Serum Ferritin and Transferrin as Biomarkers for Liver Fibrosis Appreciated using Transient Hepatic Elastography (TE) by Fibro Scan in Egyptian Patients with Chronic Hepatitis C Virus

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ABSTRACT

Exact determination of liver fibrosis stage in chronic hepatitis C patients is of great importance for estimation of anticipation, as well as for evidence of antiviral treatment. This study was designed to investigate whether the serum iron indices is a conceivable mean for determination of liver fibroses stage. Hepatitis C virus (HCV) infected untreated patients (n=88) were admitted mainly for evaluation of HCV infection. Twenty healthy individuals negative for HCV disease were included in this study as a control group. All infected subjects had active HCV confirmed by real time PCR, liver fibrosis stages was appreciated using transient hepatic elastography (TE) by Fibro Scan, the activities of serum liver function biomarker enzymes and iron indices were determined by automated analyser. Concentration of serum ferritin was increased with the evolution of fibrosis in all stages from F0 to F4 and this increase was significant (P<0.01) in cirrhotic patients (F4). There was a positive high correlation between serum level of ferritin and progression of fibrosis (0.983911) (R²= 0.968). Transferrin concentration was significantly elevated (P<0.01) with the progression of fibrosis (F1-F4). There was a significant positive correlation between serum concentration of transferrin and progression of fibrosis (P<0.05) and this correlation was highly positive from stage 0 (F0) to stage 2 (F2) (0.976) (R²=0.953). Serum transferrin concentration may be used as liver fibrosis biomarker in the early stages of fibrosis. Serum ferritin concentration may be used as liver fibrosis biomarkers especially for end stage fibrosis.

Keywords: Liver fibrosis biomarkers, Ferritin, Transferrin, HCV.

INTRODUCTION

The hepatitis C disease (HCV) is a noteworthy general health problem¹. It has been reported that about 130-210 million individuals are chronically infected with HCV around the world². Chronic hepatitis C virus (HCV) infection is an overall health problem, and inflammation is believed to be an imperative player in disease pathogenesis. HCV disease once in a while prompts severe fibrosis/cirrhosis and hepatocellular carcinoma³.

Liver fibrosis is a transformation in the histological structure of the liver because of liver inflammation⁴. Fibrosis is a scar tissue in the liver parenchyma, characterized by accumulation of over value of extracellular matrix (ECM), the backbone of all tissues. ECM is a complex grid consisting of multiple structural proteins that plays a vital role for maintaining the normal tissue function⁵. Although the fibrosis is a part of the pathogenesis of an infection, the fibrogenesis is a part of the ordinary healing response to various kinds of injuries⁶. Estimation of the amount of fibrosis is called staging. There are five stages of fibrosis ranging from no fibrosis (F0) to cirrhosis (F4) and including, mild fibrosis (F1), moderate fibrosis (F2) and severe fibrosis (F3)⁷.

Determination the stage of liver fibrosis in chronic HCV patients is of great importance for estimation of anticipation, as well as for decision-making in treatment. Patients with no fibrosis or with only mild fibrosis at the season of analysis have more favourable outcomes and a lower possibility of reaching end-stage liver illness than patients with severe fibrosis or cirrhosis⁸-⁹. Therefore, exploration of new methods for determination of fibrosis stage of liver in HCV patients became of great interest.

Liver biopsy has been taken into consideration as the best standard for determining the degree of liver fibrosis, because fibrosis denotes morphological changes⁹-ⁱ⁰. Many recent studies clearly highlight a few urgent disadvantages of liver biopsy, including variable accessibility, auxiliary expense, testing incorrectness and inaccuracy because of inter- and intra-observer variability of pathologic elucidationsⁱ¹.

On the other hand, remarkable achievements have been made in determining the stage of fibrosis by non-invasive methods, this is a developing field and there is still room for improvement⁵. The non-invasive evaluation of hepatic fibrosis has been a prominent subject sentence about talk over the past decade due to its ideal properties which includes widespread availability, straight forwardness for use, cost-efficiency, reproducibility, and capacity with recognized changes in fibrosis over timeⁱ². The fibro scan is a non-invasive, painless, fast and objective technique to quantify liver fibrosisⁱ³. Fibro Scan device, is a choice...
short time ago for the evaluation of liver disease confirmed by the Food and Drug Administration. In the last two years, transient elastography has been used as a non-invasive method for staging of liver fibrosis by liver stiffness measurement.

The liver is the fundamental iron stockpiling organ. About 33% of the body's iron aggregate is kept in hepatocytes, in the entry tracts, sinusoidal mesenchymal cells, and reticuloendothelial cells. In addition, liver plays an essential role in iron digestion system, as both transferrin (the principle transporting protein for iron) and ferritin (the main stockpiling protein) are synthesized in it. Several studies reported that elevated serum iron indices (iron, ferritin and transferrin) and iron accumulation in the liver are frequently occurred in individual with chronic hepatitis C infection and may worsen liver injury.

Exploration of conceivable connections between serum iron digestion markers and severity of liver disease in chronic hepatitis C infection is very important to be used as new non-invasive liver fibrosis marker. Therefore, the goal of the present study was to evaluate the relationship between iron indices (iron, transferrin and ferritin) and degree of fibrosis in chronic hepatitis C patients and its possibility to be used as liver fibrosis biomarkers.

MATERIALS AND METHODS

Subjects

This study was accomplished during January 2014 and March 2015 at the National Liver Institute, Menofia University, Shebin Al-Kom, Egypt. Eighty-eight HCV-infected untreated patients were admitted mainly for evaluation of HCV infection. Twenty healthy individuals negative for HCV infection were included in this study as a control group. Individuals’ with positive results for screening tests for other viral hepatitis infections (anti-HAV IgM, HBs Ag, anti-HBs IgM) or HIV disease, hepatic cancer, other types of malignancies, haematological or non-viral chronic liver diseases, and patients treated for HCV infection were excluded from this work. However, this study was approved by the Institutional Ethics Committee and was carried out in accordance with The Code of Ethics of the World Medical Association for experiments involving humans. Informed assent was taken from all individuals; patient privacy was respected over the testing and analysing data. All data exchanged and stated by the patients remained confidential.

Methods

All subjects included in HCV group are positive for HCV RNA as confirmed by quantitative real time PCR.

Detection of HCV-RNA in patient’s sera by real-time PCR

The serum HCV viral RNA was extracted using QIAamp viral RNA mini kit (Qiagen, USA) and subjected to a quantitative HCV viral evaluation by real-time PCR according to the procedures in the instruction manual of artus® HCV RG RT-PCR Kit (Qiagen, CA USA) performed on a Rotor gene 5 plex instrument.

Measurement of liver fibrosis stages

In the present study liver fibrosis stages was appreciated using (TE) by Fibro Scan; with values from F0 to F4 for liver fibrosis. Hepatic flexibility is communicated in kilopascals (kPa), within a range of 2.5-75.0 kPa. The severity of fibrosis can be predicted by utilizing ranges of values rather than a single cut-off value should be used, for instance: mild or absent fibrosis present when liver stiffness values range between 2.5 and 7 kPa. Significant Fibrosis (F2) when stiffness values range between 7.1 and 9.4 KPa. Severe Fibrosis (F3) when stiffness values range between 9.5 and 12.4 KPa, whereas cirrhosis (F4) is likely when values are above 12.5 kPa.

Biochemical analysis of liver enzymes and iron biomarkers

The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the concentrations of serum iron, unsaturated iron-binding capacity (UIBC) and transferrin were determined by automated analyser (Beckman coulter chemistry analyzer AU 480). Serum ferritin concentration was monitored with an automated analyser (Advia centaur cp immunoassay analyser). Total iron binding capacity (TIBC) was determined as the total of serum iron concentration and UIBC values.

Statistical analysis

Data are expressed as mean ± SEM., and were analysed by using one-way ANOVA with Duncan’s post hoc test to determine the significant differences among data in this study. The differences between means were analysed at the 5% probability level (P ≤ 0.05) was considered statistically significant.

RESULTS

Patients’ characteristics according to fibro scan results

The average age of patients was 43±2.5 years (ranging from 19 to 69 years), and females number was equal to males number (male/female index=1). According to fibro scan results, 18 subjects infected with HCV with age ranged from 19 to 58 years had no liver fibrosis (F0). Twenty subjects with age ranged from 20 years to 58 years, had mild fibrosis (F1). Fifteen subjects with age ranged from 30 to 67 years showed moderate liver fibrosis (F2). Fifteen subjects with age ranged from 38 to 58 years had severe liver fibrosis (F3). Twenty subjects with age ranged from 28-69 years suffered from liver cirrhosis (F4).

The correlation between the degree of liver fibrosis and age of patients

In our study age has been clearly demonstrated to be connected with fibrosis progression, as there was a significant (P<0.05) high positive correlation (0.955) (R²=0.91) (Figure1a) between age and progression of liver.
fibrosis. The average of age of HCV infected patients with no fibrosis was 37.11, mild fibrosis was 41.9, moderate fibrosis was 47.47, severe fibrosis was 54 and with cirrhosis was 52.6 (Table 1).

Figure 1a: The correlation between the age (year) of patients and the progression of fibrosis in HCV hepatic patients

Figure 1b: The correlation between serum iron concentrations (µg/dl) and the progression of fibrosis in HCV hepatic patients

HCV hepatic patients was confirmed with real time PCR and liver fibrosis stages represented by (F0) stage of no fibrosis, (F1) mild fibrosis stage, (F2) moderate fibrosis stage, (F3) severe Fibrosis stage, (F4) end stage of fibrosis (cirrhotic stage)

The activities of serum liver function biomarkers are increased in different fibrosis stages in HCV patients

The activities of serum AST and ALT were significantly increased (p<0.05) in HCV patients suffering from fibrosis from F0-F4 stage compared to control group. Also, the magnitude of AST and ALT activities were significantly increased with progression of fibrosis as their activities at stage 4 (F4) were significantly elevated (p<0.05) than those at other stages of fibrosis (F0-F3) (Table 1).

Serum iron indices were positively correlated with the progression of fibrosis in HCV patients

Serum Iron concentration was increased with the progression of fibrosis except at F2 stage. Serum iron concentration was significantly increased (P<0.05) in patients suffering from severe fibrosis and cirrhosis (F3-F4) compared to healthy control individual, mild and moderate subjects Table 2. Furthermore, there was a positive correlation (0.812926) (R²=0.66) between serum iron concentration and progression of fibrosis in HCV patients Figure (1b). Serum ferritin level was elevated with the progression of fibrosis in all stages from F0 to F4 and this increase in serum ferritin was significant (P<0.05) in cirrhotic patient (F4) in comparison to healthy control subjects and mild fibrotic patients (F0-F1) Table (2). There was a significant (P<0.05) positive high correlation (0.983911) (R²= 0.968) between serum ferritin concentration and progression of fibrosis (Figure 2a). Therefore, serum ferritin concentration may be used as liver fibrosis biomarker especially at the end stage of fibrosis (cirrhotic stage F4).

Transferrin concentration was significantly elevated (P<0.05) with the progression of liver fibrosis from mild fibrotic stage to cirrhotic stage (F1-F4) compared to healthy control individual and HCV patients without fibrosis (F0). The magnitude of serum transferrin level was increased with increasing of the degree of fibrosis Table 2. The correlation between the serum transferrin level and the progression of fibrosis was positive correlation (0.523)(R²=27) (Figure 3a). This correlation was significant (P<0.05) and highly positive from stage 0 (F0) to stage 2 (F2) (0.976) (R2=0.953) (Figure 3b). TIBC concentration was increased with the progression of fibrosis from F0 to F2 but in severe fibrosis and cirrhosis (F3-F4) there was significant (P<0.05) decrease with correlation (0.976753) (R2=0.073) (Figure 2b).
stage, (F3) severe Fibrosis stage, (F4) end stage of fibrosis (cirrhosis)

![Graph A](image1)
![Graph B](image2)

**Figure 3a:** The correlation between serum transferrin concentrations (g/l) and the progression of fibrosis in HCV hepatic patients

**Figure 3b:** The correlation between transferrin concentrations (g/l) and the progression of fibrosis in HCV hepatic patients

HCV hepatic patients was confirmed with real time PCR and liver fibrosis stages represented by (F0) stage of no fibrosis, (F1) mild fibrosis stage, (F2) moderate fibrosis stage, (F3) severe Fibrosis stage, (F4) end stage of fibrosis (cirrhosis).

**DISCUSSION**

Our study was performed to elucidate the utility of serum concentrations of transferrin and ferritin in predicting liver fibrosis and cirrhosis as well as the correlation between the age and the progression of fibrosis in HCV patients. Few literatures have proposed a relationship between the severity of chronic hepatitis and the degree of iron deposition in liver. In addition, the association of atypical serum iron tests with the advanced stage of liver disease is characterized by increased fibrosis and/or cirrhosis. A relation between iron accumulation and fibrosis, sign of hepatic cirrhosis, has been indicated in many studies.

Ferritin is a multi-subunit protein contains a light and heavy chain. Ferritin from distinctive tissues can shift in the proportion of heavy chains compared to light chains. Liver ferritin contains generally the light chain and can keep up to 4500 atoms of iron. Hepatocytes are a noteworthy site for ferritin synthesis, however most cells that have been assessed can synthesize a minimal amount of ferritin. Liver disease is the cause of increased serum ferritin because injured hepatocytes spill ferritin into the serum. Thus, SF can be considered as another sort of liver function test, alongside with aspartate aminotransferase (AST), alanine aminotransaminase (ALT) and gamma-glutamyltransferase. Serum ferritin levels are elevated in about the half of the chronic viral hepatitis cases.

Transferrin, a glycoprotein with a solitary polypeptide chain and two iron-binding sites, is an 80 kDa plasma glycoprotein produced mainly by hepatocytes with little amounts synthesized in brain and testes. It plays a crucial role in iron digestion-system. TF is the most noteworthy serum iron binding protein and is crucial for systemic circulation of iron. It comprises of two spherical lobes of α-helices and β-sheets, which have a significant amount of homology between the N- and C-terminal halves of the molecule. This homology is thought to be a direct result of a gene duplication emerged from a 40 kDa genealogical protein containing a solitary lobe. Each lobe contains metal binding amino acid residues (2 Tyr, 1 His, and 1 Asp). It binds to ferric iron reversibly and with high affinity, notwithstanding, does not bind to iron in the second oxidation state. Moreover, TF can bind other metals with lower affinity. The affinity of iron is additionally regulated by pH, as the pH drops the iron binding to TF diminishes, with no perceivable amounts observed below pH 4.5. Recently, Cho reported that serum transferrin could be promising serum marker for anticipating advanced liver fibrosis in patients with chronic hepatitis.

The present study indicated that increased serum levels of iron, ferritin, and transferrin may represent early markers for the severity of liver disease. Elevated levels of serum iron and ferritin concentrations were correlated with degree of fibrosis with significant elevation of ferritin in the end stage of fibrosis. In addition, serum transferrin levels were increased in all stages compared to the control however, with progression of fibrosis into cirrhosis serum transferrin level was decreased. These elevations could be explained by that damaged hepatocytes can release iron and ferritin in concomitant with high serum levels of ALT. Furthermore, iron accumulation in the liver of CHC induces oxidative stress which results in liver damage and subsequently increases necrosis/apoptosis of hepatocytes and hepatic stellate cells together with induction of fibrogenesis and proliferation of actin and collagen.

Elevated serum levels of iron were strongly associated with liver diseases, emphatically HCV infection. Liu reported that patients with CHC present mild to moderate hepatic iron accumulation, which fundamentally worsens clinical outcomes, prompting an expanded danger of hepatocellular carcinoma. After excluding the possible influences of age, transaminases and gender on liver fibrosis to stay away from...
confounders influence, utilizing numerous direct relapse investigations, our study suggested that ferritin levels were the independent predictors for end stage of fibrosis (cirrhosis). While transferrin and serum iron levels were the independent predictors for mild and moderate fibrosis. These results were in line with report of Guayader50 who suggest that serum iron markers can represent surrogate markers for the severity of liver disease; yet these perceptions should be carefully interpreted, and the level of serum iron markers ought to be observed in progress, as other interferences cannot be excluded.

Table 1: Age and serum levels of liver function biomarkers in different stage of fibrosis in control and hepatic patients

<table>
<thead>
<tr>
<th>Item</th>
<th>Control n=20</th>
<th>Stage of fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F0 n=18</td>
<td>F1 n=20</td>
</tr>
<tr>
<td>Age (year)</td>
<td>27.3±1.62</td>
<td>37.11±3.15</td>
</tr>
<tr>
<td>AST U/ml</td>
<td>14.65±1.22</td>
<td>31.11±2.56</td>
</tr>
<tr>
<td>ALT U/ml</td>
<td>15±0.68565</td>
<td>38.88±3.71</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM, Values carrying different letters in the same ROW are significantly different at (P< 0.05)

Table 2: Serum iron indices in different stage of liver fibrosis in control group and hepatic patients

<table>
<thead>
<tr>
<th>Item</th>
<th>Control n=20</th>
<th>Stage of fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F0 n=18</td>
<td>F1 n=20</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>66.55±3.94</td>
<td>67.47±7.17</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>43.38±9.44</td>
<td>68.11±8.05</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>2.18±0.03</td>
<td>2.35±0.14</td>
</tr>
<tr>
<td>TIBC (µg/dL)</td>
<td>272.55±4.04</td>
<td>293.82±17.47</td>
</tr>
</tbody>
</table>

Data are expressed as Means ± SEM, Values carrying different letters in the same ROW are significantly different at P< 0.05. Total iron binding capacity (TIBC)

CONCLUSION

This study indicated that severe liver fibrosis was present in a high rate of HCV patients.

There was a positive correlation between the progression of liver fibrosis and serum concentrations of iron, ferritin and transferrin.

This study suggested that serum transferrin concentration may be used as liver fibrosis biomarker in hepatic patients particularly in the early stages of fibrosis (mild and moderate stages) while serum ferritin concentration may be used as liver fibrosis biomarkers especially for end stage fibrosis.

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