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Original Article

Assessment of Zinc and Copper Levels in Patients with Different Causes of Liver Cirrhosis

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Abstract

Background: Liver cirrhosis is a worldwide health problem in general and in Yemen in particular, mainly due to the environmental and nutritional health problems that lead to many liver diseases. It is a slowly progressing disease in which a healthy liver tissue is replaced with scar tissue. This change in liver structure prevents liver from carrying out its proper functions and disturbances in the antioxidant system and oxidative stress may play a role in the pathogenesis of chronic liver diseases. Aim: is to assess plasma trace elements (zinc and copper) in patients with liver Cirrhosis due to chronic hepatitis B and C, chronic non-viral hepatitis (Bilharziasis and autoimmune hepatitis). Methods: Liver cirrhosis was diagnosed on the basis of clinical, biochemical, imaging methods and endoscopic signs. Sixty plasma samples were collected from Yemeni cirrhotic patients aged (48 ± 3) and forty samples were collected from persons aged (39 ± 5), as control group. Zn and Cu were analysed by Atomic Absorption Spectrophotometer. The data were statistically analysed using SPSS version 18.0 computer program and results were presented as mean ±SD. The differences in results between patients and control subjects were compared using t-test. Results: Our result indicated a significant ($p < 0.001$) higher levels of Cu in plasma of liver cirrhotic groups by 9.1 folds when compared to control group, being highest in the HCV group (4.455 ± 1.139 ppm). In contrast, plasma concentration of Zn was significantly ($p < 0.001$) lower in liver cirrhosis groups by 90.7% as compared to control group, being lowest in the HCV liver cirrhosis patients. Conclusion: Liver cirrhosis is a health problem in Yemen, mainly due to the environmental and nutritional health problems that lead to many liver diseases.

Key words: Liver cirrhosis, Zinc, Copper, Free radical and antioxidant

Introduction:

Liver cirrhosis is a worldwide health problem in general and in Yemen in particular, mainly due to the environmental and nutritional health problems that lead to many liver diseases. Liver cirrhosis is the end stage of liver fibrosis. It is characterized by nodule formation1. It is commonly associated with abnormalities in the systemic circulation and impaired primary hemostasis2. Cirrhosis is a slowly progressing disease in which healthy liver tissues are replaced with scar tissues. This change in liver structure prevents the liver from carrying out its proper functions, and disturbances in the antioxidant system and oxidative stress may play a role in the pathogenesis of chronic liver diseases3,4. However, Cirrhosis often is a silent disease, with most patients remaining asymptomatic until decompensating occurs. Quantity and duration of alcohol consumption is an important factor in the early diagnosis of cirrhosis3. Other risk factors include those for hepatitis B and C transmission, as well as transfusion history and personal or family history of autoimmune or hepatic diseases3,4.

Liver plays a central role in zinc homeostasis, removing zinc from albumin in blood and distributing it to the body as needed. Hormonal stimuli such as glucocorticoids and epinephrine up-regulate hepatic zinc uptake by hepatic Metallothionein levels5,6. Zinc is an essential trace

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element necessary for a broad range of biological activities, such as, cell proliferation, normal protein metabolism, membrane integrity, and for the function of more than 200 Zn metalloenzymes1,7,8. Zinc functions as the natural defense of reactive oxygen radicals by Zn-enzyme Cu-Zn superoxide dismutase9. Zinc acts as an antioxidant, a membrane and cytosceletal stabilizator, an anti-apoptotic agent, and as an important co-factor in DNA synthesis10. Also, zinc inhibits the activity of the enzymes involved in fibrogenesis (fibrosis) in the liver11,12. Several studies in humans suggest that zinc may have a protective effect against free radical generation and oxidative stress. Zinc may exert protective antioxidant by stabilizing lipid membranes and preventing lipid peroxidation by free radicals11,12. Many of the clinical features of liver cirrhosis have been linked to Zn deficiency including loss of body hair, testicular atrophy, poor appetite, immune deficiency, and distorted protein metabolism1,7. The blood zinc concentration decreases with progression of the disease from chronic hepatitis (CH), to liver cirrhosis (LC), to hepatocellular carcinoma (HCC)1,13-17.

On the other hand, copper (Cu) is a transition metal and its physiological roles serve to provide many functions: including transport of oxygen and electrons, catalysis in oxidation reduction reactions and protection of the cell against damaging oxygen radicals (18). At least ten enzymes are known to be dependent upon copper for their function, such as cytochrome C oxidase, dopamine B hydroxylase, Lysyl oxidase and superoxide dismutase. Superoxide dismutase is required to prevent the accumulation of the superoxide radicals which cause cellular damage; the enzyme responsible is a copper/zinc metalloenzyme, found in the cytosol of all cells19. Copper is mainly stored in the liver, but is also found in the skeletal system (bone marrow) as well as in muscle, brain and spleen tissue. The serum proteins, albumin & transcuprein transport Cu in blood to the liver, which is the site of several studies have shown that antioxidant enzymes that contain zinc and copper decrease in liver cirrhosis, accompanied with increase in lipid oxidative stress.

Aim of the study

The aim of our study was to determine the levels of zinc and copper as antioxidants, in viral and non-viral causes of liver cirrhosis.

Subjects and Methods

Liver cirrhosis was diagnosed on the basis of clinical, biochemical, imaging methods (Ultrasound, CT) and endoscopic signs. Sixty patients (42 men and 18 women with cirrhosis and 40 healthy control group (22 men and 18 women were included in this study; in the Department of Gastroenterology in Azal Hospital, Sana'a City. Cirrhosis was related to post-hepatitis C in 20 patients, post-hepatitis B in 20 patients and non-viral cirrhosis in 20 patients. Serum total bilirubin, amino transferase enzymes, albumin, gammaglutamyl transferase (GGT), prothrombine time (PT), Zn and Cu levels were measured in all patients and control. The exclusion criteria of the study were; patients with acute or chronic diarrhea, pregnant women, patients with other chronic diseases and blood transfusion within previous two weeks.

Three milliliters venous blood was collected in heparinized tube, where sample was centrifuged at 3000 rpm for 10 minutes within 3 hours of collection. The plasma in heparinized tubes was stored at -20oC until time of analysis of copper and zinc by Atomic Absorption Spectroscopy (AAS), Perkin Elmer 2380 Model. Zinc was measured at wavelength of 213.9 nm, and copper at 324.8 nm.

Results of analysis of blood for Copper (Cu) and Zinc (Zn) were statistically analyzed using SPSS computer program (version 18.0) and the results were presented as mean ±SD. The differences in results between patients and control subjects were compared using the t-test. The ANOVA test was used for intergroup comparisons. Significant differences were taken in account if p-value was <0.05.

Results

The mean age 48 ± 3 years with cirrhosis and the mean age 39 ± 5 years of healthy control. The result presented in Table 1 reveals changes in Cu and Zn parameters in liver cirrhosis groups and control. Cu was observed to be significantly ($p < 0.001$) higher in liver cirrhosis groups by 9.1 folds when compared with control group. In contrast, zinc was significantly ($p < 0.001$) lower in liver cirrhosis groups by 90.7% as compared with the control group. No significant differences were found among the different groups of patients in terms of plasma Zn levels ($p = 0.245$) (Table 2). Plasma copper levels were highest in Hepatitis C virus (HCV) patients than other study groups (4.455 ± 1.139 ppm), followed by Hepatitis B virus (HBV) cases (3.830 ± 0.796 ppm). Among non-viral cases the copper level was higher in autoimmune hepatitis (AIH) cases than those with bilharziasis liver cirrhosis (Table 2).

Table 1: Comparison between mean levels (±SD) of Cu and Zn in cirrhotic patients and control.

Variables	Study groups	N	Mean ±SD	P-Value
Cu [ppm]	Case	60	3.672±1.24	< 0.001*
	Control	40	0.402±0.553	
Zn [ppm]	Case	60	0.393±0.343	<0.001*
	Control	40	4.225±1.22	

Table 2: Comparison between mean levels (±SD) of Cu and Zn in liver cirrhosis groups

Variables	Study groups	n	Mean ±SD	P-Value
Cu [ppm]	HCV	20	4.455±1.19	<0.001
	HBV	20	3.830±0.796	
	Non-viral (Bilharziasis)	10	2.160±0.783	
	Non-viral (AIH)	10	3.300±0.838	
	Total	60	3.672±1.24	
Zn [ppm]	HCV	20	0.278±0.231	0 .245
	HBV	20	0.487±0.330	
	Non-viral (Bilharziasis)	10	0.368±0.438	
	Non-viral (AIH)	10	0.461±0.425	
	Total	60	0.393±0.343	

Discussion

This study showed significant ($p < 0.001$) decrease in zinc level in cirrhotic patients due to hepatitis B, C or non-viral hepatitis, when compared to healthy volunteers. Furthermore, our study revealed that the plasma zinc levels were not statistically significant between the 4 LC groups, HCV, HBV, AIH and Bilharziasis ($p = 0.245$), as shown in Table 2. The above results of zinc levels in the present study are in agreement with Lin et al.17, who recorded a significant decrease in zinc concentration of Chinese patients group from control group. Our results were also in agreement with Nazari et al.21, who studied the zinc plasma level in Iranian cirrhotic patients and found plasma zinc levels to decrease in cirrhotic patients due to hepatitis B or C in comparison with healthy volunteer. Furthermore, Sayed et al.22, who investigated the same trace elements in hepatitis B, C and Schistosomiasis in rural population of Egypt, found a statistically significant decrease in zinc levels in patients with liver cirrhosis.

There are some explanations for the decrease of zinc content in the plasma of cirrhotic patients. During cell damage and inflammation, liver cells take up more zinc to synthesize nucleic acid, protein and enzymes related to zinc, with progression of the liver damage, due to poor appetite, impaired function of intestines and stomach and high pressure of the portal vein. As a result, zinc intake and absorption decreases, in addition to low content of serum albumin, resulting in less combination with zinc. Furthermore, because of the diffusion characteristic of blood zinc, zinc is easily lost through urine and sweat23,24, and zinc deficiency may exacerbate the complications of cirrhosis.

The most important role of copper is in redox processes. Reactive copper can

participate in liver damage directly or indirectly, through Kupffer cell's stimulation. Scientists agree that copper's toxic effects are related to oxidative stress25,26. In this study, the plasma levels of copper were significantly higher ($p<0.001$) in patients with liver cirrhosis as compared to controls. This is in agreement with those of

Rahelic et al.27, where authors studied the serum concentration of zinc, copper, manganese and magnesium in patients with liver cirrhosis, and found that serum concentration of copper was significantly higher in patients with liver cirrhosis compared to controls. Also, Nagasue et al.28 has shown that the serum copper levels were significantly higher in cirrhotic patients than those of normal subjects.

With copper's role in redox process, it can be suggested that redox cycling between Cu2+ and Cu1+ can catalyze the production of toxic hydroxyl radicals29,30. Furthermore, it is well known that redox processes and oxidative stress play an important role in the pathogenesis of liver cirrhosis. The increase in Cu concentrations was likely due to the defense strategies of the organism, and these were induced by the hormone like substance (31). It may also be due to the release of copper from damaged necrotic hepatocytes 32.

In the present study, plasma copper levels were found to be statistically significant ($p < 0.001$) in the 4 LC groups, the HCV, HBV, AIH and Bilharzias, as shown in Table 2. Plasma copper level was highest in HCV patients than that of other study groups, followed by HBV cases. Among the Non-viral cases, the copper level was higher in autoimmune hepatitis cases, than that of bilharziasis liver cirrhosis. These alterations in copper levels may be explained by variation of hepatocellular damage, according to the cause where copper released from damaged necrotic hepatic cells 33.

Conclusion

Plasma copper levels were significantly higher in all cirrhotic patients compared to the controls and were significantly higher in viral hepatitis groups than those of non- viral hepatitis patients. In contrast, plasma level of Zn was lower in all cirrhotic patients compared to control subjects. The lowest level was in HCV liver cirrhosis, but this variation was not significant.

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