familiar with and trained to assess pain of newborn through in service-training programs and nurses must be encourage to attend national, international conferences and workshops about pain assessment and management of newborns.

Key Words: Newborn – Pain indicators.

Introduction

PAIN is defined as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage” [1]. Although the newborns’ nervous system is still under developing, they are fully capable of transmitting, perceiving, responding to, and probably remembering noxious stimuli [2]. The nerves of the newborns respond more readily to noxious stimuli, with a lower threshold to stimulation, than those of adults. The neural pathways that descend from the brain to the spinal cord are not well developed in the newborns, resulting in limiting the ability of their central nervous system to inhibit nociception than the adults [3].

Newborns admitted to NICU are often exposed to pain from variety of sources, caused by diagnostic procedures such as arterial/venous puncture, heel stick, lumbar puncture, and retinopathy of prematurity (ROP) examination and bone marrow aspiration [4]. Therapeutic procedures also cause pain in newborns include nasogastric tube placement, tracheal intubation and extubation, tracheal suctioning, chest tube insertion, mechanical ventilation, suprapubic aspiration, dressing changes, suture removal, and removal of adhesive tape [4]. Frequency of invasive procedures is inversely related to gestational age and severity of illness. Therefore, the smaller and sicker newborns are exposed to the greater numbers of most painful procedures [5].

Pain in newborns cause physiological and behavioral responses. There are several parameters that used to assess physiological responses to pain. These parameters include increase sympathetic stimulation; heart rate, blood pressure, respiratory rate, changes in the level of oxygen and carbon dioxide in blood and palm sweating. Vagal tone which is manifested by pallor or flushing diaphoresis, dilated pupil and increase intracranial pressure are among the manifestation of pain [6].

Behavioral changes in newborns who have pain includes vocalizations; crying in higher-pitch,
tense, and harsh, whimpering, moaning. Moreover there are facial expressions in response to pain in newborns which includes; grimacing, furrowing or bulging of the brow, quivering chin, eye squeeze, nasal flaring, curling/curving of the tongue and facial twitching [7]. Body movements in newborns when feel pain consist of general diffuse body activity as flexing/extending extremities; extending legs; fingers play, hand on face, limb withdrawal, swiping and thrashing. The newborns have changes in tone, hypertonicity, rigidity, fist clenching then hypotonicity and flaccidity in response to painful stimuli [8].

Assessment and management of neonatal pain are an ethical duty for health care professionals and primarily focus of nursing care [9]. The assessment of pain is an essential role for the pediatric nurse and this should form a part of the routine assessment procedure for newborn assigned to the nurse [10]. Nurses, especially the pediatric nurses, should know the various neonatal pain assessment instruments, use them effectively and ensuring appropriate interventions [II].

Multidisciplinary approaches for management of pain in newborns are required in neonatal intensive care unit. Pain can be managed either pharmacologically or non-pharmacologically. The non-pharmacological strategies include; non-nutritive sucking, swaddling, oral sucrose solution, skin to skin contact and breastfeeding. Pharmacological agents are required in invasive procedures and post-operative period include; opioid, morphine, fentanyl, topical anesthetic cream [12]. It is important for nurses who care for newborns to prevent or eliminate pain as much as possible to promote positive neuro-developmental outcomes during infancy and also in later childhood and adulthood [10].

Aim of the study:
Investigate nurses’ knowledge about physiological and behavioral pain indicators of newborns.

Subjects and Methods
A descriptive study design was used, carried out at the Neonatal Intensive Care Units (NICU) of the Governmental and Health Insurance Hospitals in Port Said City. Which include 4 Hospitals.

Sample:
A sample of convenience was used in this study. Consisted of all nurses working in Neonatal Intensive Care Units, regardless of their years of experience or qualifications, comprised the study subjects with total number 70 nurses. Data collection took a period of 4 months from January to April 2012.

Tool of the study:
Nurses’ pain knowledge structured questionnaire sheet:
It was developed by the researcher guided by Jones’ pain tool [13] to identify nurses’ knowledge about physiological and behavioral pain indicators of newborn. It consisted of three parts:
Part (I):
Socio-demographic data such as; nurses’ age, level of education, years of experience care for neonates, training programs attended about neonatal care especially during pain.
Part (II):
Nurses’ knowledge about pain as; definition, causes, factors affecting pain, pain assessment and management of newborn.
Part (III):
Physiological and behavioral pain indicators. It included:
A- Physiological signs as, increase heart rate, blood pressure, respiratory rate, pallor, sweating.
B- Behavioral signs as, crying, grimace, general diffuse in body activity, sleep-wake cycle change, irritability, lethargy.

Scoring system:
Scores were used to evaluate nurses’ knowledge where each true answer of knowledge was given one mark and zero mark if not known.

Total knowledge score about newborns’ pain were 93 marks distributed as follows:
A- Nurses’ knowledge about pain in newborns (61 marks) were distributed as follows:
• Definition of pain (2 marks).
• Causes of pain in newborns (10 marks).
• Factors affecting pain sensation in newborns (4 marks).
• Types of medical and nursing interventions causing pain (12 marks).
• Nursing management of pain in newborns (11 marks).
• Non-pharmacological management to relieve pain in newborns (7 marks).
Types of medications used to relieve pain in newborns (4 marks).

Signs of pain in newborns (11 marks).

B- Nurses’ Knowledge about Physiological and Behavioral Pain Indicators in Newborns (32 marks) were distributed as the follows:

- Vocal indicators of pain in newborns (2 marks).
- Facial indicators of pain in newborns (7 marks).
- Body movement indicators of pain in newborns (6 marks).
- Physiological indicators (11 marks).
- Behavioral indicators (6 marks).

Total score of knowledge was classified as follows:

- Good 65% or more.
- Fair 50% to less than 65%.
- Poor less than 50%.

Results

Table (1) illustrates the socio-demographic characteristics of the studied nurses. It is clear from the table that 45.7% of the nurses’ aged between 20 to less than 25 years with a mean age 26.0±4.9 and 54.3% of the nurses graduated from Secondary Nursing School.

Table (2) demonstrate occupational characteristics of the studied nurse. It is revealed from the table that 67.1% of the studied nurses had experience from 1 to less than 6 years’ about newborns. Only 8.6% of the nurses attended training courses about neonatal pain, all of them attended course about the effect of pain on newborns.

Table (3) represents nurses’ assessment of pain in newborns. It is shown from the table that 58.6% of the studied nurses assessed pain in newborns. 57.1% of the studied nurses had obstacles when assess pain in newborns related to lack of time (40.5%).

Figures (1,2) illustrate knowledge of nurses about physiological indicators of pain in newborns. 98.6% of nurses knew that heart and respiratory rates would increase when newborns exposure to pain and 78.6% of the studied nurses knew that suckling reflex decreased when the newborn had pain while 62.9 of the studied nurses knew that the face of newborn become pallor when exposure to pain. Only 25.7% of the studied nurses knew that the blood glucose level would increase during pain.

Figure (3) demonstrates nurses’ knowledge about behavioral indicators of pain in newborn. 88.6 of nurses had knowledge about facial indicators of pain in newborns. 87.1% of the nurses had knowledge about both vocal and body movement indicators of pain in newborns.

Figure (4) represents nurses’ knowledge about facial indicators of pain in newborns. The most facial signs known by the studied nurses were facial redness or pallor (62.9%) and grimace of the face (32.9%). While, only 8.6% of the nurses knew that the newborn close eye firmly during pain.

Figure (5) represents nurses’ knowledge about vocal indicators of pain in newborns. It is clear from the figure that the most vocal signs known by the studied nurses were vigorous crying (81.4%) followed by groaning (21.4%).

Figure (6) revealed body movement’s indicators of pain in newborns. Body movement indicators of pain in newborn stated by one third of the studied nurses were kicking with legs (34.3%) while less than one quarter of them mentions flex legs to abdomen (22.9%), increased random movement (22.9%). Only 15.7% of the nurses knew that pain in newborns could be accompanied with convulsions.

Figure (7) describe scores of nurses’ total knowledge about pain in newborns. It revealed from the figure that 95.7% of the nurses had “poor” levels of total knowledge scores.

Figure (8) indicates that 90.9% of the nurses who are less than 25 years had “poor” total knowledge score about pain in newborns and 100% of nurses who are 25 years and more had “poor” total knowledge score about pain in newborns.

Figure (9) demonstrates from the figure that all of the studied nurses who graduated from technical institute and secondary nursing schools had “poor” total knowledge scores about pain indicators of newborns. While 85% of the nurses who have baccalaureate nursing degree had “poor” total knowledge scores.

Figure (10) revealed that 94.7% of the nurses who have experience less than 6 years had “poor” knowledge score and 100% of the nurses with experience of 6 years or more regarding pain of newborns.
Fig. (1): Nurses’ knowledge about physiological indicators of pain in newborns.

Fig. (2): Nurses’ knowledge about physiological indicators of pain in newborns.

Fig. (3): Nurses’ knowledge about behavioral indicators of pain in newborn.

Fig. (4): Nurses’ knowledge about facial indicators of pain in newborns.

Fig. (5): Nurses’ knowledge about vocal indicators of pain in newborns.

Fig. (6): Body movement’s indicators of pain in newborns.
Fig. (7): Scores of nurses’ total knowledge about pain in newborns.

Fig. (8): Total scores of nurses’ knowledge about pain in newborns according to their age.

Fig. (9): Total scores of nurses’ knowledge about pain of newborns according to their educational level.

Fig. (10): Total scores of nurses’ knowledge about pain of newborns according to their duration of experience for care of neonates.

Table (1): Socio-demographic characteristics of the studied nurses.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. n=70</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age/years:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>20-</td>
<td>32</td>
<td>45.7</td>
</tr>
<tr>
<td>25-</td>
<td>21</td>
<td>30.0</td>
</tr>
<tr>
<td>30-</td>
<td>13</td>
<td>18.6</td>
</tr>
<tr>
<td>35 or more</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Min-Max/years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-39</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean±SD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.0±4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Education:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baccalaureate nursing degree</td>
<td>20</td>
<td>28.6</td>
</tr>
<tr>
<td>Technical nursing institute</td>
<td>12</td>
<td>17.1</td>
</tr>
<tr>
<td>Secondary nursing school</td>
<td>38</td>
<td>54.3</td>
</tr>
</tbody>
</table>

Table (2): Occupational characteristics of the studied nurses.

<table>
<thead>
<tr>
<th>Occupational characteristics</th>
<th>No. n=70</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of experience about newborns/years:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>10</td>
<td>14.3</td>
</tr>
<tr>
<td>1–1-19</td>
<td>47</td>
<td>67.1</td>
</tr>
<tr>
<td>6–12</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>12 and more</td>
<td>7</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Attending training courses about pain in newborns:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>No</td>
<td>64</td>
<td>91.4</td>
</tr>
<tr>
<td><strong>Type of training courses about pain in newborns</strong>:</td>
<td>n=6</td>
<td></td>
</tr>
<tr>
<td>Assessment of pain in newborns</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>Care of newborns in pain</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>Effect of pain on newborns</td>
<td>6</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*More than one answer.
Discussion

Pain is a dynamic experience, and nurses have the responsibility for understanding that effective pain control is important [14]. It is great importance to assess the neonatal pain indicators knowledge among nurses to improve nurse’s understanding about the concept and mechanisms of pain in order to plan effective nursing intervention in clinical practice. Management of pain should be a priority for all pediatric health care providers [15].

Newborns are undergoing many painful procedures daily, which may have long-term negative effects. Painful procedures are a risk for brain damage through an increase in arterial and intracranial pressure, and oxygen desaturation [16]. Neonatal pain treatment is still far from being satisfactory [5]. Several reasons may explain why neonatal pain is underestimated; it may be due to a cultural lack of empathy with newborn and a resistance to the consideration of newborns as “real” patients.

McCaffery M. et al. [17] illustrated that, the in-service education is important and is considered as a corner stone of total quality management, moreover, the continuous improvement is impossible without it. The finding of the present study showed that the minority of the nurses attended training courses related to pain and its management (Table 2). This result may be due to insufficient time of nurses in NICU to attend educational programs or that the authority individuals of NICU department do not provide training for nurses about pain of the newborn. This result goes in line with [18] who emphasized the value of advanced educational preparation and continuing education sessions for nurses. Also, [19] carried a research about pain management in newborn and stated that few nurses reported that they received training on neonatal pain.

The results of the present study revealed that more than half of the studied nurses reported that they had obstacles hinder their role in assessment of pain (Table 3). The finding of the current study may be due to the fact that nurses who care for neonates do not have a sufficient time for assessing pain. This finding was in line with [20] who stated that nurses identified a number of barriers that concerning the organizational aspects, such as, workload, legal or institutional constraints and also analgesic prescription was sometimes inadequate or that doctors were unavailable to review medication. In this respect [21], added that perceived barrier to provide adequate pain management include lack of knowledge about pain assessment and management among nurses and physicians, lack of standardized approach to treat pain, fear of addiction and overdose.

Regarding the nurses’ knowledge about physiological indicators of neonatal pain in the present study, it was noticed that the majority of nurses mentioned that the most indicators of pain in neonates were increase in the heart and respiratory rates, decrease Pa O₂ and increase sweating (Fig. 1). These results may be due to the fact that the nurses take vital signs for newborns and notice these changes after painful procedures. The findings of the present study are supported by [22] who stated that painful procedures in newborn stimulate sympathetic nervous system and results in tachycardia, peripheral vasoconstriction, diaphoresis, respiratory rate alterations and oxygen desaturation. Glucose is essential for normal metabolism in the neonate, particularly for cerebral and cardiac metabolism [23] stated that newborns subjected to a variety of noxious stimuli have immediate hormonal responses, such as, decreased secretion of insulin. The result of the present study indicated that only a small number of nurses mentioned that increase blood glucose level is a physiological indicator of pain in newborn (Fig. 2). This may be due to the infrequent use of this method by nurses for newborns who have pain in NICU.

Behavioral indicators are important markers of pain in newborns. Three categories of pain behaviors, facial expression, vocalizations, and motor activity [24]. The highest percentage of nurses in the present study had fair knowledge regarding behavioral signs of pain in newborns includes facial, nasal and body movements indicators (Figs. 3-6). The rationale for such finding may be due to the fact that behavioral signs of pain are easily observed and easily assessed by nurses. This finding was supported by [25] who stated that behavioral responses associated with pain in newborns were

<table>
<thead>
<tr>
<th>Table (3): Nurses’ assessment of pain in newborns.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
</tr>
<tr>
<td>Assessment of pain in newborns:</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Obstacles of newborns’ pain assessment:</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Types of obstacles for pain assessment*:</td>
</tr>
<tr>
<td>Lack of time</td>
</tr>
<tr>
<td>Unavailable tools for assessment</td>
</tr>
<tr>
<td>Lack of experience</td>
</tr>
</tbody>
</table>

*More than one answer.
vocalization of sounds, changes in facial expression which included brows lowered and drawn together, eyes tightly closed, mouth opened and squarish and unexpected or unusual body movements. Understanding these behavioral pain indicators make pain assessment in newborns a little easier. In another study [26], added that these behavioral indicators may not be present in some newborns who are neurologically impaired or pharmacologically paralyzed.

The results of the present study revealed that the majority of the studies nurses were able to determine the vocal indicators of pain in newborns where a large percentage of the sample agreed that continuous vigorous crying is the most common pain indicator (Fig. 5). This result may be explained in the light of the fact that cry is easily observed and the nurses consider the newborns cry because they are in pain or hungry. This finding is congruent with [25] who show that crying is association with acute pain in neonates and it is more intense and sustained when in pain. This result was supported by [27] who also found that crying help in discriminating between the different degrees of pain and if pain intensity is low, crying features are quite different than if pain intensity is high.

The current study results regarding nurses’ knowledge related to newborns’ pain are alarming. The majority of nurses had “poor” knowledge scores regarding newborns’ pain (Fig. 7). In this regard [28], who assessed knowledge and practices of nurses about pediatric pain, found that nurses have lack of knowledge about pain in children. The poor knowledge score findings in the present study may be attributed to the fact that nurses do not emphasize for updating their knowledge regarding pain in newborns. These results may be attributed to the facts that nurses in NICU may have a false perception about pain in newborns and that newborns do not feel pain and do not need treatment. The finding of the current study illustrated by [29] where they found that nurses lacked knowledge about pain. In this respect [30] indicated that nurses had misconceptions and inadequate knowledge regarding pain.

In studying the relationship between nurses’ knowledge scores and their level of education, it was found that nurses who have high level of education had “good” score level of knowledge (Fig. 9). This result may be attributed to their curriculum which might include pain and pain assessment. This finding is supported by [31]; who found that there was a positive correlation between the education level of the nurses and their knowledge regarding pain.

It is revealed from the findings of the present study that nurses who have of experience less than 6 years had “Good” knowledge score compared to nurses with experience of 6 years or more (Fig. 10). This finding may be attributed to the fact that the young nurses may still remember the knowledge they had at school. This finding was not supported by [25] who studied the pain management practices in children after surgery and mentioned that nurses who have lack of experience, have lack in their knowledge. In this respect [12] illustrated that there was no relationship between increased clinical experiences and overall pain scores of knowledge and practice.

**Conclusion:**

Based on the findings of the current study, it is concluded that, the majority of the studied nurses who are working in the Neonatal Intensive Care Units (NICU) in Port Said lack of knowledge regarding physiological and behavioral indicators of pain in newborns. In addition, there is no relation between nurses’ knowledge about newborns’ pain and their age, level of education or years of experience in caring for newborns.

**Recommendations:**

- Nurses, especially those caring for newborns, should be familiar with and trained to use pain assessment tool for newborns’ through in-service training programs.
- Hospital policy should include the application of pain assessment in NICU.
- Adequate pain assessment facilities should be available to encourage nurse to assess pain in newborns.
- Simple Arabic handout about newborns’ pain indicators, causes, methods of assessments and non-pharmacological pain management should be available in all NICU.
- Encourage nurses to attend national and international conferences, and workshops about pain assessment and management for newborns.

**References**

3. HOWARD R., CARTER B., CURRY J., MORTON N., RIVETT K. and WILLIAMS: Good practice in postoper-


Review Article:
Diabetic Foot in the Arab World: An Update

ELSAYED ALSALAMONY, M.D.
The Department of Internal Medicine, Faculty of Medicine, Mansoura University

Abstract
While diabetic foot problem was discussed in many papers throughout the world, unfortunately it has not discussed well in the Arab World. The global epidemic of diabetes has not spared the Arabic-speaking countries; this has not been fully appreciated in the world's literature. In this paper, we clarify why it is more prevalent, less managed and has been associated with worse health outcomes in diabetic patients in the Arab world.

Key Words: Diabetic foot – Arab world – Diabetes.

Introduction
DIABETES mellitus, long considered a disease of minor significance to world health [1], is now considered a global epidemic of the 21st century. The International Diabetes Federation (IDF) estimates show that for the year 2012, 371 million people are living with diabetes, representing a prevalence rate of 8.3%. Estimates also show that 50% of diabetics do not know that they have the condition, as it has not been diagnosed yet [2]. By 2030, the global burden of diabetes is projected to reach 552 million people [1], with a 69% increase in the number of adults with diabetes in developing countries and a 20% increase in developed countries [3].

The Arab world refers to Arabic speaking countries expanded from the Atlantic Ocean in the west to the Arabian Gulf in the east and from the Mediterranean Sea in the north to the horn of Africa and Indian Ocean in the southeast, the prevalence of type II diabetes has increased dramatically in the Arabic-speaking countries over the last three decades, a trend that parallels increased industrial development. The wealth generated by oil-rich resources in countries of the Arabian Gulf have led to improved living standards, while there have also been accelerated urbanization, drastic changes in nutrition, reduced physical activity, and a greater reliance on mechanization and migrant workers. As many as six Arabic-speaking countries are among the world’s leaders in terms of type II diabetes prevalence: These countries are SA is ranked with the 6th highest prevalence of diabetes worldwide, and is expected to hold this position for the next 20 years, with a prevalence rate of 20.0% among 20-79 year-old adults [5]. Other countries ranked in the top 10 include Kuwait (21.1%), Lebanon (20.2%), Bahrain (19.9%) and the United Arab Emirates (19.2%) [4]. The prevalence of diabetes mellitus DM in SA varies among studies [5-7], principally because of differences in research methodologies. Al-Nozha et al., [6] conducted a well-designed national survey and reported a prevalence rate of 23.7% in adult Saudis aged >30 years (26.2% in males versus 21.5% in females). Table (1) provides the 2010 International Diabetes Federation (IDF) statistics for type II diabetes prevalence in developed and developing countries. An estimated 9.1% of the populations from the Middle Eastern/North African region have type II diabetes (32.8 million) in 2011, and this is projected to reach 60 million in 2030. As for type I diabetes in the Middle Eastern/North African region, Saudi Arabia has the largest number of cases (65,000) of T1DM in children aged 0-14 years, while Kuwait has the highest incidence rate (22 cases per 100,000 per year) [4].

Table (1): Arab countries located in the East have among the top ten highest diabetes prevalences in the list published by the IDF (Table 2).

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Nauru</td>
<td>1 Nauru</td>
</tr>
<tr>
<td>2 United Arab of Emirates</td>
<td>2 United Arab of Emirates</td>
</tr>
<tr>
<td>3 Saudi Arabia</td>
<td>3 Saudi Arabia</td>
</tr>
<tr>
<td>4 Bahrain</td>
<td>4 Bahrain</td>
</tr>
<tr>
<td>5 Kuwait</td>
<td>5 Kuwait</td>
</tr>
<tr>
<td>6 Tonga</td>
<td>6 Oman</td>
</tr>
<tr>
<td>7 Oman</td>
<td>7 Tonga</td>
</tr>
<tr>
<td>8 Mauritius</td>
<td>8 Mauritius</td>
</tr>
<tr>
<td>9 Egypt</td>
<td>9 Egypt</td>
</tr>
<tr>
<td>10 Mexico</td>
<td>10 Mexico</td>
</tr>
</tbody>
</table>

Correspondence to: Dr. Elsayed M. Alsalamony, E-mail: ssalamony2002@yahoo.com
Data accumulated over the last 30 years have confirmed that the epidemic of type 2 diabetes is mainly affecting Saudi Arabia SA and adjacent Gulf Council Countries GCC. Indeed). In Sudan knowledge of diabetes epidemic in Sudan is limited the most recent data come from small scales study that was carried out in 1996 the result of the study indicate prevalence of 3-4% but recent estimates place the diabetes population at around one million 95% of whom have type 2 diabetes [8].

Diabetic foot syndrome: Foot disorders are among the most feared chronic complications of DM. Diabetic foot disease comprise a group of disorders that often present with at least one of the following clinical manifestations: Foot ulceration, infection, neuropathy, deformity, gangrene and ischemia. Some or all of these problems may develop in the same patient, often on both feet. If not treated in a timely and appropriate, amputation will become necessary [9]. In turn, amputation is often associated with significant morbidity and mortality [10] in addition to immense social, psychological and financial consequences [11-13].

A person with diabetes has a 15% to 25% lifetime chance of developing a foot ulcer and a 50% to 70% recurrence rate over the ensuing 5 years. A foot ulcer precedes lower-limb amputation in 85% of cases [14]. The 1-year amputation rate of a person with diabetes and a foot ulcer is 15% [15]. The presence of diabetes increases the risk of a nontraumatic lower-limb amputation 20-fold, and worldwide 25% to 90% of amputations, especially nontraumatic lower-limb loss, are associated with diabetes [16].

Moreover, according to some conservative estimates, the treatment costs of these complications account for approximately 25% of total hospital costs of diabetes care, the true costs of which might be an order of magnitude higher [17].

In Sudan, Similar to other African countries, diabetes is no longer rare in Sudan. The country’s resource strained health care system is far from ready to deal with the rising burden of diabetes. Superficial heel ulcers in diabetic patients with a short history of diabetes and with good limb circulation are more likely to heal within an average duration of 25 weeks. At 3 years of follow-up, 75% showed a favorable outcome for ulcer healing, and 22 patients underwent lower extremity amputation (25%), of whom 14 were dead within 3 years [8].

Pathogenesis:

Diabetes can lead to serious complications if it is not properly managed: Most of these complications are related to complications arising from microvascular (e.g., nephropathy, neuropathy, and retinopathy) and macrovascular (e.g., coronary artery disease (CAD), peripheral artery disease (PAD), and cerebrovascular disease [19]. Studies have shown that people with peripheral neuropathy (PNP), and peripheral vascular diseases (PVD) are known to be at high risk of foot complications [20].

Diabetic neuropathy:

DPN is thought to result from multiple factors. PotENTIAL mechanisms for the development of DPN include glycosylation of neural proteins, microangiopathy, the development of neural autoantibodies, and ischemia from basement membrane thickening of the nerve capillaries (vaso nervorum). Abnormalities of the polyol pathway and defects in metabolism of myoinositol and protein kinase C3 leading to neuronal demyelination have also been described in DPN [21].

Peripheral neuropathy may cause loss of sensation in the feet, resulting in a patient’s failure to perceive foot problems, and may cause development of foot deformities that increase pressure points susceptible to ulceration. Osteomyelitis and gangrene may develop from inadequate blood supply and infection [22]. All nerve fibers (sensory, motor, and autonomic) are affected in diabetic peripheral neuropathy. Studies in the Arab world showed a prevalence range of neuropathy from 38-94% in diabetic foot cases [23].

Data from the Western part of Saudi Arabia indicates that the prevalence of neuropathy in diabetic patients is about 82% (which is considered one of the highest in the world) with another 57% being asymptomatic [24].

In a cross-sectional study from Egypt, 22% had peripheral neuropathy and 0.8% had foot ulcers
In Jordan, 5% had amputations (88). At a diabetic clinic in Libya 45% had neuropathy (26). In the UAE the overall prevalence of PN was 39%, which was higher than the equivalent rates reported in other populations (27).

Peripheral vascular disease was defined as the presence of ischemic symptoms such as, or a combination of, intermittent claudication, absence of pedal pulse, arterial occlusion, or decreased blood circulation to the foot on Doppler study (28).

Patients have a 2-4 fold greater risk of developing CAD and PAD than non diabetic individuals (29).

It has been proven by many studies that age, duration of diabetes; hypertension and smoking are the risk factors for the development of peripheral vascular disease in diabetics. It is not clear yet if hyperglycemia, hyperinsulinemia and some types of lipids are risk factors for atherosclerosis in diabetes (30). In the Arab world, peripheral arterial disease is commonly found in diabetics with a prevalence range of 50-78.7% (23).

Clinical presentation: The diabetic foot have 2 categories: The neuropathic foot and the neuroischemic foot. Both categories could be accompanied by infection with different severities.

The neuropathic foot (ulcer): It occurs at sites of high mechanical pressure on the plantar surface of the foot, commonly at the head of metatarsal bones and usually proceeded by callus formation.

Due to hot climate, the common footwear's used are slippers or sandals. These sandals or slippers has a ridge that fits between the first and the second toe. Neuropathic ulcers were commonly observed at the first web space and sometimes too advance that necessitates amputation. Neuropathic ulcers which are small and not infected are rarely seen due to its delayed presentation (7).

Charcot neuroarthropathy: Charcot deformity is a neuroarthropathy of the foot and ankle. The most common area of manifestation is in the midfoot, followed by the forefoot and ankle region. This presents as hot, red, swollen foot, ankle or lower leg that is most often confused with cellulitis, DVT or gout in the early phase. The average time of delay to proper diagnosis is 29 weeks. Prompt treatment with offloading of the foot with a removable walking boot or short leg cast and crutches, wheelchair or rollabout prevents progressive collapse of the foot. It is this collapse of the foot that can lead to callus formation, ulceration, infection and amputation (31).

The neuroischemic foot (ulcer): Ulcers often occur from localized pressure of tight shoes. One of the precipitating factors of ulcers and even infection and gangrene is wound caused by trimming of nails. The neuroischemic ulcers have a significantly poorer outcome compared to neuropathic ulcers because of the blood supply. Infection is multimicrobial. In local studies Staphylococcus aureus, Pseudomonas Argenosa, and Proteus mirabilis, were the most common bacteria (Fig. 1) (23).

Due to hot climate, the common footwear's used are slippers or sandals. These sandals or slippers has a ridge that fits between the first and the second toe. Neuropathic ulcers were commonly observed at the first web space and sometimes too advance that necessitates amputation. Neuropathic ulcers which are small and not infected are rarely seen due to its delayed presentation (7).

Charcot neuroarthropathy: Charcot deformity is a neuroarthropathy of the foot and ankle. The most common area of manifestation is in the midfoot, followed by the forefoot and ankle region. This presents as hot, red, swollen foot, ankle or lower leg that is most often confused with cellulitis, DVT or gout in the early phase. The average time of delay to proper diagnosis is 29 weeks. Prompt treatment with offloading of the foot with a removable walking boot or short leg cast and crutches, wheelchair or rollabout prevents progressive collapse of the foot. It is this collapse of the foot that can lead to callus formation, ulceration, infection and amputation (31).

The neuroischemic foot (ulcer): Ulcers often occur from localized pressure of tight shoes. One of the precipitating factors of ulcers and even infection and gangrene is wound caused by trimming of nails. The neuroischemic ulcers have a significantly poorer outcome compared to neuropathic ulcers because of the blood supply. Infection is multimicrobial. In local studies Staphylococcus aureus, Pseudomonas Argenosa, and Proteus mirabilis, were the most common bacteria (Fig. 1) (23).

![Fig. (1): Diabetes mellitus is responsible for a variety of foot pathologies contributing to the complications of ulceration and amputation. Multiple pathologies may be implicated, from vascular disease to neuropathy to mechanical trauma.](image-url)
**Investigations:** Ankle brachial index which could be falsely high. Transcutaneous oxygen (Tco2) also could be falsely normal because of shunting due to peripheral neuropathy. Toe pressure is probably the most sensitive noninvasive test because of sparing of diabetic vascular changed to the digital arteries. It has been our policy that any diabetic foot ulcer with absent palpable distal pulses should be referred to vascular surgery for further work up. Selective angiography with minimal contrast still our standard investigation. Most of the time, we carried out distal revascularization with >90% limb salvage [7].

**Diabetic foot in the Arab world:**

In the Arab world, several factors make diabetic foot prevalence higher as compared to the West (Table 2):

I- **Weather and footwear:** In most Arab countries the weather is hot and dry most of the year. This makes the habit of wearing closed shoes and socks rejected by many patients and instead they prefer to wear sandals. Sandals do not offer the protection afforded by closed foot wear since they expose feet to heat, dryness and injuries [32].

II- **Habits:** Walking bare-footed especially inside the home is still a common habit in many regions of the Arab world [32].

III- **Religion:** Ninety percent (90%) of Arab populations are Muslims. They pray five times per day where the feet have to be washed before praying. These maneuvers help patients to inspect their feet as well as clean them. Washing feet before praying and the praying itself offer some sort of physical massage to the feet.Trimming the nails is a habit encouraged by Islam, but it should be done properly so as not to harm the toes. Also, every year millions of Muslims engage in the holy practice of Hajj. Among them are many persons with diabetes who may sustain unnoticed physical harm to their feet. Diabetes education and foot care is therefore an important issue before going to do Hajj [32].

IV- **Education:** The percentage of illiterate people is higher in the Arab world than in western countries. Lack of education leads to unawareness of diabetic foot problems and their prevention. Interestingly, one study showed that 90% of screened diabetic patients had poor knowledge about their disease and 96.3% had poor awareness about its control [33].

V- **Media:** In some Arab countries, the media has an inadequate attention to the health problems in general and nothing about diabetic foot problems. Recently, few articles were published seeking medical attention promptly at the earliest onset of symptoms [7].

<table>
<thead>
<tr>
<th>Affecting factors</th>
<th>Arab world</th>
<th>Western world</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weather and foot wear</td>
<td>Hot, dry weather, sandals for shoes</td>
<td>Cold, wet, protective shoes</td>
</tr>
<tr>
<td>Habits</td>
<td>Walking bare foot still common</td>
<td>Rare</td>
</tr>
<tr>
<td>Religion</td>
<td>(Ablution) Washing for prayer five times a day</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>leads to regular foot inspection</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td>Patients information about diabetes and its</td>
<td>Patients are more educated</td>
</tr>
<tr>
<td></td>
<td>complication is still developing. High prevalence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>of illiterate old patients</td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>Poor in health education</td>
<td>Advanced</td>
</tr>
<tr>
<td>Traditional Medicine</td>
<td>Cautery, herbal medicine and blood letting, still</td>
<td>Doesn't exist</td>
</tr>
<tr>
<td></td>
<td>understudied and commonly used</td>
<td></td>
</tr>
<tr>
<td>Surgery Phobia</td>
<td>Poor education is leading to surgery phobia</td>
<td>Patients are more educated and less anxious to seek medical advise early</td>
</tr>
<tr>
<td>Health care system</td>
<td>Patients have to be referred by primary care</td>
<td>Diabetic foot clinics are more available</td>
</tr>
<tr>
<td></td>
<td>physician to the specialist</td>
<td></td>
</tr>
<tr>
<td>Health care providers</td>
<td>Low awareness of the magnitude of the problem</td>
<td>More knowledge about the problem</td>
</tr>
<tr>
<td></td>
<td>and standard management</td>
<td></td>
</tr>
<tr>
<td>Community factor</td>
<td>Strong believe in traditional medicine, delayed</td>
<td>Only modern medicine is practiced, early</td>
</tr>
<tr>
<td></td>
<td>presentation</td>
<td>presentation</td>
</tr>
<tr>
<td>Rehabilitation</td>
<td>Still sub optimal</td>
<td>Advanced</td>
</tr>
</tbody>
</table>
VI- Traditional medicine: This is one of the most important factors in my opinion that led to high prevalence of diabetic foot problems in the Arab world. A) Herbal medicine: herbal medications are still commonly used and most of the time they complicate or obstruct the treatment of modern medicine. Local healers use different kinds of local herbs of a broad spectrum to cure many illnesses [34]. With all the explanation and education spent on trying to convince the public to avoid this kind of traditional medicine; still it is a very common practice. Cautery-since ancient times it is a common belief that cautery is the treatment of choice or the last resort to many diseases. Local healers use heated iron rods of various sizes and shapes with either sharp or pointed ends while they are glowing red. The heated end of the instrument is used by either employing the fine touch or firm pressure. The site of the application varies with different disease. In the diabetic foot, it is commonly seen in the dorsum of the foot or the lateral aspect of the lower leg. We observed some cases complicated with wound infection due to delayed presentation, often leading to amputation.

C- Blood-letting—there is a belief that in certain diseases the blood is bad and the body must get rid of this evil blood. Like in cauterization, there are different sites of blood-letting according to the disease. In the diabetic foot, it is carried out at the ankle. The skin is cut into small multiple cuts then an inverted cup is applied with a match burning inside, immediately before it is applied the match is put off. This will create suction on the skin. An average of 60-120ml of blood is let [34].

VII- Surgery phobia: A very common reason for the delayed presentation of the diabetic foot even in educated patients, is the fear of surgery or amputation. People believe in our part of the world that they should not lose any part of their body even if this leads to death [7].

VIII- Health care system and health care providers: Health resources available for diabetes care and diabetic foot management differ considerably among Arab countries and still the management of the diabetic foot is not based on a multidisciplinary team approach. Due to the frequency and long hospital stays, diabetic foot cases usually consume a considerable part of the health care budgets. For this reason the hospitals’ administrative staff and health care providers are somewhat reluctant to admit patients with diabetic foot problems in their early presentation. This of course results in more complicated problems and subsequently, more amputations [32].

IX- Community factor: Due to a single story for example about a patient who went to the hospital to treat his diabetic foot and ended up by a major amputation or death and in the other hand another patient tried traditional medicine first and got cured, the repercussion would be that most of the patients will try traditional medicine first and will take recourse to the hospitals at a late stage of the disease and end up with an amputation, such as the vicious cycle! [7].

X- Rehabilitation: Physical and social rehabilitation is still an underdeveloped field in Arab countries. Patients with amputations may wait for a long time before they can be provided with an orthotic device. Frequently the cost inhibits the patient from seeking appropriate help. Unfortunately, patients isolate themselves after amputation and live a lonely, depressed life. In addition to this, a lack of employment for amputees has a very negative impact on their life and that of their families [32].

Management:

International studies and guidelines show that targeted foot care and proper screening of risk cases can result in a reduction in the incidence of foot ulcers in patients with diabetes [35].

National Diabetes Programme was established in June 2010 under the Clinical Strategy and Programmes Directorate. In 2011 funding was received to establish a national multidisciplinary foot care service for people with diabetes [36].

Foot care management in diabetes is based on three categories of risk:

1- Patients “at low risk of diabetic foot disease” will be managed preventatively through annual screening and regular foot inspections/examinations by primary care nurses*.
(Definition: A low risk foot patient has normal foot pulses, normal vibration and sensation to 10g monofilament, no history of foot ulceration, no significant foot deformity, or no visual impairment).

2- Patients “at risk of diabetic foot disease” may be stratified as either moderate risk or high risk. All patients will be under regular surveillance by primary care nurses/general practitioners#.

Moderate risk patients will be referred by the GP to the podiatrist, either in the community or in the hospital, for an annual review. These patients will remain under the clinical governance of the GP and podiatrist #.

(Definition: The moderate-risk patient has either impaired peripheral sensation or impaired circulation or significant visual impairment or a structural foot deformity).

High risk patients will be called to be seen at least annually by the diabetes foot protection team in one of the 16 designated centres, and will be under the governance of the foot protection team for their foot care.

(Definition: The high-risk patient has an abnormality that predisposes them to foot ulceration. This can be impaired sensation and impaired circulation, or a previous foot ulcer, previous lower limb amputation or previous Charcot foot).

3- Patients with “active diabetic foot disease”, defined as patients with an active foot ulcer (defined as a full thickness skin break) or a Charcot foot, will be actively managed by a multidisciplinary specialist foot care service, in conjunction with vascular surgery, orthopaedics and orthotics input as required.

Nonetheless, the future is looking promising as there are many efforts to improve the outcome of diabetes and its complications in many Arab countries.

References

20- SHOJAIEFARD A., KHORGAMI Z. and LARIJANI B.:...


Case Report:
Vaginal Neurofibroma
WAEL S. NOSSAIR, M.D. and MOHAMMED S. FARAG, M.D.
The Department of Obstetrics & Gynaecology, Faculty of Medicine, Zagazig University

Abstract
Background: The causes of masses protruding at vulva are many, but vaginal neurofibroma is very rare cause. The manifestations of solitary primary neurofibroma differ as a mass or pain or acute urine retention up to obstructed labour. Pain is one of manifestation that cannot be ignored especially if severe pain that interrupts patient life like physical activity, sexual intercourse, and may lead to psychological disorder. In addition to the repeated attacks of urine retention that may need hospital transfer and repeated catheterization.

Case(s): The case presented with mass protruding at vulva, with attacks of acute urine retention relieved by repeated difficult catheterization, in addition to severe pain that partially relieved by analgesia and hypnotics with disturbed sexual relation and secondary infertility, and at same period with psychological upsets. By Cusco speculum examination using reverse technique i.e. insertion of Cusco speculum in form of blade latterly instead of one blade up and another down, the mass discovered to be pedunculated arising from anterior vaginal wall, the pedicle was long allowing this mass to be entered inside the urethra which was dilated to degree as if vagina is divided transversely in equal manner. Surgical excision was done, neurofibroma detected. Patient became pregnant and delivered safely.

Conclusion: Neurofibroma is rare but should be considered in any painful mass at vulva. By using Cusco speculum in reverse manner we can examine anterior and posterior vaginal wall.


Introduction
neurofibroma differ as a mass or pain or acute urine retention up to obstructed labour. Pain is one of manifestation that cannot be ignored especially if severe pain that interrupts patient life like physical activity, sexual intercourse, and may lead to psychological disorder. In addition to the repeated attacks of urine retention that may need hospital transfer and repeated catheterization.

Case(s)

A 30 year old patient, P2+0, with 3 years 2ndy infertility, previous 2 caesarian sections, presented with mass protruding at vulva, for about 2y with attacks of acute urine retention relieved by repeated difficult catheterization, repeated urinary tract infection in addition to severe pain that partially relieved by analgesia and hypnotics with disturbance of sexual intercourse at same period with psychological upsets. There were offensive vaginal discharge, improved partially by metronidazole and vaginal douches. No family history of medical or surgical disorders. During examination the mass is very painful, by inspection the mass was about 4*3cm with some necrotic areas and offensive discharge. By speculum examination there was difficulty in insertion but by using reverse technique i.e. insertion of Cusco speculum in form of blade latterly instead of one blade up and another down, the mass discovered to be pedunculated arising from anterior vaginal wall 3cm from urethral orifice, the pedicle was long (5cm) enough allowing this mass to enter inside the external urethral meatus which became dilated to degree as if vagina is divided transversely in equal manner. Husband was attending examination and noted that there were partial intercourse at upper opening as he cannot complete the act and cannot enter inside the lower opening containing the mass (vagina), and there are 2ndy infertility for 3 years.

The investigations revealed that, urine pus was over 100/HPF, bilateral back pressure changes of both kidneys, thick wall of urinary bladder, renal gravels.

The decision was removal and histopathology. After routine investigations, and correction of urinary tract infection, under general anesthesia, after catheterization, with use of scalpel, elliptical incision was done, and by use of diathermy the pedicle completely removed with the mass, few stitches were taken after confirmation of intact urethra. After patient become fully conscious of anesthesia, there were dramatic improvement of pain and patient did not use analgesics.

Histopathology diagnose the case as neurofibroma with associated ulceration and septic granulation tissues negative for atypia. Benign soft tissue tumor of peripheral nerve origin. Tumor is formed neural tissue.

After 3 months, patient came with missed period and she was pregnant and followed during pregnancy and delivered safely at term.

Comment:

The interesting presentations of neurofibroma in this patient were; 2ndy infertility, disturbed sexual relation with dilated urethra that allow partial intercourse and leads to repeated urinary tract infections, in association with infected tumor and repeated catherization. In addition to the repeated urethral obstruction by the mass lead to bilateral back pressure changes in kidneys. Also the pedunculated anterior position of the tumour near urethra inside vagina leads to difficulty in diagnosing the case by many gynecologists, but by using reversed Cuso position allow me to examine the anterior in addition to posterior vaginal wall in this very painful lesion without using anesthesia, as outpatient examination. Dramatic improvement after removal of neurofibroma, no pain, no repeated urinary tract infections, no back-pressure changes on both kidneys, and getting pregnant after establishment of normal sexual relation lead to improvement of psychological condition of patient.

Few authors all round world reports cases with genital neurofibroma; Gómez-Laencina, et al., [1], described the case of a 71-year-old patient with pelvic pain and a uterine mass who underwent a
hysterectomy after having been diagnosed with an 11 cm neurofibroma occupying the myometrium of the entire uterine corpus. But our patient site of neurofibroma is vagina and patient was 30 years.

Mourali, et al., [2] and Yayli et al., [20] reported a 71 year old patient with vaginal neurofibroma with Von Recklinghausen disease. Our patient was at 30 years old, and hasn’t Von Recklinghausen disease.

Baulies, et al., [8], reported a 20-year-old woman with a history of type-1 neurofibromatosis with a vaginal nodule neurofibroma. But our patient not have history of type-1 neurofibromatosis and not just a nodule.

Wei, et al., [4], described a case of plexiform neurofibroma affecting the uterine cervix. In our report the vagina was site of the lesion.

Sharma, et al., [5], reported Huge localized vaginal neurofibromatosis as an unusual cause of postmenopausal bleeding. In our report, the bleeding wasn’t a manifestation and patient was young.

Gordon, et al., [6], reported Plexiform neurofibromatosis involving the uterine cervix, endometrium, myometrium, and ovary in a patient without a family history of von Recklinghausen’s disease and without other clinical manifestations of the disease. In our report vagina only was site of lesion.

Ilok, et al., [7], a case of neurofibroma affecting the uterine cervix and endocervix, vagina, and vulva, the urinary bladder, urethra, and one ureter were also extensively affected. In our report vagina only was site of lesion.

Eusebi and Schönauer, [11], reported Pigmented vaginal neurofibroma. In our report there were no pigmnetations.

Gold, [12], published about Neurofibromatosis of the bladder and vagina. In our report the vagina only was involved.

Drescher and Herzog, [15], published about On neurofibromatosis of the vulva and vagina. In our report vagina only was involved.

Marmey and Lacroix, [16], published about Recklinghausen disease and pregnancy; cesarean section in vaginal neurofibromatosis. But our patient was not pregnant at presentation and the neurofibroma was solitary, and pain interfer with intercourse and no pregnancy achieved except after excision of lesion.

Some authors describe single solitary vaginal neurofibroma; Azzopardi, et al., [8], described Neurofibroma with rhabdomyomatous differentiation: Benign “Triton” tumour of the vagina. Imperato, et al., [9], reported Anatomo-clinical observations on a case of solitary neurofibroma of the vagina. Belvederi, et al., [10], reported Anatomo-clinical findings on a case of neurofibroma of the vagina. De Jorio and Belfiore [13], reported rare case of vaginal localization in the course of Recklinghausen’s disease. Stingl, published about contribution to the knowledge of primary neurofibroma of the vagina [14]. Norris and Cooper, [17], reported about Primary neurofibroma of the vagina.

References


