Evaluation of Serum Zinc Level in Egyptian Patients with Hepatitis C-associated Cirrhosis

ABSTRACT

Hepatitis C viral infection is a common health problem in Egypt. The complications of HCV include cirrhosis and hepatocellular carcinoma. Zinc is a micronutrient that plays a role in immune system and antioxidant system. Zinc is thought to be associated with HCV-related hepatic complications.

The aim of the present study was to evaluate the level of serum zinc in patients with different stages of hepatitis C associated liver cirrhosis and HCC.

This cross-sectional study was carried out in 75 patients with various stages of HCV-associated liver affections. They included 25 early cirrhotic patients on top of HCV with positive U/S and laboratory tests for cirrhosis with positive viral markers for HCV, 25 patients with advanced cirrhosis on top of HCV and 25 patients with primary hepatocellular carcinoma on top of HCV with a positive U/S and triphasic CT for malignant focal lesions with positive viral markers for HCV. In addition, 25 healthy subjects were recruited as a control group. Blood samples were obtained from each subject for full laboratory studies for biochemical liver functions tests, prothrombin time and Zinc determination.

There were statistically significant differences in late cirrhosis, HCC and control groups regarding albumin (ALB) and total bilirubin (T. Bil) (P < 0.001). Prothrombin time had statistically significant prolonged time (P < 0.001) in early cirrhotic group, 14.75 ± 0.75 seconds, late cirrhotic group was 16.7 ± 2.2 seconds and in HCC group was 17.5 ± 2.9 seconds than the standard value of prothrombin time (11–13 seconds) and INR (0.9–1.1). There were statistically significant (P < 0.001) differences between early cirrhosis (103.8 ± 9.7) and control groups (122.4 ± 7.1) as regard to serum zinc. There were statistically significant differences (P < 0.001) between late cirrhosis (94.0 ± 12.7), HCC (94.0 ± 12.7) and control groups (122.4 ± 7.1) with regard to serum zinc. There were insignificant correlations between ALT, AST, bilirubin, albumin and serum zinc level.

From the present study we can conclude that serum zinc level decreases significantly in chronic HCV infection with liver cirrhosis and hepatocellular carcinoma. The decrease of serum zinc can be used as a laboratory parameter for evaluation of liver status in cases of liver dysfunction on top of HCV. It is recommended to evaluate the role of zinc supplementation in treating clinical manifestation of liver cirrhosis and liver cell failure associated with HCV.

KEYWORDS: HCV, HCC, liver cirrhosis, zinc

INTRODUCTION

Hepatitis C virus (HCV) is a member of Hepacivirus genus of the Flaviviridae family. It is a major cause of chronic viral hepatitis worldwide. The sequel of this viral infection ranges from mild acute viral hepatitis to chronic liver disorders involving cirrhosis and hepatocellular carcinoma (HCC)¹. In Egypt, hepatitis C infection represents a major health problem with its complications.

Hepatitis C viral infection is usually associated with exaggerated immune reactions leading to oxidant stress. This oxidative stress leads to change in micronutrients level and demolishing their effects as protective elements in HCV infection². Patients with chronic liver disease exhibit metabolic imbalances of trace elements such as high levels of iron and copper, and low levels of zinc, selenium, phosphorus, calcium and magnesium³. Among trace elements, zinc (Zn) is a micronutrient influencing growth and affecting

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the development and integrity of the immune system. Zinc is an essential trace element required for normal cell functions. It is integrated in molecular regulation at cellular level such as DNA synthesis, RNA transcription, and cell division. It is a fundamental component in many zinc protein/enzymes, including critical zinc transcription factors. Zinc altered metabolism is observed in many types of liver disease, including alcoholic liver disease (ALD) and viral liver disease.

Zinc also is the most effective metal inhibitor for propyl hydroxylase, an enzyme that plays a central role in collagen synthesis. Zinc also helps in the degradation of collagen and preventing liver fibrosis through activating collagenase enzyme which is a zinc metalloenzyme. So, zinc deficiency may result in hepatic fibrosis and increased collagen synthesis. Therefore, zinc supplementation has a favourable effect on the inhibition of hepatic fibrosis.

The aim of the present study was to evaluate the level of serum zinc in patients with different stages of hepatitis C associated liver cirrhosis and HCC.

**MATERIALS AND METHODS**

This cross-sectional study was carried out in 75 patients with various stage of HCV associated liver affections. They included 25 early cirrhotic patients on top of HCV with positive U/S and laboratory tests for cirrhosis with positive viral markers for HCV, 25 patients with advanced cirrhosis on top of HCV and 25 patients with primary hepatocellular carcinoma on top of HCV with a positive U/S and triphasic CT for malignant focal lesions with positive viral markers for HCV. In addition, 25 healthy subjects were recruited as a control group. The study excluded patients with hepatitis B virus infection and patients with diseases affecting of serum zinc level such as chronic renal failure, chronic heart failure and malnutrition.

The patients were selected from Gastroenterology and Tropical Department and outpatient clinic of Al-Azhar university hospital, in New Damietta, during the period from November 2012 to June 2013. The study was approved by the ethical committee of the university and signed written consent was obtained from each participating in the study.

The subjects of the study were subjected to full history taking, clinical examinations and ultrasound examinations.

Ten millimetres of blood samples were obtained from each subject and distributed on citrated and plain tubes. For the citrated blood sample plasma was separated immediately and prothrombin time and INR was measured by using blood coagulometer (SEAC S2) and commercial kit (Biostec Liquiplustin, Egypt).

The samples in plain tubes were kept in water bath for 20–30 min after collection; then centrifuged and serum was separated and divided into two aliquots, one for liver function tests determination including alanine amino transferase (ALT), aspartate amino transferase (AST), albumin and total bilirubin on the day of blood collection. The other aliquot of serum was stored in deep freezing at −70°C for Zinc measurement. Liver function tests were performed by automatic analyzer (Hitachi 902).

**RESULTS**

Zinc measurement (Bio-diagnostic, Egypt)

Zinc present in the sample is chelated by zincon (2-caboxy-2-hydroxy-5-sulfoformazyl-benzene) in the reagent at alkaline pH. The formation of this complex was measured at a wavelength of 610 nm. Normal value of zinc in serum and is (109–167 microgram/dl).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n = 25)</th>
<th>Early cirrhotic (n = 25)</th>
<th>Late cirrhosis (n = 25)</th>
<th>HCC group (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.2 ± 7.18</td>
<td>51.9 ± 4.34</td>
<td>53.7 ± 4.24</td>
<td>61.2 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>17 (68%)</td>
<td>21 (84%)</td>
<td>22 (88%)</td>
<td>19 (76%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>8 (32%)</td>
<td>4 (16%)</td>
<td>3 (12%)</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>ALT IU/ml</td>
<td>25.3 ± 4.5</td>
<td>52.7 ± 25.9</td>
<td>47.5 ± 30.67</td>
<td>87.04 ± 25.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST IU/ml</td>
<td>23.8 ± 5.39</td>
<td>52.2 ± 32.0</td>
<td>52.2 ± 32.0</td>
<td>101.7 ± 26.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALB g/dl</td>
<td>4.7 ± 0.34</td>
<td>3.7 ± 0.3</td>
<td>2.3 ± 0.22</td>
<td>2.7 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T.BIL mg/dl</td>
<td>0.7 ± 0.15</td>
<td>2.1 ± 1.72</td>
<td>4.01 ± 0.93</td>
<td>3.33 ± 1.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT (second)</td>
<td>14.75 ± 0.75</td>
<td>16.7 ± 2.2</td>
<td>17.5 ± 2.9</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>1.25 ± 0.09</td>
<td>1.6 ± 0.23</td>
<td>0.9–1.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(61.2 ± 3.9) of patients with HCC was significantly higher (P < 0.001) than cirrhosis mean age ± SD (51.9 ± 4.34) and control mean age ± SD (37.2 ± 7.18).

There were also a high percentage of males in all groups. Males (84%) were more predominant than females (16%) in early cirrhotic group (males were 88% and females were 12%), in late cirrhotic group and HCC group, males were 76% and females were 24%.

Regarding liver functions tests, there was statistically significant difference between early cirrhosis and control groups as regard to ALT, AST, bilirubin, albumin and serum zinc level (Table 3).

Moreover, there were statistically significant differences in late cirrhosis, HCC and control groups regarding albumin (ALB) and total bilirubin (T. Bil) (P < 0.001). Prothrombin time had statistically significant prolonged time (P < 0.001) in early cirrhotic group, 14.75 ± 0.75 seconds, late cirrhotic group was 16.7 ± 2.2 seconds and in HCC group was 17.5 ± 2.9 seconds than the standard value of prothrombin time (11–13 seconds) and INR (0.9–1.1), Table 1.

There were statistically significant (P < 0.001) differences between early cirrhosis (103.8 ± 9.7) and control groups (122.4 ± 7.1) with regard to serum zinc (Table 2).

There were statistically significant differences (P < 0.001) between late cirrhosis (94.0 ± 12.7), HCC (94.0 ± 12.7) and control groups (122.4 ± 7.1) with regard to serum zinc (Table 3).

There were insignificant correlations between ALT, AST, bilirubin, albumin and serum zinc level (Table 4).

Fig. 1 demonstrates highly significant (P < 0.001) difference between serum zinc between control subjects, patients with early cirrhosis, patients with late cirrhosis and patients with HCC.

DISCUSSION

Hepatitis C is a major health problem in Egypt9. Hepatitis C virus is a major cause of chronic liver diseases, cirrhosis and HCC10. The duration of HCV infection is directly related to HCC development11.

In the present study, the mean age in HCC was statistically significant higher (P < 0.001) than cirrhosis mean age and control mean age. This result is similar to that found by Wang et al. Also Elzayadi et al. (2005)12 reported that HCC in Egypt is significantly more prevalent among older age groups than younger age groups and suggested that HCV infection in old patients induces a rapid progression to HCC independent of HCV genotype. Old age is a risk factor for HCC, especially in areas where HCV infection is endemic as Egypt13.

This is attributed to the fact that older age reflects a longer exposure to HCV, thus increasing the probability of malignant transformation11.

Regarding sex distribution among patients, there were a high percentage of males in all groups. This finding is similar to that found by Johnson et al. (2003)14 who reported that the rate of cirrhosis was higher for males than females. Male to female ratio differs among countries as greater ratios were noticed in the high incidence regions such as Africa, China, Taiwan and Japan15.

This finding may be attributed to more exposure to risk factors like HCV among male patients. However, sex hormones and other x-linked genetic factors may also be important12.

In the present study, there were statistically significant differences (P < 0.001) in late cirrhosis, HCC and control groups as regard to lower albumin level and prolonged prothrombin time.

### Table 2

**Serum zinc in early cirrhotic group compared to control group.**

<table>
<thead>
<tr>
<th>Zinc (microgram/dl)</th>
<th>Control group</th>
<th>Early cirrhotic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>137</td>
<td>119</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimum</td>
<td>112</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>122.4</td>
<td>103.8</td>
<td></td>
</tr>
<tr>
<td>Std. deviation</td>
<td>7.1</td>
<td>9.7</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

**Serum zinc in late cirrhosis and HCC groups compared to control group.**

<table>
<thead>
<tr>
<th>Zinc (microgram/dl)</th>
<th>Control group</th>
<th>Late cirrhosis</th>
<th>HCC group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>137</td>
<td>111</td>
<td>112</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimum</td>
<td>112</td>
<td>70</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>122.4</td>
<td>94.0</td>
<td>95.4</td>
<td></td>
</tr>
<tr>
<td>Std. deviation</td>
<td>7.1</td>
<td>12.7</td>
<td>10.1</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4

**Correlation between serum zinc and laboratory data in different groups.**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Early cirrhotic</th>
<th>Late cirrhosis</th>
<th>HCC group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>ALT</td>
<td>−0.024</td>
<td>0.908</td>
<td>0.215</td>
<td>0.303</td>
</tr>
<tr>
<td>AST</td>
<td>0.118</td>
<td>0.575</td>
<td>0.174</td>
<td>0.406</td>
</tr>
<tr>
<td>TB</td>
<td>0.126</td>
<td>0.548</td>
<td>−0.172</td>
<td>0.412</td>
</tr>
<tr>
<td>ALB</td>
<td>0.007</td>
<td>0.973</td>
<td>−0.050</td>
<td>0.812</td>
</tr>
<tr>
<td>INR</td>
<td>−0.316</td>
<td>0.124</td>
<td>−0.327</td>
<td>0.111</td>
</tr>
</tbody>
</table>
It is hypothesised that zinc supplementation may contribute to inhibition of liver fibrosis and improvement in hepatic encephalopathy. Though, little is known about the anti-inflammatory effect of zinc on HCV related chronic liver disease, there were supporting data that zinc administration in patients with HCV related chronic liver disease exerts an anti-inflammatory effect by the reducing iron overload and reducing fibrosis).

In the study of the correlation between serum zinc, AST and albumin there was insignificant correlations between them and all groups. In contrary, data indicated significant correlations between serum zinc level and AST and albumin. The difference in the findings may be due to the difference in the studied number of the patients.

From the present study we can conclude that serum zinc level decreases significantly in chronic HCV infection with liver cirrhosis and hepatocellular carcinoma. The decrease of serum zinc can be used as a laboratory parameter for evaluation of liver status in cases of liver dysfunction on top of HCV. It is recommended to evaluate the role of zinc supplementation in treating clinical manifestation of liver cirrhosis and liver cell failure associated with HCV.

**REFERENCES**