Evaluation of Interleukin-18 as a Non Invasive Marker of Liver Fibrosis among Chronic Hepatitis C Virus Patients

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ABSTRACT

Background: The World Health Organization has declared hepatitis C a global health problem, with approximately 3% of the world’s population (roughly 170-200 million people) infected with hepatitis C virus (HCV). In Egypt, Chronic hepatitis C is the main cause of liver cirrhosis and liver cancer. Liver biopsy is currently the gold standard method for ascertaining the presence of cirrhosis, and scoring the severity of necroinflammation and fibrosis, but it is invasive, expensive and associated with rare but serious complications. There has been increasing interest in noninvasive assessment of liver fibrosis by the use of surrogate serum markers; one of them is interleukin-18 (IL-18).

Objectives: The aim of this study was the estimation of the levels of interleukin-18 in chronic hepatitis C patients and comparing between IL-18 levels and results of liver biopsy, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

Material and Methods: It included fifty chronic viral hepatitis C patients who were subjected to liver biopsy as well as fifty healthy blood donors as a control group. Sera were tested for estimation of ALT and AST levels and were subjected to enzyme-linked immunosorbent assay (ELISA) for determination of IL-18 levels. Results: The mean level of IL-18 was significantly higher in chronic hepatitis C patients (347.22pg/ml) compared to the controls (209.61pg/ml), and there was significant
relation between levels of IL-18 and the stage of liver fibrosis. **Conclusion:** IL-18 could be used as an additional non invasive marker for monitoring the degree of liver fibrosis in chronic hepatitis C patients.

**Key words:** HCV, IL-18, liver biopsy.

**INTRODUCTION**

HCV is a serious viral infection that causes acute and chronic liver diseases in humans. The World Health Organization (WHO) has declared hepatitis C a global health problem, with approximately 3% of the world’s population (roughly 170–200 million people) infected with HCV. HCV infection is hyper endemic in Egypt and represents the most important threat to the Egyptian liver. Chronic hepatitis C is the main cause of liver cirrhosis and liver cancer in Egypt, and indeed, one of the top five leading causes of death.

In patients with chronic viral hepatitis, liver biopsy is the traditional gold standard method to establish the diagnosis. The biopsy stage (degree of fibrosis) and grade (inflammatory activity) predict the course of the disease (progression to cirrhosis) and response to interferon (IFN) therapy.

However this procedure has many disadvantages, it is invasive, costly and difficult to standardize. Patients with chronic HCV are often anxious regarding undergoing a liver biopsy. Biopsy results show significant variability up to 40% for fibrosis diagnosis which can lead to a wrong diagnosis, indeed the result depends on the representativity of the punctured sample. That is why there has been increasing interest in noninvasive assessment of liver fibrosis by the use of surrogate serum markers.
IL-18, previously known as interferon-gamma-inducing factor, is a pleiotropic proinflammatory cytokine that is expressed mainly by peripheral blood mononuclear cells and macrophages. In the liver, besides its expression in Kupffer cells, IL-18 can also be synthesized by injured hepatocytes. (8)

IL-18 plays a strategic role in inflammation through the induction of inflammatory cytokines and chemokines. Besides, IL-18 increases the susceptibility of liver endothelial cells to undergo apoptosis. (9) The fundamental function of IL-18 is an enhancement of type 1 T helper (Th1) cytokine production. (10) Through interferon-gamma (IFN-γ) production, IL-18 augments perforin dependent cytotoxicity of liver natural killer cells and Fas ligand-mediated cytotoxicity of Th1 cells. (11,12)

The fact that the liver is responsible for IL-18 clearance and excretion into bile may partly explain the increased IL-18 concentrations in cirrhotic and fulminant hepatic failure. (13) Increased circulating levels of IL-18 is likely to play a pathogenic role in patients with chronic liver disease. (14)

This study was aiming to evaluate IL-18 as non invasive marker of the severity of liver damage in chronic hepatitis C patients by estimating its levels in those patients and comparing between them and results of liver biopsy and levels of liver enzymes.

MATERIALS AND METHODS

The study was carried out during the period from January to August 2008. It included fifty consecutive, unselected patients proved serologically as having chronic viral hepatitis C. They were admitted to the National Liver Institute, Menoufia
University and subjected to liver biopsy prior to IFN therapy. The necroinflammatory activity (grading) and the degree of fibrosis and cirrhosis (staging) of the liver were carried out using the modified Knodel scoring system for the liver biopsy sample. (15)

Patients suffering from renal failure, diabetes mellitus, coronary heart disease, hepatitis B virus infection or any other cause of viral liver cirrhosis were excluded. Informed consent was obtained from each patient. Fifty healthy persons from Alexandria Regional Blood Bank, having normal liver enzymes and free from viral hepatitis markers were included as a control group.

Five ml of venous blood were collected aseptically from each person. Serum was separated by centrifugation and divided into two aliquots: one aliquot was immediately used for estimation of ALT and AST using test kits from Chem Bio for in vitro diagnostic use, Egypt. The other aliquot was stored deep frozen till sera were subjected to ELISA technique for detection of IL-18.

Quantitative determination of IL-18 by ELISA technique:

Test kit was purchased from Medical & Biological Laboratories CO., LTD Nogoya, Japan.

ELISA was performed according to the manufacturer’s instructions. The Human IL-18 ELISA Kit measures human IL-18 by sandwich ELISA. The assay uses two monoclonal antibodies against two different epitopes of human IL-18. In the wells coated with anti-human IL-18 monoclonal antibody, samples to be measured or standards were incubated. After washing, a peroxidase conjugated anti-human IL-18 monoclonal antibody was added into the microwell and incubated. After another washing, the peroxidase substrate was mixed with the
chromogen and allowed to incubate for an additional period of time. An acid solution was then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density (OD) of each well was then measured at 450 nm using a microplate reader. The concentration of human IL-18 was calibrated from a dose response curve based on reference standards.

Statistical analysis:

Data was analyzed using SPSS (Statistical Package for Social Sciences) program version 13.0. Simple regression analysis was used for prediction of IL-18 concentration from OD values. The prediction equation was as follows:

- Concentration of IL-18=1071.4x+47.937 (where x is the OD value).

Descriptive statistics were calculated, which included: arithmetic mean, standard deviation and median.

Normally distributed data were analyzed using parametric tests (t test), data that did not follow normal distribution were analyzed using non parametric tests (H test of Kruskal Wallis, Mann Whitney test and Spearman rho correlation. (16)

RESULTS

Levels of AST and ALT were measured in both cases and controls, and they were significantly elevated in cases than in controls but there was no significant statistical relation between their levels and the grading or staging scores of liver biopsy or the levels of IL-18.

Table (1) shows that the mean level of IL-18 was higher in chronic hepatitis C patients (347.22pg/ml) compared to the
controls (209.61pg/ml). This difference was statistically significant ($Z = 5.823$, $p = 0.001$).

**Table (1): Levels of IL-18 among Chronic Hepatitis C Patients and Healthy Controls**

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-18 (pg/ml)</th>
<th>Min.</th>
<th>Median</th>
<th>Max.</th>
<th>Mean</th>
<th>Mann-Whitney Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>124.01</td>
<td>308.82</td>
<td>933.98</td>
<td>347.22</td>
<td></td>
<td>$Z = 5.823^{**}$</td>
</tr>
<tr>
<td>Control</td>
<td>140.08</td>
<td>204.89</td>
<td>300.79</td>
<td>209.61</td>
<td>p = 0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Significant.**

Table (2) shows that the levels of IL-18 increased as the stage of fibrosis increased and this was statistically significant. $X^2 = 8.633$, $p = 0.035$.

**Table (2): Levels of IL-18 among Chronic Hepatitis C Patients in Relation to the Stages of Liver Fibrosis**

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. (49)*</th>
<th>%</th>
<th>Min.</th>
<th>Median</th>
<th>Max.</th>
<th>Mean</th>
<th>Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18</td>
<td>36.70</td>
<td>179.72</td>
<td>244.00</td>
<td>480.78</td>
<td>278.10</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>17</td>
<td>34.70</td>
<td>124.01</td>
<td>320.07</td>
<td>933.98</td>
<td>374.84</td>
<td>$X^2 = 8.633^{**}$</td>
</tr>
<tr>
<td>3-4</td>
<td>9</td>
<td>18.40</td>
<td>230.08</td>
<td>314.71</td>
<td>795.77</td>
<td>398.04</td>
<td>p = 0.035</td>
</tr>
<tr>
<td>5-6</td>
<td>5</td>
<td>10.20</td>
<td>291.14</td>
<td>458.28</td>
<td>524.71</td>
<td>417.99</td>
<td></td>
</tr>
</tbody>
</table>

* One case of the 50 patients had HCC and was excluded from this table. IL-18 level of the HCC case was 310.43pg/mL.
** Significant.

0: No fibrosis. 1-2: partial fibrosis 3-4: bridging fibrosis 5-6: cirrhosis.
Table (3) demonstrates that the levels of IL-18 increased as the histological activity grade increased. However, this was not statistically significant ($\chi^2 = 3.23, p = 0.199$).

Table (3): Levels of IL-18 among Chronic Hepatitis C Patients in Relation to Necroinflammatory Activity Grades

<table>
<thead>
<tr>
<th>Grade</th>
<th>IL-18 (pg/ml)</th>
<th>No. (49)*</th>
<th>%</th>
<th>Min</th>
<th>Median</th>
<th>Max</th>
<th>Mean</th>
<th>Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td></td>
<td>17</td>
<td>34.70</td>
<td>174.36</td>
<td>245.07</td>
<td>568.64</td>
<td>299.65</td>
<td>$\chi^2 = 3.23$</td>
</tr>
<tr>
<td>5-8</td>
<td></td>
<td>26</td>
<td>53.00</td>
<td>186.15</td>
<td>316.85</td>
<td>933.98</td>
<td>377.14</td>
<td></td>
</tr>
<tr>
<td>9-13</td>
<td></td>
<td>6</td>
<td>12.30</td>
<td>124.01</td>
<td>382.21</td>
<td>533.28</td>
<td>358.46</td>
<td></td>
</tr>
</tbody>
</table>

* One case of the 50 patients had HCC and was excluded from this table. IL-18 level of the HCC case was 310.43 pg/mL.

Table (4) shows that the geometric means of IL-18 levels were higher among the cirrhotic patients (417.99pg/ml) than among the non-cirrhotic patients (340.01pg/ml). However this was not statistically significant. ($Z = 1.784, p = 0.074$).

Table (4): Levels of IL-18 among Non-cirrhotic Chronic Hepatitis C Patients and Cirrhotic Patients

<table>
<thead>
<tr>
<th>Cirrhosis</th>
<th>IL-18 (pg/ml)</th>
<th>No. (49)*</th>
<th>%</th>
<th>Min.</th>
<th>Median</th>
<th>Max.</th>
<th>Mean</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cirrhotic</td>
<td></td>
<td>44</td>
<td>89.80</td>
<td>124.01</td>
<td>293.82</td>
<td>933.98</td>
<td>340.01</td>
<td>$Z = 1.784$</td>
</tr>
<tr>
<td>Cirrhotic</td>
<td></td>
<td>5</td>
<td>10.20</td>
<td>291.14</td>
<td>458.28</td>
<td>524.71</td>
<td>417.99</td>
<td>p = 0.074</td>
</tr>
</tbody>
</table>

* One case of the 50 patients had HCC and was excluded from this table. IL-18 level of the HCC case was 310.43 pg/mL.
DISCUSSION

HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. About 80% of newly infected patients progress to develop chronic infection. Cirrhosis develops in about 20% of persons with chronic infection, and liver cancer develops in 1% to 5% of persons with chronic infection over a period of 20 to 30 years.\(^4\)

Liver biopsy is the traditional gold standard method to establish the diagnosis and to determine the extent of inflammatory changes and the extent of fibrosis and cirrhosis. However this procedure is invasive, costly and prone to intra and interobserver variation and to sampling variability. \(^5\) There is increasing interest in non invasive markers to assess inflammatory activity and degree of fibrosis in chronic hepatitis C infection. IL-18 is a pleotropic proinflammatory cytokine related to the IL-1 family, and is proposed to be one of these non invasive markers.\(^11\)

In this study, it was found that there were elevations in serum ALT and AST levels by increasing the scores of the liver biopsy grades and liver biopsy stages; however these results were not statistically significant. The same findings were proved in many studies reporting that liver enzymes in patients with HCV do not help to assess disease severity as inflammatory and fibrosis scores were nearly similar in patients with normal and elevated enzymes.\(^17-19\)

To validate the up regulation of IL-18 levels in chronic HCV patients, these levels in serum were measured and compared to those of controls. Serum IL-18 levels were significantly higher in patients than in controls (the mean level of IL-18 in chronic HCV
cases was 347.22 pg/ml versus the mean IL-18 level in controls 209.61 pg/ml, p=0.001).

These data are in accordance with earlier findings observed by Mc Guineness et al. (2000) who noted that IL-18 mRNA was up regulated in chronic and cirrhotic HCV patients. The same findings were observed by Ludwiczek et al., (2002). Stanislawksa et al. (2008) and Bouzgarrou et al., (2008) have also demonstrated a significant increase in plasma IL-18 levels in HCV patients when compared to healthy controls.

In this study, serum IL-18 levels were significantly related to liver biopsy staging (Kruskal Wallis test X2=8.633, P=0.035). Yamano et al. (2000) showed that serum IL-18 in primary biliary cirrhosis had increased significantly according to the severity of the cirrhosis. Falasca et al (2006) described IL-18 as a marker of both inflammation and hepatic injury and so did Neuman et al. (2007) who found a positive correlation between IL-18 level and the severity of inflammation in hepatitis C patients.

These findings confirm that disease progression is accompanied by an increase in plasma IL-18 and strongly support the involvement of IL-18 in causing liver injury. Furthermore this hypothesis is reinforced by a significant decrease of plasma IL-18 levels after treatment followed by a decrease in histological liver damage. (21,26)

Although in this study the serum levels of IL-18 increased with increasing the grade of liver biopsy, this was not statistically significant. Plasma IL-18 levels were significantly positively correlated with hepatic histologic activity in the study of Schwoerer et al. (2003) in which 53 patients were investigated and divided into two groups: one group included cases with
mild activity and the second group included cases with moderate activity and cases with severe activity. In the present study, most of the cases had minimal or mild grade of activity and only 6 cases had moderate grade activity, and this may influence the statistical results of the relation between IL-18 concentration and the grading of liver biopsy.

In this study, it was found that the mean IL-18 level in cirrhotic patients was higher than that in non-cirrhotics (417.99pg/ml versus 340.01pg/ml) however, this increase was not statistically significant. Bouzgarrou et al. (2008) (22) found that patients with cirrhosis and HCC presented a higher increase in plasma IL-18 concentration than chronically infected patients. The same finding was observed by Ludwiczek et al. (2002)(20) who found that disease progression from noncirrhotic to cirrhotic disease was accompanied by an increase in plasma IL-18 level, he also found that the deterioration of cirrhosis from Child-pugh stage A to B and C further increased IL-18 levels.

**CONCLUSION AND RECOMMENDATIONS**

It can be concluded that IL-18 levels are elevated in chronic hepatitis C patients than in healthy subjects. IL-18 level is significantly increased with the increase in the histological stage of fibrosis and its concentration may predict the degree of hepatocellular damage. Thus IL-18 could be used and nominated as an additional non invasive marker for monitoring the degree of liver fibrosis in chronic hepatitis C patients and as a monitoring tool to assess response to therapy.
REFERENCES


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