THE RELATIONSHIP BETWEEN TRACE ELEMENTS AND CERULOPLASMIN WITH SEVERITY OF FASCIOLIASIS PATIENTS

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/Rapporto tra oligoelementi e ceruloplasmina in pazienti con gravità d fascioliasi/

ABSTRACT

The aim of our study is to evaluate the relationship between trace elements, as zinc (Zn) and copper (Cu), and acute phase reactant ceruloplasmin (CP) levels during fascioliasis hepatic infection.

45 patients with fascioliasis and 38 healthy controls were enrolled in the study. Fascioliasis was diagnosed with ELISA IgG, stool examination and radiologic imaging. Number of females and males were determined as 34/11 and 30/8 in patient and control groups respectively. Mean age was 38.1±11.7 and 35.8±16.9 years in patient and control groups, respectively. Blood samples were collected to determine plasma Zn, Cu, and CP levels. As a result of the study serum Cu (from 1.4 to 1.8) and CP (from 26.7 to 33.4) levels were rised in while Zn (from 1.1 to 1.9) level was decreased in patient group. We determinated significant correlation (r: 0.781) of fasciola titers with ceruloplasmine that increased by acute phase in the patients with fascioliasis.

In conclusion, an high correlation between CP and Cu levels in patients suffering from fascioliasis can be noted. On the other hand, low Zn levels, especially in endemic areas of disease, suggested that Zn can be given in addition to medical treatment, or as a dietary supplement. This situation may lead the way in terms of increasing the efficiency of treatment of fascioliasis.

Key words: Fascioliasis, zinc, copper, ceruloplasmin.

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Introduction

Fascioliasis is a zoonotic infection caused by Fasciola sp. trematodes. The parasite has a enlarged host population including ruminants, rodents and humans. Transmission of humans develops by means of raw consumption of metacercariae contaminated water plants and drinking waters(1,2). Fascioliasis is a disease that may be acute or chronic due to changes in liver parenchyma and in bile ducts. This disease shows with different clinical findings varying from asymptomatic infection to severe hepatic cirrhosis(3). Transition of the parasite in liver parenchyma explains for basic pathologic changes. Parasites digestion of liver tissue results in significant hemorrhagic lesions, immunologic and inflammatory responses, parenchymal destruction. Hemorrhage and reactive hepatitis develop with traumatic effect of the parasite. Small infarction areas may also develop due to vascular injury. These small areas in liver parenchyma may regenerate or may change with fibrosis(6).

Levels of serum metal have been reported to be highly sensitive in the diagnosis of hepatic disorders(5). Many evidence proves the antioxidant role of zinc(8). Trace metals play an important role in liver disease especially liver degeneration.

Among the significant trace elements Zn is a trace element essential for cell proliferation and differentiation. It is an important element in preventing free radical formation, in protecting biological structures from damage and in correcting the immune functions(9). Under certain conditions, it may have antioxidant properties(10) and component to several key functions in human metabolism(11). Also, Zn is a critical part of biomembranes and is essential for exact membrane structure and function and the activity of numerous enzymes(12), and plays...
an important role in regulation of cellular glutathione that is vital to cellular antioxidant defence\textsuperscript{(13)}.

CP is an abundant plasma protein that contains seven copper atoms per molecule and accounts for 95\% of the total circulating Cu in healthy adults\textsuperscript{(14)}. The most important role of CP is Cu transport\textsuperscript{(15)}. CP is a positive acute-phase protein, which means that its level in plasma is elevated in disorders accompanied by inflammation\textsuperscript{(16)}. Higher serum levels of CP are associated with different pathological states are demonstrated Table 1\textsuperscript{(17)}.

Cu is an essential trace metal found in all living organisms in the oxidized Cu (II) and reduced Cu (I) states. It is necessary for survival and serves as an important catalytic cofactor in redox chemistry for proteins that perform fundamental biological functions that are required for growth and development\textsuperscript{(18)}. The regulation of Cu excretion appears to be the main mechanism for homeostasis\textsuperscript{(19)}. Most Cu return to the liver for excretion. Most CP enters hepatocytes after desialylation in endothelial cells\textsuperscript{(20)}. The primary pathway for the excretion of Cu from the body is from hepatocytes, via the bile. The importance of maintaining mechanisms for proper Cu homeostasis in the liver is underlined by the existence of Wilson’s disease\textsuperscript{(18)}.

In this study; we aimed to the relationship between serum levels of zinc and copper with severity of disease at patients with fascioliasis and zinc levels that is an important trace element in this disease were affected in what direction.

**Materials and methods**

A total of 45 fascioliasis patients who were referred to Parasitology Laboratory of Dicle University Medical Faculty Research and Training Hospital from various outpatient clinics between November 2010 and June 2011 and 38 healthy controls were included in the study. Fascioliasis was diagnosed through ELISA IgG (DRG International Inc., USA), stool examination native and sedimentation [midi Parasep\textregistered Faecal Parasite Concentrator tubes (Diasys company)], radiologic imagings (ultra-sonography, computed tomography), clinical and laboratory parameters. Demographic characteristics and ELISA titers presented at the following table (Table 2).

### Table 1: Pathological states associated with increased levels of ceruloplasmin.

<table>
<thead>
<tr>
<th>Pathological states</th>
<th>Fold increase in serum levels of ceruloplasmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic syndrome, insulin resistance</td>
<td>1.17</td>
</tr>
<tr>
<td>Nonalcoholic steatohepatitis</td>
<td>1.30</td>
</tr>
<tr>
<td>Alcoholic liver steatosis</td>
<td>1.39</td>
</tr>
<tr>
<td>Lupus</td>
<td>1.51</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>1.62</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>1.61</td>
</tr>
<tr>
<td>Diabetes with diabetic complications</td>
<td>1.66</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>1.83</td>
</tr>
<tr>
<td>Lymphocytic leukemia</td>
<td>2.25</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>3.3</td>
</tr>
</tbody>
</table>

### Table 2: Some characteristics of patients.

<table>
<thead>
<tr>
<th>Number of patient (n)</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>34/11</td>
</tr>
<tr>
<td>Age (year)</td>
<td>10-63 (11 ± 1.75)</td>
</tr>
<tr>
<td>Rural/Urbant</td>
<td>27/18</td>
</tr>
<tr>
<td>*ELISA titer Cut off = 10 (DU/ml) 11&gt;Positive</td>
<td></td>
</tr>
<tr>
<td>12-20</td>
<td>6</td>
</tr>
<tr>
<td>21-30</td>
<td>25</td>
</tr>
<tr>
<td>31-40</td>
<td>14</td>
</tr>
</tbody>
</table>

*DU: Value of patient absorbance x 10 / Cut off (C1 + C2/2)
Spectrophotometer AA-6800 SHIMADZU, 2000). CP levels were assayed by rate nephelometry on Immage 800 analyzer (Beckman Coulter, Fullerton, CA, USA).

SPSS 15 (SPSS Inc. Chicago, IL) statistical package program was used for statistical analysis. Distribution pattern of data was assessed by Kolmogorov-Smirnov test. Student’s t test and Pearson’s correlation were used for analysis.

Results

While Cu (1.8±1.4 ppm) and CP (33.4±11.4 mg/dL) levels were found significantly higher in patients with fascioliasis than control groups, Zn (0.9±0.3 ppm) levels were found lower (Table 3). Especially, a strong correlation between ELISA levels and serum CP levels in patients were fonud (r: 0.781) (Figure 1).

Zn is an essential trace element for normal cell function and metabolism. Zn ions are key structural components of a large number of proteins with highly specific functions; i.e. removal of fibrosis in chronic liver disease (22). The role of Zn is extremely concerning in all manners of cell metabolism, regulating the function of a large variety of metabolic processes. In fact, even minor changes in Zn concentration in the human body are likely to have multiple biological and clinical effects, particularly in older subjects (23). For the complete of liver function, Zn is necessary and the pathogenesis of multiple liver diseases is related with Zn deficiency. Especially, the reduction of Zn concentration is associated with liver cirrhosis has shown different studies (24). On the other hand, liver plays a key role in Zn homeostasis, regulating the incorporation of Zn ions in a large variety of enzymes which play a central role in the metabolism of the human body. In several disease that has different etiology, characterized by fibrosis and degeneration in liver has shown Zn decreases death of hepatocytes (25).

Davtyan et al, Damyanova & Gabrashanska, Tsocheva & Gabrashanska showed at experimental studies performed on animals; decreased Cu and Zn levels during acute phase of fascioliasis (26-28). In our study Cu levels were increased, Zn levels were decreased.

Patients with alcoholic cirrhosis who has acrodermatitis enteropathica like symptoms: dry eczematous skin lesions, stomatitis, cheilitis, and alopecia, response to Zn supplementation (29).

Discussion

The Fasciola hepatica that causes fascioliasis is cause of hepatobiliary system infections. This trematode infects cattle, sheep and goats. Human beings are accidental hosts. After ingestion of infective form metacercaria, they spread in the intestine, perforate the intestinal wall, enter the peritoneum and then pass through the liver capsule to enter the biliary zone (21).

![Figure 1: Correlation of CP and fascioliasis ELISA titer (r: 0.781, p<0.001)](Pearson correlation used in this figure)
On the other hand, nutritional zinc deficiencies also predispose to the pathogenesis and or continuance of liver damage, given the pivotal role of Zn ions as powerful antioxidants, in the imbalance between the formation of reactive oxygen species and antioxidant defense mechanisms. The role of Zn in the evolution of chronic liver disease of different aetiology is mainly related to its influence on the remodelling of liver fibrosis and on cirrhosis, the end stage consequence of liver fibrosis\(^3\). Liver cirrhosis has been correlated, in different human studies\(^3\) to reduced serum and hepatic Zn levels, compared with healthy individuals.

Our study confirms the above-mentioned studies, which found lower Zn levels at patients with fascioliasis than control groups.

Excessive liver Cu levels have been reported in cholestatic syndromes and in alcoholic cirrhosis\(^3\). Cirrhotic patients showed higher liver Cu levels, which is in relation with the amount of liver fibrosis and also lower liver Zn levels. In patients with primary biliary cirrhosis, excessive Cu accumulation in the liver secondary to cholestasis has been well documented\(^3\). In a variety of other clinical and experimental liver diseases, Cu levels are also elevated, probably due to impaired excretion\(^3\).

In our study Cu levels were found higher at patients with fascioliasis than control groups. But we didn’t find any information related to high Cu levels in patients with fascioliasis in the literature.

CP is a glucoprotein that transports serum copper 90-95%. CP is synthesized in the liver, from whence it transports copper to tissues that need this element for metalloenzyme functioning. Also CP shows some enzyme activity as ferro-oxidase, amino-oxidase and superoxide dismutase\(^3\). It can mobilize copper and act as an acute phase reactant\(^3\).

Conforti et al.\(^3\) reported a clear correlation between increases in CP and serum Cu concentrations in normal rats and animals with experimental inflammation.

In our study, we were found that significantly increased the levels of CP at patients with fascioliasis than control groups. More importantly, we showed a significant correlation between the titers of Fasciola and CP levels. This situation reinforces the impression that CP values can be used at patient with fascioliasis take place follow-up of patients with the disease.

Conclusion

We believe that in addition to medical treatment giving Zn and especially in endemic areas of this disease in the addition of Zn-rich foods in their diet should not be ignored. Do the new experimental studies, particularly the degree of accumulating-copper in the liver can be determined and to avoid the possible toxicity of copper; we think it is important that using of Cu-binding agents can be increase efficiency of treatment. High correlation of CP and fascioliasis is suggested that CP values may be used as monitoring marker in patient with fascioliasis but this statement need more detailed studies.

### Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Name</th>
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<tbody>
<tr>
<td>Zn</td>
<td>Zinc</td>
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<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>CP</td>
<td>Ceruloplasmin</td>
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</tbody>
</table>

### References

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