Original Article

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The Relationship between Serum Zinc Level and Liver Elastrography Using Fibroscan in Non-Alcoholic Fatty Liver Patients

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ABSTRACT

BACKGROUND AND OBJECTIVE: Zinc is one of the essential elements for many vital functions of the body, including regulation of gene expression, anti-inflammatory and antioxidant activity, protein and nucleic acids metabolism. Since, many people develop advanced liver disease, including liver cirrhosis, they have zinc deficiency. The aim of this study was to investigate the relationship between liver stiffness and serum zinc levels in patients with non-alcoholic fatty liver disease.

METHODS: This cross-sectional study was performed on 40 non-alcoholic fatty liver disease Patients referring to Imam Khomeini Hospital in Ahvaz in 1395. Serum zinc levels and liver Stiffness (based on METAVIR scale) were measured by fibroscan, and Age, sex, serum zinc level and liver fibroscan were recorded. Serum zinc level at different ages and scores of Liver stiffness was compared and analyzed.

FINDINGS: This study was conducted on 40 patients with non-alcoholic fatty liver disease. The number of men was 26 patients (65%). Mean age of patients was 45.077±9.4 years, mean serum zinc level was 81.4±8.1 and mean liver stiffness was 6.5±2.1 kPa. Serum zinc level had a reverse and strong correlation with liver stiffness (p=0.0001, r=0.9). Also, with age increasing, liver stiffness increased (p=0.01, r=0.5) and serum zinc level decreased (p=0.01, r=0.5).

CONCLUSION: According to findings of the present study, the serum zinc level significantly decreased with fibrosis progression in patients with non-alcoholic fatty liver.

KEY WORDS: *Non-Alcoholic Fatty Liver, Zinc, Elasticity Imaging Techniques.*

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Introduction

Zink is one of the vital elements of the body, which is required for the activity of more than 300 enzymes, the body of a healthy person has about 2 grams Zink (1). Recommended dietary intake is 11 mg per day for men and 8 mg per day for women. Getting low levels of zinc from the diet reduces its serum levels. But there are mechanisms that increase absorption and reduce body excretion, which will lead to modulation and adjustment of serum levels of zinc. Among the vital functions of the body in which zinc is involved, the regulation of gene expression, anti-inflammatory and antioxidant activity, the metabolism of protein and nucleic acids can be mentioned. The metabolism of zinc is mainly done in the liver (2).

Zinc deficiency can lead to a wide range of disorders and diseases, including impairment of hepatic cell regeneration (3). In progressive fatty liver disease and cirrhosis, Zinc adsorption is disturbed and and its excretion increases in the urine (4). Zinc in the body is usually reported as serum and plasma levels. The normal serum level of zinc in an adult and healthy person is 110-70 µg/ml, and levels below 70 mg/ml are considered as deficiencies (5).

Fatty liver is one of the most important causes of mortality associated with liver disease. Alcohol consumption, fatty foods, overweight, lack of mobility, malnutrition, hypertension, pregnancy, and the use of drugs such as aspirin and tetracycline are the mainstay of fatty liver disease. The progression of disease and liver cell damage can lead to complications such as progressive liver fibrosis, cirrhosis, liver cancer, and an increased risk of cardiovascular disease (6). Studies show that out of every 10 Iranians, three persons have fatty liver, and the prevalence of this disease in Iranian children is increasing (7).

The diagnostic method of vibration-controlled transient elastography in patients with fatty liver is a tolerable, reliable and low-cost method for liver biopsy to evaluate liver rigidity. In this method, the 50-Hz elastic waveguide detector is transmitted to the liver and the velocity of the waves is measured by a transducer. The measured speed will also be converted to liver stiffness measurement by Hook's law. The higher measured values indicate the higher risk of advanced liver disease.

The 10.3 kPa with AUROC (Area under receiver operating characteristic) 0.95 is considered for cirrhosis (8). In a study in Egypt on 297 individuals with chronic hepatitis C, serum levels of serum zinc had a reverse and significant correlation with progression of liver fibrosis (1). In another study in Spain, in which 74 patients with chronic hepatitis C were diagnosed, there was a significant relationship between low serum zinc levels and hepatic fibrosis in advanced stages (9).

The results of some recent studies suggest that zinc deficiency has been observed in patients with cirrhosis. The cause of zinc deficiency can be due to diuretic use, inadequate protein intake, disorder of zink intestinal absorption, hypoalbuminemia, increased urinary excretion (11, 10).

Regarding the prevalence of zinc deficiency and non-alcoholic fatty liver disease in Iran and the lack of a study on the relationship between serum zinc level and severity of liver fibrosis, the present study was aimed to investigate the effect of fibrosis due to non-alcoholic fatty liver disease on serum zinc levels and its association with an increase in the degree and severity of hepatic fibrosis in patients.

Methods

This cross-sectional study after obtaining permission from the ethics committee of Ahvaz University of medical sciences with registration code 328.1396IR.AJUMS.REC was performed on 40 patients with non-alcoholic fatty liver referring to specialized clinic or gastrointestinal department of Imam Khomeini Hospital, Ahwaz, after obtaining history and clinical examination by gastroenterologist. Diagnosis and confirmation of non-alcoholic fatty liver disease was done with liver ultrasonography (with 100% sensitivity) and increased alanine aminotransferase and aspartate aminotransferase enzymes.

Patients were evaluated for other liver diseases including viral hepatitis, autoimmune, drug, hemokromatosis, Wilson and all bile duct disorders. Patients aged 18 years and over, 33% involvment of liver tissue, ultrasound, serum alanine aminotransferase and aspartate aminotransferase were taken at intervals of 3 to 6 months more than 30% in women and 45% in men were entered in the study. Individuals under the age of 18 years old, zinc intake for the past 6 months, taking over-the-counter medications including cefalexin, tetracycline and its derivatives, quinolone antibiotics, ceftibuten, deferiprone, alcohol consumption of more than 20 g per day in women, and more than 30 grams in men for at least 3 consecutive

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months, patients with autoimmune hepatitis, hepatitis B, hepatitis C or other chronic liver disease, heart failure patients, patients who recieved steatohepatitis-inducing drugs such as tamoxifen, Nonsteroidal anti-inflammatory drugs, amiodarone, statins and fibrates, diabetes patients and lack of consent to participate in the study were excluded. The considered variables included age, serum zinc level and insertion of elastography. Ellography was performed with an assessment of serum zinc level in one day.

The used fibroscan device was the EcoSens 500 made in France (Echosens, France), which reported the degree of stiffness of the liver based on kilopascal. Fibrosis classification system with the help of fibroscan according to the METAVIR (meta-analysis of histological data in viral hepatitis) scoring system is as follows:

F0-1 (1.7-1.4) 5.5, F2: (6.9-8.4) 6.6, F3: (9.12-6.7) 3.10, F4: (48-3.16) 8.30 kPa (12). According to previous studies, 13.9 kPa has a sensitivity and specificity of 100% for determining F3 and F4 (8). Samples of blood were collected at a rate of 5 ml in fasting state and after clotting, they were isolated by centrifugation and kept at a temperature below 20 °C. Determination of blood levels of zinc was done by colorimetric method.

The used kit was provided by Greiner company (Germany) and reference values were set at 100-70 µg/ml. Data were analyzed by SPSS software version 22 to describe qualitative data the frequency and percent of relative frequency and to describe quantitative variables, mean and standard deviation were used.

Correlation between quantitative variables was determined by regression test. To compare the median of mild and moderate groups with advanced group fibroscan scores, Boxplot was used and p <0.05 was considered significant.

Results

This study was performed on 40 patients with non-alcoholic fatty liver. The number of women in this study was 14 patients (35%) and the number of men was 26(65%). The mean age of the patients was 45.07±9.4 years and the minimum and maximum age was 31-66 years. The mean serum level of zinc was 81.4±8.1 and the minimum and maximum values were 81.2-109.2. There was no statistically significant difference between mean serum zinc level between male and female groups (94.5±7.8 and 96.1±8.8 µg/dl) .The mean of liver stiffness was 6.5±2.1 kPa and the minimum and maximum values were 11.5 to 11.6 kPa (Table 1). Patients were divided into two groups: mild to moderate (7.3≥), 26 (65%) and advanced (7.3<) 14 persons (35%). There was a significant relationship between age and serum zinc level (r=-0.5, p<0.0001). These data suggest that the serum level of zinc decreases with age. There was a statistically significant relationship between age and serum zinc level (p=0.001 and r=-0.552), as well as between age and elastography of the liver with the help of fibroscan (p=0.001, r=0.5).

Table 1. Mean variables of age, serum zinc level and liver stifness and patient category based on METAVIR scoring system

Variables	Mean±SD	
Age (year)	45.07±9.4	
Serum zinc level (µg/dL)	81.4±8.1	
The degree of liver stiffness (kpa)	6.5±2.1	
Serum zinc level in different degrees of liver stiffness based on METAVIR system (µg/dl)	F0-1(4.1-7.1)	95.9±4.1
	F2(4.8-9.6)	91.4±4.5
	F3(7.9-12.9)	82.7±1.3
	N(%)	
Patient category according to the degree of stiffness of the liver (kpa)	Mild to moderate(≤7.3)	26(65)
	Advanced (>7.3)	14(35)
Patient categorization according to METAVIR grading system	F0-1(4.1-7.1)	56.25
	F2(4.8-9.6)	25
	F3(7.6-12.9)	18.75

These findings suggest that the degree of elastography increases with age. The findings also showed that 56.25% of our patients had F0-F01, 25% F2 and 18.75% F3. None of the patients were F4. The mean serum zinc level decreased with increasing degree of fibrosis. There was a statistically significant difference between the two groups of F0-F1 (95.9±1.4 µg/dl) and F2 (91.4±4.5 µg/dl) in the mean serum zinc level (p=0.037) and the mean serum level of F2 was significantly (p=0.001) different compared to F3(82.7±1.03 µg/dl). Significant, strong and reverse correlation was observed in regression analysis between degree of elastography and serum zinc level (p<0.0001, r=-0.908). These findings indicate that the level of serum zinc decreases with increasing liver rigidity (Fig. 1).

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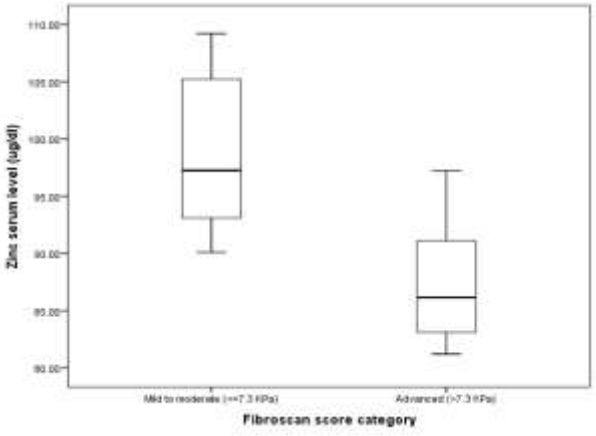


Figure 1. Relationship between serum zinc levels and the stiffness of the liver

Discussion

According to the findings of this study, in patients with non-fatty liver, serum zinc level significantly decreases with progression of fibrosis and age. In the study of Omran et al., the majority of patients had hepatitis C virus deficiency and had a mean of 55±30.7 µg/dl, and serum zinc levels had a significant negative correlation with hepatic fibrosis at severe degrees (>F2) compared with mild to moderate degrees (≤F2) (1). In our study, the mean serum level was 81.4±8.1 and negative, inverse and significant correlation was observed between serum level of serum and severity of liver rigidity in all grades.

The difference in the results of the mentioned study with our study was a larger sample size and, in parallel, increased sensitivity of the study, as well as differences in serum levels in patients, so that the patients had zinc deficiency in the study. In the study of Iwata et al., which was performed on patients with hepatitis, cirrhosis, there was a reverse relationship between the serum zinc level and the severity of liver fibrosis based on METAVIR score.

The results of this study showed that serum zinc level significantly decreases with increasing severity of hepatic fibrosis, varicose veins and patients with high risk of bleeding. According to the results of this study, serum levels of zinc are associated with the complications of hepatitis C as a parameter, including fibrosis degree (13). In the current study, the results of the study were similar to those of all phases of fibrosis, the mean serum levels of F0-F1 group in comparison with F2 and F2 compared to F3 were significantly different. In the study of Anber et al. with the progression of hepatitis C, serum zinc levels decreased in the early stages of cirrhosis (103.8±9.7 µg/dl) and in the final stage of cirrhosis (94.3±12.7 µg/dl) which has a significant difference in serum concentrations in healthy subjects (122.7±2.1 µg/dl) (14).

The results of this study were similar to those of our study in terms of the progression of fibrosis and the reduction of serum levels of zinc. However, in this study, control group was compared and serum zinc values were compared with the control group. In a study by Guo et al., the level of zinc in patients with hepatitis C without non-alcoholic fatty liver was compared with non-alcoholic fatty liver and the healthy group. Plasma concentrations of zinc in patients with hepatitis C were lower compared to controls. In addition, serum zinc levels in hepatitis C patients with non-alcoholic fatty liver were lower in patients with hepatitis C without non-alcoholic fatty liver. The results of this study showed that the distribution of mineral elements in non-alcoholic fatty liver disease, including zinc, is effective on the treatment of chronic hepatitis C (15).

In the study of Mikhail et al., the relationship between zinc deficiency and hepatic steatosis in the animal model of fatty liver by tetracycline has been shown. They conclude that lowering the synthesis of high-density lipoprotein (HDL) cholesterol leads to accelerated hepatic steatosis in animal models due to zinc deficiency (16). There are very limited studies about the association between the serum zinc level and the degree of fibrosis of the liver by the non-invasive fibroscan method in non-fatty liver and even hepatitis C patients. Zinc is essential for the activation of about 300 enzyme-metal complexes and metal-activated enzymes. Therefore, it is one of the most important elements of the body (17).

Serum and liver concentrations of zinc in chronic liver patients are reduced, and this reduction accelerates in the process of liver fibrosis (18). In explanation of the involved mechanisms, it can be explained that one of the structural components is PPAR-α (Peroxisome proliferation-activated receptor-α) and zinc deficiency interferes with lipid metabolism (19). Also, patients with nonalcoholic fatty liver have low levels of zinc intake, and zinc deficiency is associated with the progression of non-alcohol fatty liver (20). This element has a potential impact on the exacerbation of lipid peroxidation in animal models and in the elderly (22, 21).

The excessive accumulation of lipid in the liver is associated with the formation of reactive oxygen species (ROS) and increases the expression of Zn-

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finger ZNF-267 mRNA (23). Positive regulation of the importing proteins due to pro-inflammatory cytokines and oxidative stress reduces plasma concentrations of zinc (24). PPAR-α plays a regulating role in fat homeostasis, and on the other hand, zinc is involved in regulating PPAR-α activity and it is necessary to connect PPAR-α to DNA. Therefore, zinc deficiency reduces the activity of binding to DNA and thus increases the fat peroxidation. Zinc supplementation therefore restores liver steatosis in animal models of alcohol fatty liver by activating PPAR-α and a 4α liver nuclear factor (25). Baltaci et al. have reported that zinc supplements increase zinc levels in the liver tissue (26). In patients recieving zinc supplements, maintainin zinc level above 80 µg.dl is the most important factors in the survival of free-cancer survival (26-28, 3). Our findings suggest that non-alcoholic fatty liver disease is an effective factor in serum zinc levels in patients, so that with a significant increase in degree of fibrosis from the results of elastography, serum zinc levels have decreased and this factor also leads to increased oxidative stress, inflammation and fibrosis in patients. The results of this study could be the basis for introducing zinc as a supplement to reduce the progression of fibrosis in the treatment program for patients with non-alcoholic fatty liver. Multi-center studies and increase of sample size are proposed to generalize the correlation results for future studies. It is also suggested more researches in future clinical trials on the effect of zinc supplementation and liver fibrosis obtained from fibroscan in non-alcoholic fatty liver patients.

Conflict of Interest: No conflicts of interest.

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