SERUM ZINC LEVEL IN CHRONIC HEPATITIS C PATIENTS: DOES IT HAVE A ROLE IN THE PROGRESSIONOF LIVER DISEASE?

Sahar A. El-Nemr, Sherif M. Galal*, Randa H. Mohamed**, Mahmoud W. Emara****

Tropical Medicine,Medical Biochemistry** and Pathology***Departments,Faculty of Medicine, Zagazig University*

ABSTRACT

Introduction: Chronic hepatitis C virus (HCV) is the most frequent disease states responsible for the induction of liver fibrosis. Zinc(Zn) has a protective effect against liver fibrosis. **Aim of study:** To estimate serum zinc levels in patients with chronic HCVand determine the correlation between their levels and clinical, laboratory and histopathological profilesto assess its role in the pathogenesis of chronic HCV. **Subjects and methods:**Seventy five patients with chronic HCV. Their age ranged from 22 to 58years with a mean age ± SD of 40.5 ± 6.5. They were 48 (64%) males and 27(36%) females. In addition to twenty-five healthy individuals matched in age and sex as a control group. All subjects were submitted to full clinical and laboratory investigations, serum zinc levels estimation by atomic absorption spectrophotometry. Liver biopsy was done for patients to assess liver fibrosis.**Results:** A statistically significantly lower serum levels of zinc was observed among patients with chronic HCV when compared with control group (46± 3.1 and 67± 7.2, respectively).There was an inverse correlation between serum zinc level and age. No significant correlation was found between amounts of HCV RNA,duration of interferon therapy.The serum zinc concentration of the patients on interferon therapy group was significantly higher than that of the untreated group. A high significant difference was found between the different grades of histopathological changes in liver biopsy findings as regard serum zinc levels. **In conclusion:** The serum concentration of zinc decreased significantly in chronic HCV and has been implicated in liver disease progression.

***Keywords*:** Zinc, chronic hepatitis C virus, HCV, chronic liver disease.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major health problem in Egypt, where the seroprevalence is 10–20-fold higher than that in the United States[1,2].HCV is the most frequent disease state responsible for the induction of liver fibrosis.Understandingthe mechanisms that contribute to liver fibrosis will prove helpful in designing more efficacious diagnostic and treatment strategies[3].

Zinc is an essential trace element, exerting important antioxidant, anti-inflammatory, and antiapoptotic effects[4]. The liver is important for the regulation of zinc homeostasis, while zinc is necessary for proper liver function. Decreased zinc levels have been implicated in both acute and chronic liver disease states, and in the pathogenesis of liver diseases[5].zinc supplementation enhances the response to interferon therapy in patients with intractable chronic hepatitis C[6].

It has long been speculated that Zn has a protective effect against liver fibrosis[7]. Zn could directly affect the fibrotic process by influencing the activity of proteins and enzymes that participate in collagen synthesis and degradation (e.g., prolyl-hydroxylase, collagenase, and matrix metalloproteinases)[8]. Indirectly, Zn could inhibit the fibrotic process by its anti-inflammatory, antiapoptotic, and antioxidant properties and by controlling the function of the hepatic stellate cells (HSCs) that play a central role in collagen metabolism in the liver[9].

Although Zn is a redox inert molecule, it has important antioxidant properties. The most important of the antioxidant properties of Zn are the protection through the antagonism of

redox-active transition metals like copper (Cu) and iron (Fe) and the protection of protein sulfhydryl groups from oxidative damage[10,11].

SUBJECTS AND METHODS

The present study was carried out in the departments of Tropical medicine (outpatient clinic) and Biochemistry, Pathology, Faculty of Medicine, Zagazig University Hospitals during the period from December 2009 to June 2010.

This study included 75 patients with chronic hepatitis C. They were 48 (64%) males and 27 (36%) females. Their age ranged from 22 to 58 years with a mean age ± SD of 40.5 ± 6.5 years,in addition to 25healthy individualswho matched patient groups for age and sex as a control group.

Forty-four patients were on interferon therapy for a duration ranging from 1month to 12 months with a mean duration ± SD of 6.2 ±0.9and 31patients not on interferon therapy.

An informed written consent was signed from each subject prior to participation in this work.

All patientsand controls were subjected to:

1- Full medical history and complete physical examination.

2- Laboratory investigation including; urine and stool analysis, complete blood count, Liver and kidney function tests.

3- Abdominal ultrasound; was done after an overnight fast using Aloka SSD-200 (a 3.5 MHz transducer).

4- Viral markers: Hepatitis hepatitis B surface antigen (HBs Ag) and HCV antibodies by enzyme linked immunosorbant assay and HCV RNA – PCR (All of the patients were positive for serum HCV RNA).

5- Estimation of plasma concentrations of Zn was performed using conventional atomic absorption spectrometry using a Z-6100 polarized Zeeman Atomic Absorption Spectrophotometer (HITACHI, Tokyo, Japan) within 48 h of collection of blood[12].Reference range **(**Adults 60-130 µg/dL) and detection limit 10 µg/dL.

6- Liver biopsy was done for patients only.

Exclusion criteria: All conditions that affect zinc homeostasis are excluded from the study examples:

- Advanced renal disease, kidney dialysis.
- Severe malnutrition, tube feeding, tracheostomy or chronic ventilator use.
- Heavy parasitic infestation.
- Neoplastic diseases.
- Decompensated liver cirrhosis.
- Chronic steroid treatment >10 mg/d, use of immunosuppressive drugs, use of antibiotics, diuretics within the prior 2 weeks.
-More than the Recommended Dietary Allowance (RDA) level supplements of vitamins E, C, B_6, selenium, zinc, β-carotene, or fish oil[13].
- Stop intake of supplements within 1 week of the study.

Recommended Dietary Allowances (RDAs) for Zinc[14]

Age	Male	Female	Pregnancy	Lactation
0–6 months	2 mg	2 mg		
7–12 months	3 mg	3 mg		
1–3 years	3 mg	3 mg		
4–8 years	5 mg	5 mg		
9–13 years	8 mg	8 mg		
14–18 years	11 mg	9 mg	12 mg	13 mg
19+ years	11 mg	8 mg	11 mg	12 mg

STATISTICAL ANALYSIS

Analysis of data was performed using SPSS windows (Version 12). All data were presented as the mean±SDand were compared using independent t test. Analysis of variance test (ANOVA) was used when comparing between more than two means, and the least significant difference (LSD) was calculated. Correlation coefficient (r) was calculated to measure correlation between variables.A P value> 0.05 was considered non-significant.

RESULTS

Table (1) shows a high significant difference of serum zinc level between cases and controls. Controls had a higher serum zinc level than cases.

There was an inverse correlation between serum zinc levels and age, while there was no significant correlation between duration of interferon therapy and serum zinc levels as shown in table (2).

Regarding gender, there was no significant difference between the mean value of serum zinc level in males and females, while statistical analysis indicated that there was a highly significant difference between serum Zn levels in smokers and nonsmokers as shown in table (3).

There was an inverse high correlation between serum zinc level and liver enzymes, bilirubin, prothrombin time (PT) and gamma- glutamyl transferase (GGT) parameters, while no significant correlation was found as regard other laboratory parameters as shown in table (4).

A high significant difference was found between the different grades of histopathological changes in liver biopsy findings as regard serum zinc level as shown in table (5).

There was no statistically significant difference of serum zinc levels between patients received interferon therapy and patients did not receive interferon therapyas shown in table (6).

Table (1) Comparison of mean value ± SD of serum zinc level (µg/dL) of cases and controls

	Serum zinc levels (µg/dL) mean± SD	*P value*
Cases (n=75)	46± 3.1	< 0.001**
Control (n=25)	67± 7.2	

**= Highly significant

Table (2) Correlation between serum zinc level (µg/dL) and age and duration of interferon therapy among HCV patients.

	R	*P value*
Age	-0.45	< 0.001 **
duration of interferon therapy	0.20	> 0.05

**= Highly significant

Table (3): The mean serum zinc levels (µg/dL) in relation to gender and smoking habit.

	Serum zinc levels (µg/dL) Mean± SD	*P value*
Gender		
Male(n=48)	44 ±7.1	>0.05
Female (n=27)	47 ± 6.0	
Smoking Habit		< 0.001**
Smokers (n=39)	43 ± 7.3	
Non-smokers (n=36)	51 ± 3.2	

Table (4) Correlation between serum zinc level (µg/dL) and different laboratory parameters.

	R	*P value*
Haemogloubin	0.20	>0.05
Platelets	0.12	>0.05
ALT	-0.39	< 0.001**
AST	-0.38	< 0.001**
Bilirubin	-0.70	< 0.001**
Albumin	0.20	>0.05
GGT	-0.38	< 0.001**
ALP	-0.09	> 0.05
PT	-0.70	< 0.001**
RBS	-0.11	> 0.05
Serum creatinine	-0.15	>0.05
PCR (quantitative)	-0.16	>0.05

ALT, alanine aminotranferease; AST,aspartate aminotransferase GGT, gamma-glutamyl transferase;ALP, alkaline phosphatase; PT, prothrombin time; RBS, random blood sugar; PCR, polymerase chain reaction

**= Highly significant

Table (5) Mean serum zinc level (µg/dL) and liver biopsy findings in patients.

	No	Mean±SD
1/6	21	51 ± 3.2
2/6	18	46 ± 1.2
3/6	15	41 ± 4.1
4/6	12	35 ± 4.0
5/6	9	31 ± 2.0

P < 0.001 = Highly significant

Table (6) Relation between serum zinc level (µg/dL) and interferon therapy among patients.

	No	Mean ± SD
Patients on interferon therapy	44	44±7.2
Patients not on interferon therapy	31	45±5.3

P >0.05 = Significant

DISCUSSION

Zinc is an essential trace element, exerting important antioxidant, anti-inflammatory, and antiapoptotic effects. The liver is important for the regulation of zinc homeostasis, while zinc is necessary for proper liver function. Zinc deficiency has been implicated in the pathogenesis of liver diseases[4,5].

In this study, a statistically significantly lower serum level of zinc was observed among cirrhotic patients with chronic HCV when compared with control group[15]. It can be explained by inadequate dietary intake and impaired intestinal mucosa with the development of liver disease, with resultant decreases in absorption of zinc through the mucosa. In addition to the reduced intrahepatic content of zinc secondary to decreases in the number of viable hepatocytes, which finally results in decreases in the serum zinc concentration[16].

There are an inverse correlation between serum zinc level and agebecause Egypt is considered one of the developing countries with low socioeconomic standards and diet contains few animal sources, in addition to the absence of programs of geriatric care, bad nutritional habits of elderly. There was a negative correlation between Zn concentrations and smoking habit. This may be due to deficient absorption of Zn caused by a tobacco chelation effect[17].

Although most of zinc that exists in blood bound to albumin, there was no correlation between serum zinc and albumin levels because all patients were compensated with normal serum albumin. On the other hand, the serum concentration of zinc inversely correlated significantly with liver enzymes, bilirubin, prothrombinPT and GGT. The previous parameters were increased with disease progression[18].

In this study, there was no correlation between the serum zinc concentration and the amount of HCV RNA. This suggests that the presence of HCV itself has no influence on the serum zinc concentration. This result was in agreement with Himotoet al[19]who found that administration of zinc in patients with hepatitis C virus-related chronic liver disease improved serum zinc level but did not affect HCV-RNA load.

Moreover, no significant correlation was found between duration of interferon therapy and serum zinc level. Also, no statistically significant difference was found between the serum zinc concentration of the patients on interferon therapygroup and that of the untreated group.However, these results need further studies to determine Zn level before, during and after treatment from every patient on interferon therapy as well as between responder and non responderto compare effect of interferon on serum zinc level.

A high significant increase was found among different histopathologicalgrades in liver biopsy as regard serum zinc level. This result denotes close relations between zinc and fibrosis of the liver as reported by Kojima-Yuasa et al.[9]. Zn deficiency induced by chronic liver disease enhances oxidative stress in the liver and, thus, the fibrotic process[11,20]. Zn deficiency could have a direct effect on HSCs activation that play a key role in liver fibrosis induction [9].

In conclusion, the serum concentration of zinc decreased significantly in chronic HCV and has been implicated in liver disease progression

REFERANCES

1- Medhat A, Shehata M, Magder LS, Mikhail N, Abdel-Baki L, Nafeh M, Abdel-Hamid M, Strickland GT, Fix ADHepatitis C in a community in Upper Egypt: Risk factors for infection. *Am. J. Tropical Med*, 2002; 66: 633-638.

2- el-Sadawy M, Ragab H, el-Toukhy H, el-MorAel-L, Mangoud AM, Eissa MH, Afefy AF, el-Shorbagy E, Ibrahem IA, Mahrous S, Abdel-Monem A, Sabee EI, Ismail A, Morsy TA, Etewa S, Nor Edin E, Mostafa Y, Abouel-Magd Y, Hassan

MI, Lakouz K, Abdel-Aziz K, el-Hady G, Saber M. Hepatitis C virus infection at Sharkia Governorate, Egypt: seroprevalence and associated risk factors. *J Egypt SocParasitol*,2004; 3:367-84.

3- Pinzani M, Rombouts K Liver fibrosis: from the bench to clinical targets. *Dig Liver Dis*, 2004;36:231–242.

4- Prasad AS, Beck FW, Bao B, Fitzgerald JT, Snell DC, Steinberg JD, Cardozo LJ. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am J ClinNutr. 2007; 85:837-44.

5- Stamoulis I, Kouraklis G, Theocharis S. Zinc and the liver: an active interaction. *Dig Dis Sci*, 2007; 52: 1595-612.

6- Takagi H, Nagamine T, Abe T, Takayama H, Sato K, Otsuka T, Kakizaki S, Hashimoto Y, Matsumoto T, Kojima A, Takezawa J, Suzuki K, Sato S, Mori M. Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. *J Viral Hepat*. 2001; 8:367-371.

7- Capocaccia L, Merli M, Piat C, Servi R, Zullo A, Riggio O. Zinc and other trace elements in liver cirrhosis.*Ital J Gastroenterol*, 1991; 23:386-91.

8- Takahara T, Furui K, Funaki J, Nakayama Y, Itoh H, Miyabayashi C, Sato H, Seiki M, Ooshima A, Watanabe A. Increased expression of matrix metalloproteinase-II in experimental liver fibrosis in rats. *Hepatology*, 1995; 21:787–795.

9- Kojima-Yuasa A, Ohkita T, Yukami K, Ichikawa H, Takami N, Nakatani T, Opare Kennedy D, Nishiguchi S, Matsui-Yuasa I. Involvement of intracellular glutathione in zinc deficiency-induced activation of hepatic stellate cells. *ChemBiol Interact*, 2003; 146:89–99.

10- Stehbens W. Oxidative stress, toxic hepatitis, and antioxidants with particular emphasis on zinc. *ExpMolPathol*,2003; 75:265–276

11- Prasad AS, Bao B, Beck FW, Kucuk O, Sarkar FH Antioxidant effect of zinc in humans. *Free RadicBiol Med*, 2004; 37:1182–1190.

12- Oka S, Ogino K, Matsuura S, Yoshimura S, Yamamoto K, Okazaki Y, Takemoto T, Kato N, Uda T. Human serum immuno-reactive copper, zinc-superoxide dismutase assayed with an enzyme monoclonal immunosorbent in patients with digestive cancer. *Clin. Chim. Acta.* 1989;182:209–219.

13- National Research Council (U.S.) *Recommended dietary allowances.* 10th ed. National Academy Press; Washington, D.C.: 1989. Subcommittee on the Tenth Edition of the RDAs. National Institutes of Health (U.S.), National Research Council (U.S.). Committee on Dietary Allowances

14- Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academy Press, 2001.

15- Kalkan A, Bulut V, Avci S, Celik I, Bingol NK. Trace elements in viral hepatitis, *J. Trace Elem. Med. Biol.* 2002; 16: 227–230.

16- Moriyama M, Matsumura H, Fukushima A, Ohkido K, Arakawa Y, Nirei K, Yamagami H, Kaneko M, Tanaka N, Arakawa Y. Clinical significance of evaluation of serum zinc concentrations in C-viral chronic liver disease. *Dig Dis Sci*. 2006; 51: 1967-1977.

17- Uz E, Semsettin S, Ibrahim FH, Var A, Sadik S, Omar. A The relationship between serum trace element changes and visual function in heavy smokers. *ActaOphthalmol Scand*. 2003; 81:161–164.

18- Goode HF, Kelleher J, Walker BE Relation between zinc status and hepatic functional reserve in patients with liver disease. *Gut*.1990; 31:694–697.

19- Himoto T, Hosomi N, Nakai S, Deguchi A, Kinekawa F, Matsuki M, Yachida M, Masaki T, Kurokochi K, Watanabe S, Senda S, Kuriyama S. Efficacy of zinc administration in patients with hepatitis C virus-related chronic liver disease. *Scand J Gastroenterol*. 2007; 42:1078-1087.

20- Klotz LO, KrönckeK.D, BuchczykDP,SiesH. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress, *J. Nutr.*2003;133:1448–1451