Biol Trace Elem Res (2017) 178:189–193
DOI 10.1007/s12011-016-0919-5

Elemental Zinc Is Inversely Associated with C-Reactive Protein and Oxidative Stress in Chronic Liver Disease

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Received: 29 October 2016 / Accepted: 20 December 2016 / Published online: 5 January 2017

Abstract Chronic liver disease (CLD) is associated with the destruction of liver parenchyma cell. It is the main cause of morbidity and mortality in most of the developed countries. Oxidative stress and altered levels of different trace elements in serum have been documented for different diseases including inflammation and many liver diseases. This study aims to evaluate the serum level of malondialdehyde (MDA), nitric oxide (NO), antioxidant vitamin C, C-reactive protein (CRP), and zinc (Zn) in CLD patients and to establish a correlation among the study parameters with the severity of inflammatory conditions of CLD. In this study, CLD patients and healthy volunteers were recruited. Total cholesterol and triglyceride were determined by colorimeter using enzymatic method. Serum non-enzymatic antioxidant vitamin C, reactive oxygen species nitric oxide (NO), and malondialdehyde (MDA) were determined by UV-spectrophotometric method. Trace element (Zn) levels were determined by graphite furnace atomic absorption spectroscopy. Independent sample *t* test and Pearson's correlation test were performed for statistical analysis using the statistical software package SPSS, Version 20. Studies showed that the MDA ($p < 0.001$), NO ($p < 0.001$), and CRP levels were significantly higher in CLD patients than in control subjects. The antioxidant vitamin C ($p < 0.001$) and trace element zinc ($p < 0.001$) were comparatively lower in the CLD patients than in control subjects. Elemental Zn showed an inverse relationship with MDA, NO, and CRP but positively correlated with antioxidant capacity, whereas MDA showed a positive correlation with CRP level. Thus, we conclude that attenuated level of Zn and antioxidant in serum play an important role in the inflammatory status of CLD patients by elevating the concentration of MDA, NO, and CRP.

Keywords Chronic liver disease · Inflammation · Malondialdehyde · C-reactive protein · Zinc

Introduction

Chronic liver disease (CLD) is associated with the destruction of liver parenchyma cell. It is the main cause of morbidity and mortality in most of the developed countries and is generally caused by viral hepatitis and due to alcohol abuse [1]. CLD includes a wide range of health problems such as inflammation, liver cirrhosis, and hepatocellular carcinoma. In a CLD patient, liver failure is characterized by progressive and irreversible defects in the hepatocellular metabolism [2]. When the reactive oxygen species is generated from either exogenous or endogenous sources and are not balanced by antioxidants with a similar rate, the status of oxidative stress in developed [3]. Oxidative stress not only is responsible for the destruction of different cellular components such as DNA, proteins, and lipids/fatty acids leading to cell damage and apoptosis but also releases some proinflammatory cytokines resulting in hepatic inflammation, fibrosis, and cirrhosis [4, 5]. Malondialdehyde (MDA) is the end product of lipid peroxidation and is used as a biomarker of oxidative stress [6]. Peripheral venous MDA levels showed significant correlation with hepatic venous MDA levels in chronic liver disease [7]. Along with MDA, nitric oxide (NO) has also been studied in the pathogenesis of distinct patterns of liver diseases [8, 9].

Though trace element present in a minor amount in the body, it plays a key role in various biochemical enzymatic reactions. Zinc, being a member of the essential trace

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elements, is playing an important role in various biological processes such as cellular metabolism and immunological response [10, 11]. Altered level of Zn may influence the liver functions specially regeneration of hepatic cells [12]. Imbalance of Zn is responsible for various health problems including dermatitis, delayed wound healing, alopecia, nodular goiter, and diabetes [13–15]. Oxidative stress due to the deficiency of trace elements such as Zn has been studied in psoriasis and diabetes [16, 17]. Moreover, Zn supplementation has been considered to attenuate the production of C-reactive protein (CRP), an inflammatory biomarker, in acutely ill elderly patient [18]. CRP, the acute phase protein, is produced particularly by hepatocytes under the transcriptional control of IL-6 [19]. Although several studies had been conducted to explore the role of oxidative stress in different liver diseases, but there are limited information about the correlation among oxidative stress, elemental Zn, and inflammation in chronic liver diseases. Taking all these in consideration, we aimed to study the status and correlation of oxidative stress and Zn in manifestation of inflammation in chronic liver disease.

Materials and Methods

This case-control study was carried out with adult CLD patients attending the Department of Medicine, Jononeta Nurul Hoque Adhunik Hospital, Noakhali, Bangladesh. Patients and control group were matched by age, sex, and socioeconomic conditions. Children, non-cooperative volunteers, and volunteers having comorbid diseases and mental disorders were excluded from the study. Institutional ethical committee approved the study protocol (# BS58/2015). Detailed patient history was taken with a predesigned questionnaire. Every volunteer was informed about the purpose of this study and written consent was taken before data collection. Twenty healthy volunteers as control and 20 CLD patients were recruited in this study.

Fasting venous blood was drawn from each CLD patient and control group. The samples were allowed to clot within 30 min, and after centrifugation, serum was collected. Extracted serum stored in −80 °C. All the steps were carried out in an aseptic condition to avoid the possible interference in the test readings. These samples were then used for determining the serum level of antioxidant (vitamin C), lipid peroxide (MDA), nitric oxide (NO), C-reactive protein (CRP), triglyceride (TG), serum total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and zinc. Serum MDA was measured according to the modified method described by Satoh [20]. Serum vitamin C level was estimated by phenylhydrazine spectrophotometric method [21]. Serum nitric oxide (NO) was estimated by the Griess reagent method [22]. Serum zinc concentration is detected by graphite furnace atomic absorption spectroscopy (Shimadzu AA6800) developed by Sultana [23] with slight modification with the instrumental settings of wavelength: 213.9 nm; lamp source: hollow cathode lamp; lamp current: 15 mA; diluents: 0.5% HNO_3; rinsing solution: 0.5% HNO_3; matrix modifier: $[Mg(NO_3)_2]$; background correction: the Zeeman effect; and furnace setting: atomize (1800 °C), cleaning (2450 °C). Standard reference material was used to maintain the accuracy and precision of analysis. Serum CRP was estimated as described by Pfeiler and Molinari [24]. Other experiments were carried out using respective assay kit according to the manufacturer's instruction adapted in the hospital.

The statistical analysis of data was calculated using SPSS software statistical computer package, Version 20. All results were expressed as mean ± SD. Correlation coefficient was measured to test a positive or a negative linear relation between two variables. p values of <0.05 and <0.01 were considered as significant and highly significant, respectively.

Results

Finding on the assessed parameters in CLD patients and healthy control subjects are shown in Tables 1 and 2.

Significant elevated levels of ALT and AST ($p < 0.001$) were estimated in CLD patients compared to control group, whereas serum TG and TC in CLD patients were less than control.

The average concentration of MDA (8.82 ± 1.58 mmol/L) and NO (68.56 ± 2.53 μmol/L) in serum of patients with CLD was higher ($p < 0.001$) in comparison with the serum of healthy people.

As a function of antioxidant capacity, level of vitamin C was estimated and the average value was 0.78 ± 0.08 mg/dL in serum of CLD patients that was significantly less ($p < 0.001$) in comparison with the serum of healthy volunteers (0.95 ± 0.07 mg/dL).

Table 1 Characteristics of the study subjects

Parameters	Control (n = 20)	CLD (n = 20)	p value
Age (years)	49.4 ± 11.72	50.3 ± 16.58	0.89
Sex (M/F)	15/5	14/6	
Alcoholic/non-alcoholic	0/20	6/14	
Smoker/non-smoker	4/16	13/7	
Disease duration (years)	–	>2	
BMI (kg/m^2)	21.53 ± 1.58	18.59 ± 2.06	0.002[a]
Serum AST (U/L)	14.63 ± 6.28	42.4 ± 10.24	0.001[a]
Serum ALT (U/L)	27.6 ± 7.25	62.12 ± 26.87	0.001[a]

Values are expressed as mean ± SD

[a] Highly significant result

CLD chronic liver disease, *BMI* body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Table 2 Triglyceride, cholesterol, MDA, NO, vitamin C, and Zn levels in CLD and control groups

Parameters	Control (n = 20)	CLD (n = 20)	p value
Serum triglyceride (mg/dL)	137 ± 27.72	121.6 ± 28.34	0.235
Total serum cholesterol (mg/dL)	187.27 ± 8.93	145.94 ± 16.72	0.001[a]
Serum MDA (mmol/L)	3.13 ± 0.44	8.82 ± 1.58	0.001[a]
Serum NO (μmol/L)	25.32 ± 3.41	68.56 ± 2.53	0.001[a]
Serum vitamin C (mg/dL)	0.95 ± 0.07	0.78 ± 0.08	0.001[a]
Serum Zn (μg/mL)	17.86 ± 0.69	14.73 ± 1.05	0.001[a]

Values are expressed as mean ± SD

[a] Highly significant result

CLD chronic liver disease, *MDA* malondialdehyde, *NO* nitric oxide

The mean content of Zn in serum of patients with CLD (14.73 ± 1.05 μg/mL) was lower ($p < 0.001$) compared to control group (17.86 ± 0.69 μg/mL).

We measured the C-reactive protein (CRP) level as a maker of inflammation. Our studies found a high level of CRP in most of the serum of CLD patients with a value of 48 mg/dL, but, in the case of healthy controls, the CRP level was less than 6 mg/dL.

Discussion

The prevalence of CLD in Bangladesh is endlessly increasing and represents a major clinical threat. Injury to the liver, whether chronic or acute, results in an increase in serum concentrations of aminotransferase. Both the aminotransferases (ASL and ALT) are highly concentrated in the liver, and the rise in serum concentration of ALT is more specific for liver damage [25]. Here, along with the elevated concentration of AST, prominent increase in ALT was observed indicating the liver malfunction in CLD. Severity of CLD was assessed with the values of ALT.

We found a lower level of total triglyceride and total cholesterol in the serum of patients with CLD compared to healthy volunteers. There was a positive correlation between the decrement in serum triglyceride and cholesterol with the severity of chronic liver disease. Although our finding is slightly different from the result of Ghadir et al. [26], where they showed that liver damage is correlated with reduced cholesterol but not with triglyceride, our data is similar with the result of Mehboob et al. [27]. Moreover, a higher concentration of MDA and NO was found in the serum of all patients compared to healthy controls in this study. Nitric oxide is considered as a source of reactive oxygen species (ROS), and MDA is the end product of lipid peroxidation. The result of our study revealed the presence of more ROS and lipid peroxidation in patients with CLD compared to control in a positive correlated manner. Taking all these in consideration, we assume that the reduced level of triglyceride and cholesterol in CLD patients may be attributed by the failure of liver lipid biosynthesis and in some extent with more lipid peroxidation by free radicals produced compared to control group. Elevated MDA level has been reported in alcoholic liver disease by Pujar et al. [28], and in some part, long-term alcohol abuse is responsible for the generation of chronic liver disease, thus elevated MDA level in CLD patients is more likely.

Oxidative stress in the body is encountered by different enzymatic and non-enzymatic antioxidants. Among the different non-enzymatic antioxidants, vitamin C is the important one. Besides the role of antioxidant, vitamin C is inversely associated with inflammatory marker [29]. In this study, we reported the depleted level of vitamin C in patients with CLD compared to control. Our findings are similar with the results of some other studies [30, 31] with liver diseases. This depleted level of vitamin C fails to neutralize the elevated ROS and resulting in the higher level of MDA as lipid peroxidation product and promotes the inflammation in chronic liver disease. An inverse relationship was reported between vitamin C with MDA and CRP in our study.

Along with antioxidant, some essential trace elements like Zn are considered to be useful in neutralizing the excess ROS in body by inhibiting NADPH oxidase [32]. Altered level of Zn may influence the liver functions specially regeneration of hepatic cells [12]. Decreased amount of Zn may contribute to activation of monocytes and macrophages to generate inflammatory cytokines and increase oxidative stress [33]. An attenuated concentration of serum trace element Zn is found in the serum of all patients compared to healthy control in this study. Decrease of Zn level in chronic liver disease indicates the severity of liver damage. We found a positive correlation between Zn and vitamin C ($r = 0.215$) but negative correlation between Zn and MDA ($r = -0.094$).

C-reactive protein (CRP) is an acute phase plasma protein that is produced by hepatocytes, and its concentration increased in inflammatory condition. Previous study showed that serum CRP is mainly produced in hepatocytes and under transcriptional control of IL-6 [19]. A high level of serum CRP in the blood indicates that there may be an inflammatory process occurring in the body. A little data is available for the association of CRP with liver disease mortality and incidence.

Recent study observed the association of CRP in short-term outcomes of cirrhosis [34], but elevated level of CRP is not associated with non-alcoholic liver disease [35]. Furthermore, oxidative stress and depleted antioxidants are associated with inflammatory response. Hence, we aimed to determine the level of CRP in serum of patients with CLD to access the inflammatory response associated with depleted Zn and elevated oxidative stress and found elevated level of CRP in serum in all patients with CLD than that of control. A negative correlation between Zn and CRP ($r = -0.325$) was reported. There was also positive correlation between MDA and CRP ($r = 0.639$), whereas negative correlation between NO and CRP ($r = -0.110$) was reported in our study.

Conclusion

Our study explored the dyslipidemia; depleted Zn; vitamin C; and elevated level of MDA, NO, and CRP in patients with chronic liver disease. Inflammatory condition of chronic liver disease may be contributed by oxidative stress and reduced level of Zn.

Zinc homeostasis is maintained in the liver. It has been reported that inadequate dietary intake, impaired absorption, or increase clearance of zinc is associated with altered metabolism of Zn causing the decrease level of zinc in patients with liver disorders [36, 37]. Supplementation, diet with antioxidant, and elemental Zn may be beneficiary in the management of chronic liver disease, although further study with supplementation is warrant for a final conclusion.

Acknowledgments The authors are thankful to all physicians, staffs, and nurses of the Department of Medicine, Jononeta Nurul Hoque Adhunik Hospital, Noakhali, Bangladesh, for their technical and administrative support during the study.

Compliance with Ethical Standards Institutional ethical committee approved the study protocol (# BS58/2015). Written consent was taken before data collection.

Conflict of Interest The authors declare that they have no conflict of interest.

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