Abstract

Background: Pre-eclampsia is a pregnancy specific multisystemic disorder that is characterized by development of hypertension and proteinuria after 20 weeks of gestation.

Uterine artery Doppler ultrasound screening at 22-26 weeks of gestation is of great value in prediction of complications of impaired utero placental blood flow (pre-eclampsia and intra uterine growth retardation).

Serum leptin assay may allow us to predict pre-eclampsia before development of clinical manifestation.

Aim of Work: This prospective study to determine the role of the combined use of uterine artery Doppler ultrasound and serum leptin assay for prediction of pre-eclampsia.

Key Words: Pre-eclampsia – Uterine artery doppler ultrasound – Serum leptin assay.

Introduction

PRE-ECLAMPSIA is a pregnancy-specific, multisystem disorder that is characterized by the development of hypertension and proteinuria after 20 weeks of gestation. The disorder complicates approximately 5 to 7 percent of pregnancies [1].

One of the most striking physiologic changes is intense systemic vasospasm, which is responsible for decreased perfusion of virtually all organ systems [2].

Perfusion also is diminished because of vascular hemoconcentration and third spacing of intravascular fluids. In addition, pre-eclampsia is accompanied by an exaggerated inflammatory response and inappropriate endothelial activation [3].

Risk factors for pre-eclampsia include medical conditions with the potential to cause microvascular disease (e.g., diabetes mellitus, chronic hypertension, vascular and connective tissue disorders), antiphospholipid antibody syndrome, and nephropathy [4]. The clinical presentation of pre-eclampsia may be insidious or fulminant. Some women may be asymptomatic at the time they are found to have hypertension and proteinuria; others may present with symptoms of severe pre-eclampsia, such as visual disturbances, severe headache, or upper abdominal pain. From 4 to 14 percent of women with pre-eclampsia present with superimposed HELLP syndrome [5]. It has been reported that uterine artery Doppler velocimetry between 22 and 26 weeks of gestation is the “best test” for the identification of patients destined to develop pre-eclampsia [6].

Leptin is a hormone that plays an important role in several physiological processes including the regulation of endocrine function, immune function, inflammation, reproduction and angiogenesis [7].

In pregnancy it has been shown that leptin is highly expressed in the placenta [8]. Pregnant women with subsequent pre-eclampsia showed consistently elevated leptin concentration between 20 and 36 weeks of gestation [9]. Leptin has been indicated to be possible marker for early onset of pre-eclampsia [10].

The purpose of this study was to determine the role of the combined use of uterine artery Doppler diastolic notch and serum leptin assay for prediction of preeclampsia.

Patients and Methods

This study was carried on 300 primigravid women in our patient Obstetric and Gynecology Clinic and Obstetric Department, Banha University.
Hospital, from October 2013 to April 2015. Consent was taken from each patient before investigation and management.

The inclusion criteria of the study population:
- Primigravid women in the period between 22-26 weeks of gestation.

The exclusion criteria of the study population:
- Chronic hypertension.
- Chronic renal disease.
- Chronic hepatic disease.
- Fetal anomalies.
- Multiple pregnancies.

The study population included 300 primigravid women for whom both Doppler ultrasound and serum leptin assay was done.

Initial visit:
- During the initial visit, every pregnant woman was subjected to the following:
  1- Full history taking.
  2- Complete examination.
  3- Routine laboratory investigations.
  4- Ultrasonographic and Doppler examination:
    (Done by senior diagnostic radiologist).
    A- Routine trans-abdominal U/S was done to assess:
      • Biometric measurement for gestational age and fetal growth.
      • Placental location and grading.
      • Fetal wellbeing.
      • Amount of liquor amnii and its turbidity.
      • Fetal biophysical profile according to the modified manning scoring system after 28 weeks gestation.
    B- Doppler velocimetry of the uterine arteries:
      • The presence or absence of diastolic notch was noted and identified whether unilateral or bilateral. The criteria used to determine the presence or absence of a notch were those described by Bower et al., [11], notch was considered to be present "no matter how small the notch was seem to be, from either uterine artery".

According to the Doppler findings of the screening, the pregnant women were divided into:
1- Those with normal Doppler findings.
2- Those with abnormal Doppler findings.

5- Serum leptin assay:
- Blood samples were collected by sterile venopuncture an 18 gauge needle and 5ml of blood are transferred to test tube on which the name and number of the patient.

Leptin assay:
- Principle of the test: The DRG Leptin ELISA Kit is a solid phase Enzyme-Linked Immunosorbent Assay (ELISA) based on the sandwich principle.

Leptin was measured at the time of Doppler ultrasound in the period between 22-26 weeks of gestational age.

Follow-up visits:
- All candidates was followed-up regularly every 4 weeks till 28 weeks gestation then fortnightly till 36 weeks then weekly till delivery, including clinical examination and laboratory investigations to diagnose the development of preeclampsia.

Patient were studied as two groups:
- Group 1: Pregnant women who did not have preeclampsia.
- Group 2: Pregnant women who had preeclampsia.

The data was tabulated and statistically analyzed:
- t-test.
- Correlation coefficient.
- Sensitivity.
- Specificity.
- Positive predictive value.
- Negative predictive value.
- Accuracy.

Results

This prospective study carried on 300 primigravid women with single viable pregnancy.

At 22-26 weeks of gestation range of age was from 19-26 years, the results include 2 groups:

- Group 1 (non preeclamptic group) 273 pregnant ladies did not develop preeclampsia.
- Group 2 (preeclamptic group) includes 27 pregnant ladies develop preeclampsia.

There is a higher mean leptin among preeclamptic group compared to non preeclamptic group and the difference is highly significant statistically.
Albuminurria is positive in preeclamptic group compared to non preeclamptic group and the difference is highly significant statistically.

There is higher mean RI among preeclamptic group compared to non preeclamptic group and the difference is highly significant statistically.

Diastolic notch is positive in preeclamptic group except three cases while it negative in non preeclamptic group except four cases.

Table (1): Comparison between study groups according to lab. parameter.

<table>
<thead>
<tr>
<th></th>
<th>I (N=273)</th>
<th>II (N=27)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± SD</td>
<td>10.5±0.5</td>
<td>10.8±0.3</td>
<td>3.09</td>
<td>0.002*</td>
</tr>
<tr>
<td>Range</td>
<td>9.5-11.5</td>
<td>10-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± SD</td>
<td>32.2±3.6</td>
<td>69.0±8.2</td>
<td>42.9</td>
<td>0.001**</td>
</tr>
<tr>
<td>Range</td>
<td>24.1-70.7</td>
<td>29.7-73.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albuminuria:</td>
<td>No %</td>
<td>No %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–ve</td>
<td>273</td>
<td>100</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>+ve</td>
<td>0</td>
<td>0.0</td>
<td>24</td>
<td>88.9</td>
</tr>
<tr>
<td>++</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
<td>11.1</td>
</tr>
</tbody>
</table>

p<0.001 highly significant.

Table (2): Comparison between study groups according to Doppler indices.

<table>
<thead>
<tr>
<th></th>
<th>Normal group (I) N=273</th>
<th>Preclamptic group (II) N=27</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± SD</td>
<td>0.55±0.08</td>
<td>0.73±0.02</td>
<td>10.8</td>
<td>0.001**</td>
</tr>
<tr>
<td>Range</td>
<td>0.4-0.76</td>
<td>0.54-0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D Notch +ve</td>
<td>3</td>
<td>1.1</td>
<td>23</td>
<td>85.2</td>
</tr>
</tbody>
</table>

p<0.001 highly significant.
RI : Resistant Index.
D Notch : Diastolic Notch.

Table (3): Validity of Doppler and leptin in prediction of preeclampsia.

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>81.5%</td>
<td>97.1%</td>
<td>73.3%</td>
</tr>
<tr>
<td>Diastolic notch present</td>
<td>85.2%</td>
<td>98.9%</td>
<td>88.5%</td>
</tr>
<tr>
<td>Leptin</td>
<td>88.9%</td>
<td>96.3%</td>
<td>70.6%</td>
</tr>
<tr>
<td>R1 &amp; diastolic notch</td>
<td>92.6%</td>
<td>97.8%</td>
<td>80.6%</td>
</tr>
<tr>
<td>R1 &amp; diastolic notch &amp; leptin</td>
<td>96.3%</td>
<td>99.3%</td>
<td>92.9%</td>
</tr>
</tbody>
</table>

Discussion

This study tried to determine the level of serum leptin and uterine artery Doppler at (22-26) weeks of pregnancy and its prediction for preeclampsia to prove or deny that serum leptin and uterine artery Doppler may be used as a marker of preeclampsia.

This study was conducted at Banha University Hospital. The study included 300 pregnant women in the second trimester. These women were categorized into two groups according to the results Group (1) include 273 pregnant non preeclamptic women and Group (2) include 27 pregnant women with preeclampsia.

Serum leptin level were measured in every group and the persistent bilateral early diastolic notch and resistance index as abnormal finding in uterine artery doppler all data were tabulated, statistically and analyzed by Statistical Program for Social Science. The level of significance was p<0.05.

In the current study, the mean value of leptin level in Group 1 (normotensive pregnant women) was 32.2ng/dl and in Group 2 (pre-eclamptic group) was 69ng/dl. Screening of leptin among cases showed a good sensitivity of 88.9% and specificity of 96.3%, the positive predictive value was 70.6% and negative predictive value was 98.9%.

In this study, when comparing the serum leptin levels between non preeclamptic women and preeclamptic women, there was a highly statistically significant deference in between (t=42.9, p<0.001, respectively).

Other studies have reported significantly higher leptin levels in essential hypertensive patients than in controls, as well as a significant correlation between leptin levels and blood pressure [12].

On the contrary some study showing that serum leptin levels were similar in the patients with different grades of pre-eclampsia and normotensive pregnant women as in study done by (Martinez-Abundis et al., 2000) [13], this study was carried out in 14 patients with mild preeclampsia, 12 with severe preeclampsia, and in 32 normotensive pregnant women during the third trimester of pregnancy. The leptin levels were tested by an enzyme-linked immunosorben method. There were no significant differences in serum leptin concentrations between the patients with mild preeclampsia, severe preeclampsia and normotensive pregnant women (p>0.05).
Also in other study there are decreased maternal serum leptin in pregnancies complicated by preeclampsia in study done to compare the leptin level in preeclampsia and normotensive pregnant women [14]. This study was done to determine whether circulating levels of leptin differed between women with preeclampsia and women who had an uncomplicated pregnancy by measurement maternal and umbilical venous plasma leptin concentrations obtained at delivery were compared in 36 pairs of women with either preeclampsia or normal pregnancy. Median leptin concentrations were significantly lower (p<.0001) in women with preeclampsia than in normal pregnant women. Median umbilical venous leptin was not significantly different between groups.

In the present study, screening of early diastolic notch among the pregnant women at (22-24 weeks) of pregnancy showed sensitivity and specificity 85.2% and 98.9% with high PPV 88.5% and high NPV 98.5%, which is in agreement with other reports, [15,16]. Some authors have found that bilateral notches had better sensitivity for prediction of PE, [17,18]. However, others found that bilateral notching for the prediction of PE had low sensitivity, high specificity and NPV, with very low PPV, while for severe cases of PE the sensitivity was higher and the NPV was nearly 100%, [19,20].

In the current study the mean uterine artery resistance index was statistically significant. Screening of RI among cases showed a good sensitivity of 81.5 and specificity of 97.1%, with PPV of 73.3%, and high NPV of 98.1%. The same was observed in the study performed by Simmons et al., [23]. On the other hand lower values were reported in the study done by Axt-Fliedner et al., [18].

In a pilot study Coleman et al., performed uterine artery Doppler screening for 116 high risk pregnant cases at 22-24 wks gestation, a resistance index was calculated from each uterine artery and the presence or absence of a notch was determined, the sensitivity of RI >0.58 for PE was 91%. In women with bilateral notch 47% developed PE, and thus reported that in high risk women, uterine artery Doppler waveform analysis performed the best prediction of severe adverse outcome and was better than clinical risk assessment in the prediction of PE.

In other larger studies done by Albaiges et al., and Papageorghiou et al., [21,22], for prediction of PE comprising 1757 and 7851 high risk cases respectively; revealed that one-stage color Doppler screening program at 23 weeks identifies most women who subsequently develop PE.

On performing uterine artery velocimetry late in the 2nd trimester (22-24wks) as a screening test for subsequent PE development, the demographically matched groups showed a significant difference in velocimetric indices (p<0.05), with bilateral early diastolic notch occurring in one fourth of the high risk cases matching similar results achieved by others [15,16,19,23,24].

At lower rate of early diastolic notch, Zimmerman et al., [28], had a significant difference between the high risk and low risk patients. On the contrary, Nagtegaal et al., [26], failed to prove any particularly useful use of uterine Doppler study in the prediction of PE in population with a prior very high risk to develop uteroplacental insufficiency.

Similarly, the screening in low risk group was disappointing, some authors stated in their study performed on low risk women that the screening efficacy of uterine artery Doppler for adverse prenatal outcome is poor and does not justify routine screening [27-29].

The best performing index in the presence of bilateral notches was the mean resistance index [21], while others developed a limited use of resistance index for prediction of Preeclampsia as the Positive Predictive Value (PPV) of Resistance Index (RI) being as low as 12% [30].

In present study combining resistance index to the bilateral early diastolic notch increased the sensitivity to 92.6%, specificity to 97.8%, PPV to 80.6% and NPV to 99.3%. Same results were achieved by Campbell et al., [15] and Aquilina et al., [31]. In addition, Harrington et al., [28], in their more recent study of 628 pregnancies stated that in high-risk multiparous women, persistent bilateral notches with mean RI 0.55 and unilateral notches with mean RI 0.65 at 20 weeks’ gestation identified the vast majority of women subsequently developed complications secondary to uteroplacental insufficiency. By the same token, the current study showed that the prediction of PE using bilateral diastolic notch is more accurate than maternal history, which is in agreement with other studies Papageorghiou et al., [32].

In present study combining serum leptin to uterine artery Doppler study (RI & early diastolic notch) increased the sensitivity to 96.3%, specificity to 99.3%, PPV to 92.9% and NPV to 99.6%. Same results were achieved by Baumann et al., [33] who concluded that screening by uterine artery Doppler
combined with serum leptin might be the most effective method for predicting pre-eclampsia.

One of the major criticisms of uterine artery Doppler screening studies has been the excessive reliance on subjective assessment of uterine artery waveforms for presence or absence of early diastolic notches Albaiges et al., [19] and so Bower et al., [11], reported that; inspite of the clinical importance, there were no studies on the objective analysis of an early diastolic Notch. The definition of the early diastolic notch has been given as "no matter how small the notch was seen to be, either from uterine arteries" which is very subjective. In order to decrease the influence of such factor Yong et al., [16], tried to evaluate the early diastolic notch by measuring its depth, for this purpose he used the notch index, considering it as an objective measurement, having a low intraobserver and interobserver difference.

**Conclusion:**

- Combination of more than one uterine artery Doppler parameters for the prediction for preeclampsia may improve the sensitivity and so the predictive value of the screening tests.

- Combination of uterine artery Doppler and serum leptin assay increase sensitivity for prediction of pre eclampsia.

**Recommendation:**

Further studies on a wide scale needed to confirm the use of uterine artery flowmetry as a routine test for pregnant women for early prediction of preeclampsia.

**References**


الملخص العربي

فحص الشريان الرحمي بالدبوير في الفترة من 22-26 أسبوع من الحمل ذو قيمة كبيرة في التنبؤ بمضاعفات ضعف تدفق الدم الرحمي الشمي.

قياس نسبة هرمون اللبنين في نفس الفترة تسمح لنا بتوقع ما قبل الأرجاج قبل تطور المظاهر السريرية.

في هذه الدراسة استخدام الفحصين السابقين معاً يزيد من حساسية التنبؤ بما قبل الأرجاج.