ORIGINAL ARTICLE

Interleukin-10 level in Occult Hepatitis C Virus Infection in Hemodialysis Patient

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ABSTRACT

**Background:** Interleukin 10 is a critical immunoregulatory molecule, produced mainly by macrophage, Th2 and regulatory T cells, is a potent immunosuppressive cytokine with anti-inflammatory activity. **Objectives:** detection of the serum level of IL-10 in OCI in HD patients. **Methodology:** 75 HD patients from HD units in Assiut university Hospital, were enrolled in this study, and IL-10 were measured in 8 patients with OCI, 68 HD without OCI and 10 normal subjects as a control. **Results:** The level of IL-10 was highly significantly increased in HD patient with OCI than in HD patients without OCI (P< 0.001), and in HD patients than normal subject (P<0.001) There is significant increase as regarding ALT (P<0.001), AST (P<0.001), in occult HCV infection in HD patients. **Conclusion:** Our data revealed a high serum level of IL-10 in occult HCV infection in HD patients with persistently abnormal elevated levels of liver enzymes than HD patients without OCI.

INTRODUCTION

Hepatitis C virus (HCV) belongs to Flaviviridae family, and its genome is a single-stranded RNA molecule. Chronic hepatitis c virus infection is a major cause of liver disease with worldwide prevalence estimated at 3%. There were 170 million HCV patient worldwide, with 3-4 million new cases appearing each year. About 70–80 % of patients become chronic carriers who may progress to cirrhosis and hepatocellular carcinoma. HCV infection affects more than 4 million people in the united states where it represent the leading cause for liver transplantation. The most effective Current standard of care therapy in patients with CHC, a combination of PEG-INF-alpha with ribavirin. The SVR rate for individuals infected with genotype 1 CHC, the most common type in developed countries, is the lowest and requires 12 months of therapy. In those infected with other genotypes, SVRs of 75% can be achieved following 6 months of therapy.

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Occult HCV infection (OCI) means detectable HCV-RNA in the liver or peripheral blood mononuclear cells in the absence of serum HCV-RNA and, or anti-HCV antibodies. Two forms of OCI, secondary OCI in which infection has been found to persist in the presence of antibodies for HCV and normal levels of liver enzymes for years after spontaneous or antiviral therapy-induced resolution of hepatitis C infection. The other form of OCI accompanied by persistently moderately elevated liver enzymes in the absence of antibodies to HCV, which can be termed as cryptogenic OCI. Patient who participates in chronic hemodialysis units are at increased risk for hepatitis C. The prevalence of HCV antibodies in such patients reaches 15%, although it has declined in recent years. The prevalence of occult HCV infection is high among hemodialysis patients with persistently abnormal values of liver enzymes of unknown cause. It was reported that up to 57% of patients with normal renal function but with abnormal liver enzymes of unknown cause have an occult HCV infection detected by presence of HCV-RNA in liver cells. Furthermore, 70% of these patients also have HCV-RNA in the peripheral blood mononuclear cells (PBMC). In patients chronically infected with HCV, the production of inappropriate amounts of cytokines, such as IL-10, were reported to be...
associated with HCV clearance, fibrogenesis, and even resistance to interferon therapy. Interleukin 10 is a critical immunoregulatory molecule. It is a member of an expanding family consisting of cellular and viral cytokines. IL-10, produced mainly by macrophage, T-helper 2 (Th2) cells, and regulatory T cells, is a potent immunosuppressive cytokine as it inhibits the secretion of pro-inflammatory and antiviral cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). A number of studies have examined the IL-10 and their associations with HCV susceptibility as well as resistance to antiviral therapy. High serum levels of IL-10 have been correlated with poor response to interferon therapy, whereas IL-10 production has been found to be lower in responders than in non-responders. The aim of this work is to estimate the serum level of IL-10 in hemodialysis patients with abnormal levels of liver enzymes of unknown origin, and comparing these results in population with normal levels of liver enzymes.

**METHODOLOGY**

1) **Patients:**

This study was conducted on 75 chronic renal dialysis patients who were repeatedly anti-HCV Abs negative and had abnormal elevated liver enzymes of unknown cause and 10 healthy volunteers who had normal liver enzymes and kidney functions as a control.

Patients were selected from renal dialysis unit of nephrology department, faculty of medicine, Assiut university in the period from October 2012 to June 2014 after informed personal consent.

**Patient’s criteria:**

A) **Inclusion criteria:** The inclusion criteria include elevated liver enzymes (ALT&AST), anti-HCV Abs negative, HBsAg negative.

B) **Exclusion criteria:** Infection with HCV, infection with HBV, autoimmune hepatitis, drug toxicity, biliary obstruction, active schistosomiasis or malignancies.

2) **Samples**

- 3 ml venous blood for separation of peripheral blood mononuclear cells, were collected from all patients and control individuals in an EDTA-containing sterile tube. PBMCs were obtained by Ficoll-Hypaque (FH) density gradient centrifugation of sample according to the manufacturer’s instructions (Lympholot, Biotest, 63303 Dreleich, Germany).
- 3 ml of venous blood were withdrawn and left for 15 minutes to be clotted and then centrifuged at 3000 rpm for 5 minutes. The serum then used in serological tests including ELISA and biochemical tests.

3) **Methods**

1) Estimation of kidney functions, liver enzymes (ALT&AST) and serum bilirubin.

2) Detection of serum anti HCV antibodies by ELISA.

3) Detection of serum level of IL10 by ELISA:

**Using AviBion Human IL-10 ELISA kit by Organium Laboratories, Finland.**

**Principle of the assay:** Organium Laboratories, Interleukin-10 (IL-10) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-10 in cell culture supernatants, plasma (heparin and citrate), serum, urine and other body fluids. The assay recognizes both natural and recombinant IL-10. This assay employs an antibody specific for human IL-10 coated on a 96-well plate.

**Detection of HCV-RNA by Real time PCR:**

Using innuPREP Virus RNA kit, Germany for RNA extraction and RoboGene RNA Quantification kit, Supplemental protocol for Spartan Dx-12 for HCV–RNA detection and quantification, Germany.

**Procedure for HCV-RNA extraction and quantification**

A) **Blood samples:** For separation of peripheral blood mononuclear cells, were collected from all patients and control individuals in an EDTA-containing sterile tube. PBMCs were obtained by Ficoll-Hypaque (FH) density gradient centrifugation of sample according to the manufacturer’s instructions (Lympholot, Biotest, 63303 Dreleich, Germany).

B) **Extraction and purification of HCV-RNA using innuPREP RNA extraction kit.**

C) **Amplification and quantification of HCV-RNA by Real Time PCR (RT-PCR).**

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, Chi-square, and Analysis of variance [ANOVA] tests by SPSS V16.

**RESULTS**

The study subjects were classified into **group A** (patients on renal dialysis with increased liver enzymes NO. = 75) and **group B** (control persons, NO. = 10).

As regard gender, in the group A, 36 (48%) were male and 39 (52%) were female. While in the group B the number of males were 6 (60%) and that of females were 4 (40%).

The range and mean of age among the studied sample. In group A, the range was between 18 to 70 year (mean ±SD: 47.3±11.7 year), while the range of group B was between 22 to 50 years (mean ±SD: 33.4±8.1 years), with highly statistical significant difference between the two groups P value < 0.01.

When we compared both groups as regard history of blood transfusion, the difference was highly significant. In the group A, 56 (74.4%) were taken
blood transfusion and 19 (25.3%) weren't. While in the group B there was 1 (10%) was taken blood transfusion and 9 (90%) weren't. This is presented in table (1).

There is highly significant difference between both groups in Liver enzymes (P < 0.01) and there is significant difference in total bilirubin in group A than group B (P <0.01) Table (4).

Table (2) and figure (1) showing IL10 level, in group A ranged from 5.8-28.6 with a mean 10.57, while in group B ranged from 3.3-8.2 with a mean 5.6, with highly statistical difference between the two groups (P < 0.01).

Of the 75 subjects investigated for HCV-RNA by RT-PCR in the group A, 8 (10.7%) were Positive (Occult HCV patient) and named group I, while 67 (89.3%) were negative and named group II, figure(2).

As regard IL-10 level, in group I ranged from 6.8-28.6 with a mean 21.59, while in group B ranged from 5.8-13with a mean 9.26, with highly statistically difference between the two groups (P < 0.01), figure (3).

Table 1: History of blood transfusion among group A & group B

<table>
<thead>
<tr>
<th>History of blood transfusion</th>
<th>GA (n=75)</th>
<th>GB (n=10)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>56</td>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>%</td>
<td>74.7</td>
<td>10</td>
<td>67.1</td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>%</td>
<td>25.3</td>
<td>90</td>
<td>32.9</td>
</tr>
<tr>
<td>N</td>
<td>75</td>
<td>10</td>
<td>85</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

\[X^2 = 16.704\] \[P-value < 0.01^*\]

Table 2: Level of IL-10 among group A & group B

<table>
<thead>
<tr>
<th>IL10</th>
<th>GA (n=75)</th>
<th>GB (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>5.8-28.6</td>
<td>3.3-8.2</td>
</tr>
<tr>
<td>Mean</td>
<td>10.57</td>
<td>5.63</td>
</tr>
<tr>
<td>+SD</td>
<td>4.67</td>
<td>1.53</td>
</tr>
<tr>
<td>t.test</td>
<td>9.85</td>
<td></td>
</tr>
<tr>
<td>p. value</td>
<td>&lt; 0.01*</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Liver function test among group A & group B

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=75)</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin Total (mg/dl)</td>
<td>Range 0.43-1.6, Mean 0.86, SD 0.22</td>
<td>Range 0.45-91, Mean 0.86, SD 0.22</td>
<td>t = 4.89, P = 0.03*</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dl)</td>
<td>Range 0.11-0.66, Mean 0.22, SD 0.113</td>
<td>Range 0.11-0.21, Mean 0.16, SD 0.036</td>
<td>t = 2.84, P = 0.095</td>
</tr>
<tr>
<td>Indirect Bilirubin (mg/dl)</td>
<td>Range 0.3-0.94, Mean 0.64, SD 0.13</td>
<td>Range 0.34-0.71, Mean 0.54, SD 0.14</td>
<td>t = 4.64, P = 0.34*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>Range 40.5-240.4, Mean 53, SD 25.9</td>
<td>Range 12.4-21.2, Mean 17.1, SD 2.8</td>
<td>t = 18.88, P &lt; 0.01*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>Range 40.6-117.1, Mean 50, SD 12.2</td>
<td>Range 21.2-36.67, Mean 29.6, SD 5.5</td>
<td>t = 27.3, P &lt; 0.01*</td>
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DISCUSSION

Hepatitis C virus (HCV) is a commonly encountered pathogen in medical practice. It is estimated that 2-3% of the world is affected by HCV, with a prevalence of 170 million people (3% of the world's population) and incidence of 3-4 million per year.¹

Patients on regular hemodialysis have higher infection rates for HCV than the general population.²

There is suggestive evidence that T-cell immunoregulatory cytokines may play a key role in influencing the persistence of HCV infection and the extent of liver damage.³

IL10, produced mainly by macrophage, T-helper 2 (Th2) cells, and regulatory T cells, is a potent immunosuppressive cytokine as it inhibits the secretion of pro-inflammatory and antiviral cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ).⁴,⁵

In this study there were significant increase in serum level of IL-10 in hemodialysis patient than normal subjects (P <0.01), as shown in table 2.

This finding could be explained that IL-10 is a potent inhibitor of proinflammatory mediators such as IL-1, tumor necrosis factor and IL-6 and it is a classical Th2 cytokine.⁶

Regarding liver function tests There is highly statistically significant difference between group A and group B, in the levels of Liver enzymes (ALT&AST) (P < 0.01) and serum bilirubin (p < 0.03).

CONCLUSION

According to our study there were a statistical significant difference in level of IL10 in Occult HCV patient (Group I) than group II , and there is statistically significant difference as regard serum IL-10 protein level between HD patients with increased level of liver enzymes and healthy controls with normal liver and kidney functions.

REFERENCES


