Biol Trace Elem Res
DOI 10.1007/s12011-017-0938-x

Serum Zinc Deficiency and its Relation to Liver Fibrosis in Chronic HCV: a Real-Life Egyptian Study

Dalia A. Omran[1] · Samar Kamal Darweesh[1] · Hanan Fouad[2,3] · Mohamed Mahmoud[1] · Sameh Saif[4] · Azza Fared[4] · Mohamed Hassany[4] · Lamiaa Mobarak[4] · Mahmoud A. El-Tahawy[5] · Ayman Yosry[1]

Received: 15 November 2016 / Accepted: 10 January 2017

Abstract Zinc is essential for the activation of approximately 300 metallo-enzymes. Serum and hepatic zinc is decreased in chronic liver disease patients, and zinc depletion has been suggested to accelerate liver fibrosis. The study was designed to assess Zinc status in chronic HCV Egyptian patients and its relationship to fibrosis stage diagnosed by FibroScan. This was a cross-sectional study on 297 Egyptian patients with naïve chronic HCV. All patients underwent laboratory tests (including assessment of serum Zinc) and liver stiffness measurement (LSM) by Transient Elastography (FibroScan®). The study included 170 (57.2%) females and 127 (42.8%) males with a mean age 52.4 ± 10.2 years. Most of the patients had zinc deficiency as the mean zinc level was 55.5 ± 30.7 μg/dl. The FibroScan scores showed that 97 patients had mild to moderate fibrosis (≤F2), while 200 patients had advanced to severe fibrosis (F2). Zinc level was significantly lower in patients with F2 than those with ≤F2 (52 ± 30.7 vs 62.5 ± 29.7, *p* value: 0.005), as the zinc values decreased with the progression of liver fibrosis. Serum zinc level had a negative significant correlation with INR and negative significant correlation with FibroScan score but no correlation to bilirubin, ALT, AST, or albumin. Most of Egyptian chronic liver disease patients had zinc deficiency. Zinc level gets significantly lower with progression of fibrosis. Zinc supplementation is essential before and during antiviral therapy for HCV.

Keywords Serum zinc deficiency · Liver fibrosis · Chronic HCV

Abbreviations

Zn	Zinc
Fe	Iron
Mn	Manganese
Cu	Copper
Th	T helper
STAT	Signal transducer and activator of transcription 3
LSM	Liver stiffness measurement
AUROC	Area under the receiver-operating characteristics
NAFLD	Non-alcoholic fatty liver disease
IFN	Interferon

Introduction

Abnormal metal metabolism, which is related to liver disease, has long been a subject of study, particularly storage diseases such as iron metabolism in hemochromatosis [1] and copper metabolism in Wilson's disease [2].

Zinc is an essential trace element in the human body, with approximately 2 g distributed throughout the body of a healthy adult, including many organs [3–5]. In vivo, this

✉ Samar Kamal Darweesh
samarkad@hotmail.com

1 Hepato-gastroenterology and Endemic Medicine Department, Cairo University, Cairo, Egypt

2 Medical Biochemistry Department, Faculty of Medicine, Cairo University, Cairo, Egypt

3 Pharmacology Department, Faculty of Pharmacy, Hail University, Hail, Kingdom of Saudi Arabia

4 National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo University, Cairo, Egypt

5 Internal Medicine and Hepatology department, National Liver Institute, Menoufya University, Menoufya Governorate, Egypt

Published online: 16 January 2017

element stimulates the activity of as many as 300 metalloenzymes and metal-activated enzymes, and is crucial for nucleic acids and protein metabolism. Thus, deficiency of zinc causes various pathologic disorders in the human body.

In 1951, Vikbladh pointed out that the zinc content in serum was low in patients with various liver diseases [6]. In chronic HCV patients, serum and hepatic zinc concentrations were decreased, and zinc depletion was suggested to be related to liver fibrosis [7, 8]. Cases with liver failure [9] or cases of HCC [10, 11] were reported to have conspicuous hypozincemia, and zinc supplementation therapy was reported to improve liver disease.

The main zinc metabolism occurs in the liver hepatocytes. In patients with Zn deficiency, reduced Zn concentrations in the liver are one of the causes of impaired hepatocyte regeneration [12]. It has been demonstrated that Zn may play an important role as a negative regulator of HCV replication in genome length RNA-replicating cells. Thus, zinc supplementations appear to offer a novel approach for further strategies in treatment of intractable chronic hepatitis C [13]. Zinc uses are claimed to suppress Th17-mediated autoimmune diseases at least in part by inhibiting the development of Th17 cells via attenuating signal transducer and activator of transcription 3 (STAT3) activation [14].

Study Outcomes

Therefore, this study was designed to evaluate Zinc status in chronic HCV Egyptian patients and its relationship to liver fibrosis stage diagnosed by FibroScan.

Patients and Methods

Study Population

This was a cross-sectional study on 297 Egyptian patients with naïve chronic HCV infection diagnosed by positive PCR and HCV antibodies by enzyme-linked immunosorbent assay (ELISA). They were selected from outpatients and inpatients of Hepato-gastroenterology and Endemic Medicine Department, Faculty of Medicine and National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo University.

Patients were excluded when they had (1) previous history of antiviral therapy; (2) presence or history of HCC; (3) malignancy other than HCC before the study, (4) co-infection with HBV; (5) FibroScan failure or invalid liver stiffness (LS) values with fewer than ten successful acquisitions, a success rate of less than 60%, or interquartile range (IQR)/median value ratio (IQR/M) greater than 0.3; (6) presence or history of alcohol ingestion; (7) having criteria suggestive of fatty liver: body mass index (BMI) > 35, uncontrolled DM (HbA1c > 7); (8) history of hepatotoxic drugs for the previous 6 months; (9) previous or current Zinc supplementation; and (10) right-sided heart failure.

This study was approved by the Department committee (institution review board) and the institution ethics committee. This study was performed in accordance with the ethics guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each participant after explanation of diagnostic procedures.

Study Protocol

After informed consent, all included patients underwent (1) laboratory tests for liver biochemical profile (ALT, AST, bilirubin, prothrombin time, and INR), CBC, serum AFP, assessment of HCV PCR, and antibodies; (2) assessment of serum Zinc level; (3) abdominal ultrasonography; and (4) liver stiffness measurement (LSM) by Transient elastography (FibroScan®). Based on FibroScan results, patients were divided into mild to moderate fibrosis (≤F2, ≤7.3 kPa) and marked to advanced fibrosis (cirrhosis) (>F2, >7.3 kPa).

Liver FibroScan

Liver FibroScan® (Echosens, France) was performed by a single well-trained operator blinded to patients' clinical and laboratory data on the same day of the laboratory studies, including serum zinc test. Details of the technique and examination procedure have been published previously [15, 16].

The results were expressed in kilopascals (kPa). IQR was defined as an index of intrinsic variability of LS values corresponding to the interval of the LS results containing 50% of the valid measurements between the 25th and 75th percentiles.

As suggested by the manufacturer, ten successful acquisitions were performed on each patient. The median value was considered a representative of the elastic modulus of the liver. Only procedures with at least 10 valid measurements, a success rate of at least 60%, and an interquartile range (IQR/M) < 30% were considered reliable. FibroScan failure is defined when less than 10 valid measurements are obtained.

Assessment of Serum Zinc

Venous blood samples (5 mL) obtained (after overnight fasting) were allowed to clot, and sera were then separated by centrifugation (3500 rpm, 20 min, 25 °C), and then stored at −20 °C until used. Determination of zinc level was done by zinc colorimetric method; the assay was conducted according to the manufacturer specifications (kit supplied from Química Clínica Aplicada S. A, Ref. 99 28 14), references values: Serum/Plasma: 60–110 μg/dl.

Statistical Analyses

Statistical analyses were performed using SPSS software. Numerical data were presented as means ± standard deviation (SD), while categorical data were presented as number (percent). The Mann-Whitney *U* test and the chi-square test are used when appropriate. Statistical significance is considered if *p* value is less than or equal to 0.05. The performance of serum zinc was assessed by measuring the area under the receiver-operating characteristics (AUROC). Diagnostic accuracy was also evaluated by comparing the sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively).

Results

Basic Characteristics

Based on the exclusion criteria, a total of 297 patients (mean age 52.4 ± 10.2 years, 170 females, 57.2%) were included. The patients' characteristics were summarized in (Table 1). All patients had adequate liver functions with AFP values less than 150 μg/dl.

Most of the patients had zinc deficiency as the mean zinc level was 55.5 ± 30.7 μg/dl (Table 1).

The FibroScan scores showed that 97 patients had a mild to moderate fibrosis stage (≤F2, ≤7.3 kPa), while 200 patients had an advanced to severe fibrosis stage (F2, 7.3 kPa).

Table 1 Demographic and laboratory features of included patients

Characteristic	HCV patients *n* = 297 (mean ± SD)
Age, years	52.4 ± 10.2
Male	127 (42.8%)
Female	170 (57.2%)
BMI	30.3 ± 11.9
ALT (IU/ml)	62.9 ± 39.2
AST (IU/ml)	63.4 ± 34.4
Albumin (g/dl)	3.8 ± 0.5
T. Bil (mg/dl)	0.8 ± 0.3
INR	1.09 ± 0.1
AFP (μg/dl)	9 (1 – 121)
Hb (g/dl)	13.5 ± 5.7
WBC (cell/ml)	5.3 ± 2
Platelet count (cell/ml)	$169.4 \times 10^5 \pm 70.7 \times 10^5$
Viral load (IU/ml)	3.6×10^5
Median (range)	$(27 - 6 \times 10^8)$
Serum zinc μg/dl	55.5 ± 30.7

Correlation Between Variable Factors and Fibrosis Stage (by FibroScan):

From the different variables, age significantly increased, while serum albumin and platelet count significantly decreased in relation to fibrosis stage by FibroScan. While other parameters did not show significant difference between fibrosis stage F2 and stage ≤F2 Table 2.

Serum Zinc in Relation to Fibrosis Stage (by FibroScan) and Other Factors:

Serum zinc level had significant negative correlations with INR and FibroScan scores but no correlation to other lab values (Table 3), (Fig. 1).

The serum zinc level differed significantly between patients with fibrosis stage F2 and those with fibrosis stage ≤F2 (*p* value: 0.005), as the zinc values progressively decreased with the progression of liver fibrosis (Table 4).

In relation to factors associated with hepatopathy, serum zinc values did not show a significant difference between females and males or patients with low or high HCV viral load (Table 4).

Discussion

Zinc is required for the activation of approximately 300 different metallo-enzymes and metal-activated enzymes, and is therefore considered to be one of the most important trace elements [17, 18]. Deficiency of Zinc reduces various important vital functions, including protein synthesis, immunological reactions, skeletal growth and maturation, gonadal

Table 2 Relation between variable factors and fibrosis stage (by FibroScan)

Item	F2 (*n* = 200)	≤F2 (*n* = 97)	*p* value
Age	53.9 ± 9.4	50 ± 9.7	0.01[a]
BMI	31.6 ± 13.2	26.3 ± 4.2	0.06
PCR	3×10^5 $(27 - 1 \times 10^7)$	4.1×10^5 $(200 - 6 \times 10^8)$	0.3
BIL	0.9 ± 0.3	0.8 ± 0.4	0.8
AST	64.5 ± 38.8	61.8 ± 34.3	0.6
ALT	61.3 ± 38.9	61.8 ± 41.1	0.5
ALB	3.8 ± 0.5	4 ± 0.5	0.005[a]
INR	1.1 ± 0.1	1.07 ± 0.1	0.04[a]
WBC	5.3 ± 1.8	5.9 ± 2.3	0.4
HB	13.3 ± 5.1	14.3 ± 10.9	0.4
PLT	161.4 ± 68	200 ± 72	0.001[a]
AFP	9 (1 – 121)	8.5 (1 – 82)	0.2

[a] *p* value ≤ 0.05 is significant; data are expressed as mean; and SD, median (range)

Table 3 Correlation between serum zinc and laboratory findings in the studied patients

	Correlation	*p* value
Bil	0.09	0.1
AST	0.03	0.6
ALT	0.01	0.8
ALB	−0.04	0.4
INR	−0.2	0.001[a]
Hb	0.03	0.5
WBC	−0.02	0.6
PLT	0.006	0.9
AFP	0.05	0.3
HCV PCR	0.07	0.2
Fibroscan score	−0.2	0.001[a]

[a] p value ≤ 0.05 is significant

development, pregnancy, and appetite [19]. The serum and hepatic zinc concentrations are decreased in patients with chronic liver diseases, and zinc depletion has been suggested to accelerate the liver fibrosis process [20].

Interestingly, supplementation with zinc has been shown to improve the prognosis of cirrhotic patients, as well as the cirrhosis-related symptoms [17–20]. In patients receiving oral zinc supplementation, maintenance of serum zinc concentration at more than 80 μg/dl was the most important factor associated with cancer-free survival [20].

Recently, Zhu et al. [21] indicated that 12 gene groups may be related to both HCV and trace element metabolic processes, further confirming the associations between HCV and trace elements. All of the newly found 12 gene groups have been confirmed to be definitely associated with HCV infection and trace elements metabolism and may reveal the potential roles of trace elements in the biological infectious process of such a virus.

In Zhu et al. [21] study, More than 30 genes have been predicted to be associated with both zinc metabolism and HCV infection. Among them, HLA-C encodes a specific major histocompatibility complex that contributes to specific interferon (IFN)-α associated pathways and mediates the cytotoxic effect against infected cells in liver tissues. Considering that HLA-C contributes to HCV infection partially via specific IFN-α-associated pathways, while zinc has also been reported to be associated with the immune response of HLA-C associated NK cells against HCV infection, therefore, the underlying mechanism of zinc-mediated immune responses against HCV may partially depend on the HLA-C-mediated identification processes, validating the accuracy of prediction method.

In our study, most of the Egyptian patients had zinc deficiency as the mean zinc level was 55.5 ± 30.7. Mohammed et al. [22] studied trace elements as (Zn, Fe, Mn, and Cu) in 42 Egyptian patients with chronic hepatitis C. There results showed that the levels of Zinc and Manganese in HCV-infected patients were decreased compared to the healthy group, and this decrement became more after Peg/Riba treatment. As in the control group, the Zinc level was 83.6 ± 1.96, whereas Zinc values were 71.1 ± 3.38 and 50.5 ± 3.43 before and after Peg/Riba treatment in HCV group, respectively. The explanation of the decrease in serum zinc after Peg/Riba treatment is not clear.

This was in accordance with the result of Qasim et al. [23] who compared serum zinc and copper concentrations in 100 chronic hepatitis C patients with 50 healthy controls, they found that serum copper concentration was higher in HCV patients as compared to controls, while zinc concentrations were significantly lower in HCV patients than controls. Marchesini [24] found that Zinc deficiency is common in patients with advanced cirrhosis when there is a liver damage.

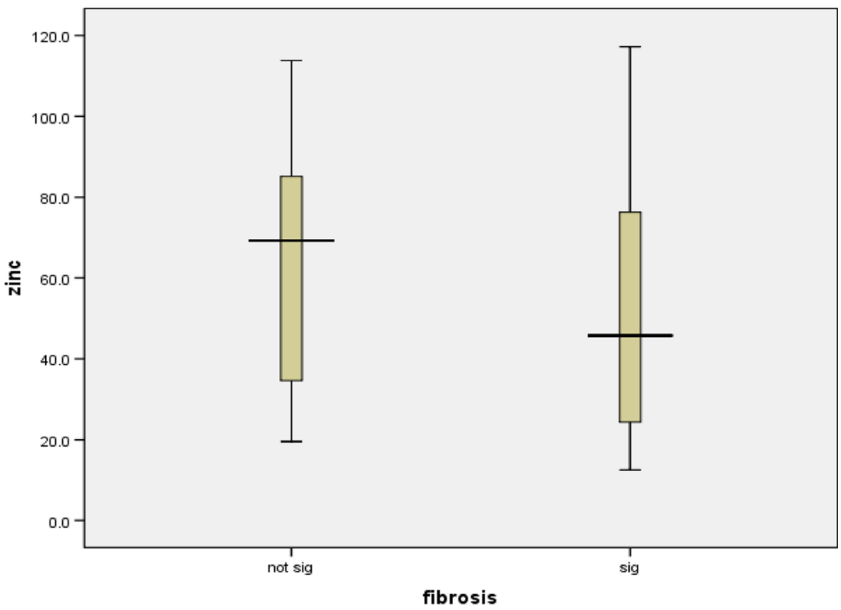

Fig. 1 Relation between zinc level and degree of fibrosis by FibroScan

Table 4 Relation between serum zinc and gender, HCV load, and fibrosis score

	Serum zinc	*p* value
Male	53.6 ± 30.8	0.4
Female	56.9 ± 30.7	
HCV RNA PCR		
Low PCR (<1 × 10^6)	54.9 ± 30.4	0.6
High PCR (>1 × 10^6)	57 ± 31.8	
FibroScan score		
F2	52 ± 30.7	0.005[a]
≤ F2	62.5 ± 29.7	

[a] p value ≤ 0.05 is significant

Similarly, Pramoolsinsap et al. [25] found that serum Zn levels decreased significantly in patients with chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. Kalkan et al. [26] and Czuczejko et al. [27] also reported that there was a decrease in the levels of Zn and Cu and an increase in the levels of Fe and Mn in sera of hepatitis cases.

However, Cesur et al. [28] compared serum zinc and copper concentrations in chronic HCV patients (n = 17) and healthy controls (n = 17), they did not find any significant difference between healthy individuals and patients with chronic hepatitis C. This could be related to the small number included in each group.

The zinc values, in our study, decreased with the progression of liver fibrosis. Zinc level was significantly lower in patients with fibrosis stage F2 than those with fibrosis stage ≤F2. Laguercia et al. [29] found that as the disease progresses from chronic hepatitis to liver cirrhosis, serum Zn, magnesium, phosphorus, and calcium concentrations decrease while the Cu concentration increases.

More recently, Kang et al. [30] stated that Zinc with a concentration of 200 μM could significantly inhibit the proliferation and the activity of hepatic stellate cell line, LX-2 which is responsible for hepatic collagen synthesis and subsequently liver fibrosis. Zinc was found to inhibit the TGFβ profibrotic signaling pathway as well as the Zinc increased expression of MMP-13 in LX-2 cells. Subsequently, Zn significantly inhibited the expression of αSMA and type I collagen in LX-2 cells.

Iwata et al. [31] evaluated the association of zinc level with the severity of liver fibrosis and esophageal varices. The mean zinc values decreased with the progression of fibrosis (METAVIR score: F0–1: 71.3 ± 11.3, F2: 68.9 ± 11.7, F3: 66.3 ± 11.8, F4: 63.9 ± 15.0). Also, zinc level was significantly lower in patients with varices than in those without varices (59.3 ± 13.6 vs 66.3 ± 12.6, $p < 0.05$). Moreover, zinc level was significantly lower in the patients with a high risk of bleeding than in those with a low risk (55.6 ± 13.0 vs 64.6 ± 13.1, $p < 0.01$).

These findings suggest that zinc level is not only an indicator of an abnormal metal metabolism, but is also a simple parameter associated with hepatitis virus-related various conditions, including the degree of liver fibrosis.

Similarly, in Anber et al. [32] study, there was a progressive decrease of zinc level with progression of HCV disease, as the zinc levels were (103.8 ± 9.7) in early cirrhosis, (94.0 ± 12.7) in late cirrhosis, and (94.0 ± 12.7) in HCC; these values were significantly different ($p < 0.001$) when compared to controls (122.4 ± 7.1).

Guo et al. [33] studied zinc levels in hepatitis C patients without non-alcoholic fatty liver disease (NAFLD) (HCV group, n = 30) and with NAFLD (HCV-NAFLD group, n = 32), and a group of 30 healthy, non-obese, non-diabetic participants (CNL group). Plasma concentrations of Zn were lower in HCV patients than controls. Moreover, Zn levels were the lowest in the HCV-NAFLD group than the HCV group.

This can be explained by the fact that Zn is a structural component of peroxisome proliferator-activated receptors, and Zn deficiency can thus interfere with lipid metabolism [34]. NAFLD patients have been shown to have a lower dietary intake of Zn, suggesting that inadequate Zn intake is associated with the development and progression of NAFLD [35]. Excessive lipid accumulation in the liver is associated with the formation of ROS and consequently increased expression of Zn-finger protein ZNF267 mRNA [36]. Upregulation of Zn importing proteins by pro-inflammatory cytokines and oxidative stress reduces plasma Zn concentrations [37].

Lin et al. [38] found that Cu/Zn ratio might serve as a biomarker for the increased severity of viral hepatic damage. Compared with copper, the decrease of serum zinc is more closely related with the development of disease from the view of the ratio of copper to zinc (the normal value is 1). The ratio increased gradually in chronic hepatitis infection.

Moreover, it appears that the nutritional status of zinc influences the effect of IFN in hepatitis C patients. This fact is supported by the study of Nagamine et al. [39] who observed that basal zinc levels are significantly lower in chronic HCV patients and administration of IFN-a to hepatitis C patients augments serum zinc reductions up to 40% in 8 h. Serum zinc level and zinc/copper ratio were significantly higher in complete responders than in non-responders to IFN therapy at each time point (before and after treatment).

Therefore, Nagamine advised Zn administration as an adjunct to interferon therapy in chronic HCV infections, they proposed that Zn-increased antiviral effect and in turns response to interferon therapy, thus, zinc has an important role in pharmacotherapy of viral hepatitis.

Similarly, Mohammed et al. [22] found that serum level of Zn was significantly higher among responders compared to the non-responder patients (56.4 ± 20.6 vs 37.5 ± 12.5, $p < 0.05$).

Ko et al. [40] also reported that serum zinc levels were significantly lower in chronic hepatitis C patients than in healthy controls and further depressed by IFN/ribavirin treatment.

In our study, serum zinc level had a positive significant relation with INR and a negative significant relation with FibroScan score but significance was not found in relation to bilirubin, ALT, AST, or albumin. Anber et al. [32] also found that there were insignificant correlations between ALT, AST, bilirubin, albumin, and serum zinc level.

Also, Mohammed et al. [22] reported no correlations between ALT and each of Zn, Fe, Cu, Cu/Zn, Mn, and Zn/Mn before treatment or after treatment, (r = 0.12, 0.11, −0.05, 0.19, 0.03, 0.11, respectively, $p > 0.05$).

The FibroScan examination for assessment of liver fibrosis is a valid and reliable method for the diagnosis and staging of liver fibrosis—this was proven in comparison to liver biopsy by many studies [41]. However, we recommend that our conclusions should be further confirmed in vivo by studying the liver fibrosis in mice models in the laboratory.

In conclusion, most of chronic liver disease patients had a significant zinc deficiency. Zinc level gets significantly lower with progression of fibrosis.

Our study raises the concerns of the potential importance of Zn as an adjunct antifibrotic therapy and novel strategy in chronic HCV treatment; it may be worthwhile exploring the benefit of zinc supplementation even with the advent of novel direct antiviral agents.

Compliance with Ethical Standards

Limitations of the Study None reported.

Ethics Approval and Consent to Participate This study was approved by the Department committee (institution review board) and the institution ethics committee. This study was performed in accordance with the ethics guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each participant after explanation of diagnostic procedures.

Consent for Publication Not applicable.

Availability of Data and Material The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing Interests The authors declare that they have no competing interests.

Funding This work was supported by the Science and Technology Development Fund (STDF), Egypt, that funded this research work; Grant No. 5380, Basic and applied research.

References

1. Granick S (1949) Iron metabolism and hemochromatosis. Bull N Y Acad Med 25:403–428
2. Evans GW, Bubois RS, Hambidge KM (1973) Wilson's disease: identification of an abnormal copper-binding protein. Science 181: 1175–1176
3. Fredricks RE, Tanaka KR, Valentine WN (1960) Zinc in human blood cells: normal values and abnormalities associated with liver disease. J Clin Invest 39:1651–1656
4. Herring WB, Leavell BS, Paixo LM, Yoe JH (1960) Trace metals in human plasma and red blood cells. A study of magnesium, chromium, nickel, copper and zinc. I. Observations of normal subjects. Am J Clin Nutr 8:846–854
5. Herring WB, Leavell BS, Paixao LM, Yoe JH (1960) Trace metals in human plasma and red blood cells. A study of magnesium, chromium, nickel, copper and zinc. II. Observations of patients with some hematologic diseases. Am J Clin Nutr 8:855–863
6. Vikbladh I (1951) Studies on zinc in blood II. Scand J Clin Lab Invest 3(Suppl 2):1–74
7. Bode JC, Hanisch P, Henning H, Koenig W, Richter FW, Bode C (1988) Hepatic zinc content in patients with various stages of alcoholic liver disease and in patients with chronic active and chronic persistent hepatitis. Hepatology 8:1605–1609
8. Milman N, Laursen J, Pødenphant J, Asnaes S (1986) Trace elements in normal and cirrhotic human liver tissue. I. Iron, copper, zinc, selenium, manganese, titanium and lead measured by X-ray fluorescence spectrometry. Liver 6:111–117
9. Nandi SS, Chawla YK, Nath R, Dilawari JB (1989) Serum and urinary zinc in fulminant hepatic failure. J Gastroenterol Hepatol 4:209–213
10. Ebara M, Fukuda H, Hatano R, Yoshikawa M, Sugiura N, Saisho H, Kondo F, Yukawa M (2003) Metal contents in the liver of patients with chronic liver disease caused by hepatitis C virus. Reference to hepatocellular carcinoma Oncology 65:323–330
11. Ebara M, Fukuda H, Saisho H (2003) The copper/zinc ratio in patients with hepatocellular carcinoma. J Gastroenterol 38:104–105
12. Cousins RJ (1985) Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 65(2):238–309 View at Scopus
13. Yuasa K, Naganuma A, Sato K et al (2006) Zinc is a negative regulator of hepatitis C virus RNA replication. Liver Int 26(9): 1111–1118 Publisher
14. Nishihara M, Ogura H, Ueda N et al (2007) IL-6-gp130-STAT3 in T cells directs the development of IL-17+ T_h with a minimum effect on that of Treg in the steady state. Int Immunol 19(6):695–702 Publisher
15. Kim SU, Ahn SH, Park JY, Kang W, Kim DY et al (2008) Liver stiffness measurement in combination with noninvasive markers for the improved diagnosis of B-viral liver cirrhosis. J Clin Gastroenterol 43:267–271
16. Castera L, Forns X, Alberti A (2008) Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 48:835–847
17. Matsuoka S, Matsumura H, Nakamura H et al (2009) Zinc supplementation improves the outcome of chronic hepatitis C and liver cirrhosis. J Clin Biochem Nutr 45:292–303
18. Himoto T, Hosomi N, Nakai S et al (2007) Efficacy of zinc administration in patients with hepatitis C virus-related chronic liver disease. Scand J Gastroenterol 42:1078–1087
19. Takahashi M, Saito H, Higashimoto M, Hibi T (2007) Possible inhibitory effect of oral zinc supplementation on hepatic fibrosis

through down-regulation of TIMP-1: a pilot study. Hepatol Res 37: 405–409
20. Katayama K, Sakakibara M, Imanaka K et al (2011) Effect of zinc supplementation in patients with type C liver cirrhosis. Open J Gastroenterol 1:28–34
21. Zhu L, Chen X, Kong X, Cai YD (2016) Investigation of the roles of trace elements during hepatitis C virus infection using protein-protein interactions and a shortest path algorithm. Biochim Biophys Acta. doi:10.1016/j.bbagen.2016.05.018
22. Mohammed MA, Abd Elmaksoud MD, Abd El Maksoud N, El-Mezayen HA, El-Garaam H (2012) Serum levels of trace elements in Egyptian patients with chronic hepatitis C under interferon therapy. Journal of Genetic Engineering and Biotechnology 10 (1:81–86. doi:10.1016/ j.jgeb.2012.03.001
23. Qasim R, Saniullah S, Muzaffar AS, Abdul Aziz M, Sohail A (2010) Serum copper and zinc concentration in patients with chronic hepatitis C. Medicine 16(1):27–30
24. Marchesini G, Fabbri A, Bianchi G, Brizi M, Zoli M (1996) Zinc supplementation and amino acid-nitrogen metabolism in patients with advanced cirrhosis. Hepatology 23(5):1084–1092. doi:10.1053/jhep.1996.v23.pm0008621138
25. Pramoolsinsap C, Promvanit N, Komindr S, Lerdverasirikul P, Srianujata S (1994) Serum trace metals in chronic viral hepatitis and hepatocellular carcinoma. Gastroenterology 29:610–615
26. Kalkan A, Bulut V, Avci S, Celik I, Bingol NK (2002) Trace elements in viral hepatitis. J Trace Elem Med Biol 16(4):227–230. doi:10.1016/S0946-672X(02)80049-9
27. Czuczejko J, Zachara BA, Staubach-Topczewska E, Halota W, Kedziora J (2003) Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. Acta Biochim Pol 50(4):1147–1154
28. Cesur S, Cebeci SA, Kavas GO, Yilmaz N, Buyukkagnici DI (2005) Serum copper and zinc concentrations in patients with chronic hepatitis C. J Infect 51(1):35–37. doi:10.1016/j.jinf.2004.08.003
29. Laguercia C, Del Girolamo V, Feng FL, Catalbi V, Del Vecchio BC et al (1997) Trace elements and chronic liver disease. J Trace Elem Med Biol 11:158–161
30. Kang M, Zhao L, Ren M, Deng M, Li C (2015) Zinc mediated hepatic stellate cell collagen synthesis reduction through TGF-β signaling pathway inhibition. Int J Clin Exp Med 8(11):20463–20471
31. Iwata K, Enomoto H, Nishiguchi S, Aizawa N, Sakai Y, Iwata Y, Tanaka H, Ikeda N, Takashima T, Saito M, Imanishi H, Iijima H, Tsuda Y, Higuchi K (2014) Serum zinc value in patients with hepatitis virus-related chronic liver disease: association with the histological degree of liver fibrosis and with the severity of varices in compensated cirrhosis. J Clin Biochem Nutr 55(2):147–152
32. Anber NH, EL-Ghannam MZ, El-Kheshen GA, Bialy MI (2016) Evaluation of serum zinc level in Egyptian patients with hepatitis C-associated cirrhosis. J Pharm Biomed Sci 06(02):81–85
33. Guo CH, Chen PC, Ko WS (2013) Status of essential trace minerals and oxidative stress in viral hepatitis C patients with nonalcoholic fatty liver disease. Int J Med Sci 10(6):730–737. doi:10.7150/ijms.6104
34. Shen H, MacDonald R, Bruemmer D, Stromberg A, Daugherty A, Li XA, Toborek M, Hennig B (2007) Zinc deficiency alters lipid metabolism in LDL receptor-deficient mice treated with rosiglitazone. J Nutr 137:2339–2345
35. Toshimitsu K, Matsuura B, Ohkubo I, Niiya T, Furukawa S, Hiasa Y et al (2007) Dietary habits and nutrient intake in non-alcoholic steatohepatitis. Nutrition 23:46–52
36. Schnabl B, Czech B, Valletta D, Weiss TS, Kirovski G, Hellerbrand C (2011) Increased expression of zinc finger protein 267 in non-alcoholic fatty liver disease. Int J Clin Exp Pathol 4:661–666
37. Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, Knutson MD, Ganz T, Cousins RJ (2005) Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc Natl Acad Sci U S A 102:6843–6848
38. Lin CC, Huang JF, Tsai LY, Huang YL (2006) Selenium, iron, copper, and zinc levels and copper-to-zinc ratios in serum of patients at different stages of viral hepatic diseases. Biol Trace Elem Res 109(1):15–24. doi:10.1385/BTER:109:1:015
39. Nagamine T, Takagi H, Hashimoto Y, Takayama H, Shimoda R, Nomura N, Suzuki K, Mori M, Nakajima K (1997) The possible role of zinc and metallothionein in the liver on the therapeutic effect of IFN-alpha to hepatitis C patients. Biol Trace Elem Res 58:65–76
40. Ko WS, Guo CH, Hsu GSW, Chiou YL, Yeh MS, Yaun SR (2005) The effect of zinc supplementation on the treatment of chronic hepatitis C patients with interferon and ribavirin. Clin Biochem 38(7):614–620. doi:10.1016/ j.clinbiochem. 2005. 04.003
41. Bonnard P, Elsharkawy A, Zalata K, Delarocque-Astagneau E, Biard L, Le Fouler L, Hassan AB, Abdel-Hamid M, El-Daly M, Gamal ME, El Kassas M, Bedossa P, Carrat F, Fontanet A, Esmat G (2015) Comparison of liver biopsy and noninvasive techniques for liver fibrosis assessment in patients infected with HCV-genotype 4 in Egypt. J Viral Hepat 22(3):245–253. doi:10.1111/jvh.12285