Prevalence of Occult Hepatitis C Virus Infection in Patients with Multiple Myeloma

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Abstract

Background: Many studies have investigated the prevalence of hepatitis C virus infections in patients with hematological malignancies. Most studies regarding the prevalence of HCV infection suggested that HCV infection has a pathogenic role in lymphoproliferative disorders, including multiple myeloma which is the second most common hematologic cancer. New form of hepatitis C virus (HCV) infection has been identified and defined as “occult” HCV infection (OCI). OCI is characterized by detection of HCV-RNA in liver tissue alone, or in liver tissue and/or peripheral blood mononuclear cells (PBMCs). In Egypt, high incidence of OCI in nonalcoholic liver disease (40.7%) was found.

Aim of the Work: To investigate the prevalence of occult HCV in multiple myeloma patients and correlate this to staging of myeloma.

Subjects and Methods: The study included 106 Egyptian multiple myeloma (MM) patients (Group I) and 100 sex and age matched apparently healthy subjects served as control (Group II). Both groups were negative for “HCV-RNA” by RT-PCR in serum. MM patients were classified according to International staging system for multiple myeloma, Group IA included stage I, Group IB included stage II & III. Diagnosis of OCI for all participants was done by extraction and detection of the “HCV-RNA Plus Strand” (advanced testing) from serum and PBMC.

Results: 24.5% of MM patients had OCI compared to 3% OCI only in control group (p-value=0.01) suggesting association of OCI with MM. On the other hand, on comparing (group IA) vs (group IB), no significant relation between the stage of MM and the presence of OCI was found (p-value=1.0).

Conclusion: Our findings suggest significant association of OCI and multiple myeloma. Further studies are needed to verify the causal relationship and also investigate the impact of OCI on the outcome of MM after chemotherapy.

Key Words: HCV infection – Multiple myeloma.

Introduction

MULTIPLE myeloma (MM) is a neoplastic plasma cell disorder and the second most common hematologic cancer [1]. MM has been considered to arise from an asymptomatic premalignant proliferation of monoclonal plasma cells that are derived from post-germinal-center B cells [2]. Previous studies have shown that multistep genetic and micro-environmental changes lead to the transformation of these cells into a malignant stage [3]. Interactions between myeloma cells and bone marrow stromal cells or extracellular matrix proteins that are mediated through cell-surface receptors (e.g., integrins, cadherins, selectins, and cell-adhesion molecules) increase tumor growth, survival, migration, and also drug resistance [4]. The adhesion of myeloma cells to hematopoietic and bone marrow stromal cells induces the secretion of cytokines and growth factors, such as interleukin-6, insulin-like growth factor I, vascular endothelial growth factor, members of the superfamily of tumor necrosis factor, transforming growth factor-β1, and interleukin-10. These cytokines and growth factors are produced and secreted mainly by bone marrow stromal cells and even by myeloma cells, and regulated by autocrine and paracrine loops [3,5]. The adhesion of myeloma cells to extracellular matrix proteins (e.g., fibronectin, laminin, collagen, and vitronectin) triggers the up-regulation of cell-cycle regulatory proteins and anti-apoptotic proteins [6]. In the past years, a new form of hepatitis C virus (HCV) infection has been identified and defined as “occult” HCV infection (OCI) [7]. OCI is characterized by detection of HCV-RNA in liver tissue alone, or in liver tissue and/or peripheral blood mononuclear cells (PBMCs), with consistently undetectable HCV-RNA in serum [8]. OCI
Prevalence of Occult Hepatitis C Virus Infection

Subjects and Methods

The current study included 106 Egyptian patients (Group I) who were diagnosed as MM based on the criteria proposed by the International Myeloma Working Group [22]. They were classified into 2 subgroups according to the International staging system (ISS) for multiple myeloma [22]. Group IA included stage I, Group IB included stage II & III. Patients were seen in the clinical hematology unit, Kasr El-Ainy Teaching Hospitals, Faculty of Medicine, Cairo University in the period between May 2009 and July 2012. Institutional ethical board approval was taken prior to the study, as well as informed consent was taken from all the participants. One hundred age and sex-matched healthy subjects served as a control group (Group II). They were selected to be negative for serum HCV-RNA. Selection criteria for patients were to being negative for serum HCV-RNA, HBsAg, HIV I & II, Ig M of Epstein-Barr virus (EBV) and cytomegalovirus (CMV). All enrolled patients were subjected to full medical history, complete clinical examination, and investigations which included complete blood picture (CBC), liver & kidney functions tests, serum uric acid, serum calcium, C-reactive protein, parameters of staging ISS (B2 microglobulin and serum albumin), HCV antibodies using the commercially available ELISA kits (DiaSorin, Torino, Italy). Patients diagnosed with monoclonal gamopathy of undetermined significance or POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome were excluded from this study. Variables regarding clinical characteristics, laboratory data, and pathology reports were retrieved from the hospitalization database via a medical chart review. Diagnosis of OCI for all participants was done by extraction and detection of the HCV-RNA Plus Strand from serum and PBMC.

RNA extraction from serum and PBMC:

RNA was extracted from serum and PBMC using Biozol (Bioflux, China, Catalogue no. BSJ0002 10001 M80) total RNA extraction reagent according to the manufacturer’s protocol.

Detection of the HCV RNA plus strand:

HCV RNA plus strand was determined by reverse transcription-polymerase chain reaction (RT-PCR). RNA was reverse-transcribed and amplified by One Step RT-PCR QIAGEN Kit (Catalogue no. 210212, sensitivity 22 viral copies) with appropriate...
primers (5'-CGC GCG ACT AGG AAG ACT TC-3') and (5'-ATA GAG AAA GAG CAAC CA GG-3') as forward and reverse primers, respectively. The lack of contamination in PCR reactions was assessed by the inclusion of a negative control containing water rather than RNA in each assay which did not show any PCR amplification in all experiments. Moreover, each sample was done in duplicate to ensure absence of false positive results. Thermal cycling conditions were denaturation: For 1 min at 94°C, annealing: for 1 min at 55°C, extension: for 1 min at 72°C for 30 cycles, and final extension for 10 min at 72°C. The PCR product (174bp) was submitted to electrophoresis by using a 1.5 agarose gel and was visualized by ethidium bromide staining under ultraviolet light.

Detection of the HCV minus strand in LPD patients with OCI:

HCV RNA minus strand was determined by a previously established and described in house RT-PCR assay [23]. Reverse transcription was performed in 25 \( \mu \text{L} \) reaction mixture containing 20U of AMV reverse transcriptase (Clontech, USA) with 400ng (3 \( \mu \text{L} \)) total PBMCs RNA, 40U of RNasin (Clontech, USA), 0.2mmol/L from each dNTP (Promega, Madison, WI, USA), and 50pmol of the forward primer 2CH (for minus strand). The reverse transcription reaction was performed at 42 C for one hour. Amplification of the highly conserved 5'-UTR sequences was done using two PCR rounds with two pairs of nested primers. First round amplification was done in 50 \( \mu \text{L} \) reaction mixture, containing 50pmol from each of 2CH (5'-AAC TAC TGT CTT CAC GCA GAA-3') forward primer and P2 (5'-TGC TGA TGG TGC ACG TGC TA-3') reverse primer, 0.2mmol/L from each dNTP, 10 \( \mu \text{L} \) from RT reaction mixture as template, and 2U of Taq DNA polymerase (Promega, USA) in a 1x buffer supplied with the enzyme. A positive control RNA of an HCV patient previously tested having no RNA at the reverse transcription step and a PCR negative control having water instead of cDNA. The thermal cycling profile was 1 min at 94 C, 1 min at 55 C, and 1 min at 72 C for 30 cycles. The second round amplification was done similar to the first round, except for use of the nested reverse primer D2 (5'-ACT CGG CTA GCA GTC TCG CG-3') and forward primer F2 (5'-GTGACGCTCAGGACCC-3') at 50pmol each. PCR (179bp) products were analyzed on 2% agarose gel electrophoresis.

Statistical analysis:

Data were analyzed using Sigma Plot software (SPSS, version 2). \( p \)-values were determined using the Chi-square test to study the association between two qualitative variables (compare between proportions); and Student’s two-tailed \( t \)-test for comparisons between two groups having quantitative variables (i.e, compare sample means). Data are presented as percentages, means, and standard deviation (SD). \( p \)-values less than 0.01 were considered highly significant, and those less than 0.05 were considered significant.

Results

The current study included 106 patients (Group I) who were diagnosed as MM. One hundred age and sex-matched healthy subjects served as a control group (Group II). Demographic and laboratory criteria of (Group I) are shown in Table (1). Table (2) demonstrate comparison of MM Patients (Group I) and control group (Group II) as regard Laboratory Data.

MM patients were classified into 2 subgroups according to stage, we considered stage I as Group IA and stages II-III as Group IB. Table (3) demonstrate comparison between Group IA and Group IB regarding demographics and laboratory data.

MM patients were reclassified according to positivity to OCI into two groups. Comparison of these groups are shown in Table (4).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group (I) (N=106)</th>
<th>Control Group (II) (N=100)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean±SD)</td>
<td>75.11±8.33</td>
<td>75.11±8.33</td>
<td>0.83</td>
</tr>
<tr>
<td>Males (%)</td>
<td>66/106 (62.3%)</td>
<td>66/106 (62.3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Females (%)</td>
<td>40/106 (37.7%)</td>
<td>40/106 (37.7%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive history of transfusion (%)</td>
<td>78/106 (73.5%)</td>
<td>78/106 (73.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>AST (IU/L) (Mean±SD)</td>
<td>38.13±6.16</td>
<td>38.13±6.16</td>
<td>1.00</td>
</tr>
<tr>
<td>ALT (IU/L) (Mean±SD)</td>
<td>40.11±5.42</td>
<td>40.11±5.42</td>
<td>0.87</td>
</tr>
<tr>
<td>Albumin (g/dL) (Mean±SD)</td>
<td>3.03±0.31</td>
<td>3.03±0.31</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive OCI (%)</td>
<td>26/106 (24.5%)</td>
<td>26/106 (24.5%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Data were expressed as mean±SD.
Table (3): Comparison of stage I MM (Group IA) and stage II, III MM patients (Group IB) regarding demographics and laboratory data.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group IA (n=42)</th>
<th>Group IB (n=64)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72.75±10.88</td>
<td>77.15±7.08</td>
<td>0.19</td>
</tr>
<tr>
<td>Males:Females</td>
<td>32:10</td>
<td>34:30</td>
<td>0.59</td>
</tr>
<tr>
<td>Positive history of transfusion (%)</td>
<td>30/42 (71.4%)</td>
<td>38/64 (59.4%)</td>
<td>0.175</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>37.84±5.18</td>
<td>39.65±7.65</td>
<td>0.174</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>34.03±7.28</td>
<td>42.12±7.97</td>
<td>0.013*</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.52±0.31</td>
<td>3.00±0.38</td>
<td>0.022*</td>
</tr>
<tr>
<td>Positive OCI (%)</td>
<td>11/42 (26.2%)</td>
<td>15/64 (23.4%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. Not significant NS (p-value >0.05). Significant* S (p-value <0.05), Highly Significant** HS (p-value <0.01).

Table (4): Comparison of MM patients with and without OCI as regard laboratory data.

<table>
<thead>
<tr>
<th></th>
<th>MM patients without OCI (N=80/106) (76.4%)</th>
<th>MM patients with OCI (N=26/106) (24.5%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gm%)</td>
<td>9±1.3</td>
<td>8±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count x 103/mm3</td>
<td>87±41.8</td>
<td>88±39.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total leucotic count</td>
<td>9.3±2.1</td>
<td>7±4.2</td>
<td>NS</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>38.44±4.76</td>
<td>40.6±3.6</td>
<td>NS</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>39.11±5.6</td>
<td>45±8.8</td>
<td>NS</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.1±3</td>
<td>0.9±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.03±0.43</td>
<td>3.1±0.52</td>
<td>NS</td>
</tr>
<tr>
<td>Positive history of transfusion (%)</td>
<td>56/80 (70%)</td>
<td>22/26 (84.6%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

Fig. (1): Comparison of MM patients (Group I) and control group (Group II) as regard OCI.

Fig. (2): Comparison of stage I MM (Group IA) and stage II, III MM patients (Group IB) as regard OCI.

Discussion

Many studies have investigated the prevalence of hepatitis C virus infections in patients with hematological malignancies. Most studies regarding the prevalence of HCV infection suggested that HCV infection has a pathogenic role in lymphoproliferative disorders, including multiple myeloma [24]. However, some reports have revealed similar incidences of HCV infection in myeloma patients and in controls [25]. Cardin et al., demonstrated that the level of 8-hydroxydeoxyguanosine, an indicator of oxidative DNA damage, in circulating leukocytes correlated well with the severity of HCV-related liver disease in a population-based study [26]. The genotoxic effects of HCV were also demonstrated in peripheral blood lymphocytes via the occurrence of DNA fragmentation [27]. Occult HCV infection (OCI) is a completely unclear entity. It has been documented in haemodialysis patients, in chronic HCV patients after Sustained virological response (SVR), in general populations, and in chronic liver disease patients of unknown etiology [28]. It have never been studied in MM patients. In Egypt, high incidence of OCI in nonalcoholic liver disease (40.7%) was found [29]. To our knowledge, this study is a pioneer one in investigating the prevalence of OCI in MM patients and to assess its relation to MM severity. The current study showed that 24.5% of MM patients had OCI compared to 3% OCI only in control group. Statistically the presence of OCI in MM patients versus controls showed a highly significant value, confirming the possible association of OCI with MM. On the other hand, on comparing Group IA vs Group IB, no significant relation between the stage of MM and the presence or absence of OCI was found (p-value 1.0). Taking into consideration that in our study OCI was detected only in PBMC (we
did not do liver biopsies) which diagnosed only 70% of patients with OCI [30], we might expect that the prevalence of OCI in MM patients may exceed this percentage if assessed in liver biopsies. Furthermore, the current study showed no statistically significant difference between MM patient whether OCI positive or negative regarding previous blood transfusion. Concerning other laboratory data like hemoglobin, platelet, total leukocyte count, AST, ALT, and albumin, all showed statistically highly significant difference between MM patient and control group. Meanwhile, no statistically significant difference between OCI positive and OCI negative MM patient was found, that is to say, no indicator within the routine workup of MM to suspect OCI. So searching for OCI might be done for all cases. Our result can be explained by the study of De Vita et al., who postulated that HCV infection leads to stimulation of B cells and hepatitis C virus may replicate in lymphocytes, [31] and can also explained by that the second portion of the HCV envelope (E2 protein) binds to CD81, suggesting that this phenomenon is associated with CD19 and CR2 as well as MHC class II molecules on lymphocytes. The binding of CD81 to B cells can activate this complex, which lowers the antigen threshold necessary for antibody stimulation, thus rendering the B cell hyper-responsive. Sequencing of the antigen-binding region of immunoglobulin produced by malignant lymphocytes demonstrates that it has a high degree of homology to both antibodies specific for E2, as well as the antibodies produced by B cells that secrete RF (rheumatoid factor). Moreover, 88% of patients with HCV infection and cryoglobulinaemia demonstrate over-expression of the anti-apoptotic bcl-2 gene, compared with 8% of patients with HCV infection, 2% of patients with other liver diseases, and 3% of individuals with other rheumatoid disorders, which cause enhanced B cell survival [32]. In addition, over-expression of NF-kB has been reported in lymphocytes and liver samples of patients with chronic HCV infection [33]. NF-kB plays a key role in virus-induced lymphomagenesis. Mutations of the NFkB gene are common in lymphoid malignancies and alterations of NF-kB could initiate changes in downstream regulatory pathways. A second mutation (e.g. myc, NF-kB) could possibly initiate the progression to lymphoproliferative disorders [34].

In Conclusion: Our interesting preliminary results suggest significant role for OCI in the pathogenesis of multiple myeloma. Further studies are recommended to verify the causal relationship between both and the impact of OCI on the outcome of MM after chemotherapy and vies versa.

References


الملخص العربي

قامت العديد من الدراسات ببحث مدى شنوه الالتهاب الكبدى الفيروسى C في مرضى أورام الدم، وقد أوضحت نتائج معظم هذه الدراسات أن الفيروس الكبدى C له دور في أورام الكتائير اللمفي متضمنة الميلياوما الممتدная والتي تعد ثانية أكثر أورام الدم شيوعاً.

ولقد سبق التعريف على نوع جديد من الأصابه بالفيروس الكبدى C يعرف بالالتهاب الكبدى الفيروسى C الخفي والذي تميز بالكشف الإيجابى عن HCV-RNA في عينة الكبد فقط أو خلايا الدم البيضاوية وحيدة النواة، أو كلاهما متصوصى بالتحليل السليبي للأجسام المضادة HCV-RNA والملص أو تحليل إيجابى للأجسام المضادة فقط. وقد تبين أن نسبة الأصابه الخفие بالفيروس الكبدى C قد تصل إلى (7.24%) في المصابين.

الهدف من البحث: دراسة مدى انتشار الفيروس الكبدى C الخفي في مرضى الميلياوما الممتدة وعلاقته بمرحلتها المرضية.

وقد ضمت هذه الدراسة مجموعتين من الأفراد تم تقسيمهم كالتالي:

- المجموعة الأولى: تضمنت عدد 101 من مرضى الميلياوما الممتدة المصريين.
- المجموعة الثانية: تضمنت 100 شخص من الأفراد الأصحاء المصريين كمجموعة ضابطة.

وقد تم اختيار أفراد كلا المجموعتين بحيث يكون نتائج الكشف عن HCV-RNA بالعمل سلبية. ولهدف تقسيم مرضى المجموعة الأولى بناءً على نتائج التربين الأولى للملياوما الممتدة إلى مجموعتين فرعتين كالتالي:

- المجموعة الأولى: وضمت مرضى المرحلة الأولى.
- المجموعة الأولى: وضمت مرضى المرحلة الثانية والثالثة.

وقد تم تشخيص الأصابات الخفية بالفيروس الكبدى C بواسطة استخلاص والكشف عن HCV RNA في الخلايا الدم البيضاوية وحيدة HCV RNA.

وقد أوضح الدراسة أن (24%) من مرضى الميلياوما الممتدة مصابين بأصابات خفية بالفيروس الكبدى C بينما نسبة الأصابات بين المجموعة الضابطة (3%) فقط (p-value=0.01) مما يرجح وجود علاقة بين الأصابات الخفية بالفيروس الكبدى C والميلياوما الممتدة. كما تبين أنه لا يوجد علاقة بين المرحلة المرضية للميلياوما الممتدة والأصابات الخفية بالفيروس الكبدى C وبناءً على ذلك فقد خلصت هذه الدراسة إلى أنه من المرجح وجود علاقة بين الإصابة الخفية بالفيروس الكبدى C ومرض الميلياوما الممتدة. إذا توجيه يعد المريض من الإصابات الخفية بالفيروس الكبدى C على نتائج العلاج الكيميائي الميلياوما الممتدة.