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Correlation of Some Trace Elements and Chronic Hepatitis B Infections in Babylon

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Abstract
Background: Hepatitis B virus (HBV) infection is a serious global public health problem with an estimated 2 billion people infected worldwide and 350 million persons with chronic HBV infection. Various trace elements play an important role in the course of HBV infection.
Aim of the study:
To determine the quantitative estimation of some trace elements [lead (Pb), iron (Fe), selenium (Se) and zinc (Zn)] in the serum of patients with chronic HBV infection and compare with serum of healthy group**,** and also to evaluate these trace elements concentrations in chronic HBV infected patients in regards to their serum transaminase levels.
Materials and Methods:
The serum concentrations of Pb, Fe, Se, and Zn were determined in 40 patients with chronic HBV infections that included 31 males and 9 females ranging between 17 and 70 years and 15 of them live in urban area while 25 live in rural areas, while the control group comprised 30 healthy volunteers that included 20 males and 10 females aged between 22 and 58 (mean of 43 ± 8.79) years, 16 live in urban area and 14 live in rural area.
Electrothermal atomic absorption spectrophotometer technique was used for this measurement, especially Se measure by cold evaporative method.
Result:
The study showed that Pb, Fe, Se and Zn concentrations in serum of hepatitis B patients with high liver function test were 88 ± 24 ppb(part per billion), 2042 ± 582 ppb, 1846 ± 345 ppb and 554 ± 292 ppb respectively. While in patients with hepatitis B virus infection with normal liver function test, these concentrations were 44 ± 22 ppb, 1692 ± 466 ppb, 2074 ± 302 ppb and 735 ± 138 ppb respectively. In healthy individuals these concentrations were 37 ± 18 ppb, 1620 ± 396 ppb, 2084 ± 155 ppb and 786 ± 333 ppb respectively. All of these results were statistically significant ($p < 0.05$).
Conclusion:
This study confirms the variation of trace elements concentration in Hepatitis B Virus affected patients compared with healthy volunteers.

Introduction

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as A,B,C,D and E. Hepatitis B is the most serious type of viral hepatitis and a major global health problem [(1)].

The hepatitis B virus is 100 times more infectious than HIV and is presented in, and usually transmitted through, bodily fluids such as blood, saliva, semen and vaginal fluid [(2)].
Infection with the hepatitis B virus can cause an acute illness that has been resolved itself quickly without causing long-term liver damage.
Hepatitis B virus infection has different clinical manifestations depending on the patient's age at infection and immune status, and the stage at which the disease is recognized. Patients may then become jaundiced although low grade fever and loss of appetite may improve. Sometimes HBV infection produces neither jaundice nor obvious symptoms [(1)].
The asymptomatic cases can be identified by detecting biochemical or virus-specific serologic alterations in their blood. They may become silent carriers of the virus and constitute a reservoir for further transmission to others[(3)].
In general, the frequency of clinical disease increases with age, whereas the percentage of carriers decreases. Worldwide, about 1 million deaths occur each year due to chronic forms of the disease [(4)].
Persistent or chronic HBV infection is among the most common persistent viral infections in humans. More than 350 million people in the world today are estimated to be persistently infected with HBV. A large fraction of these are in eastern Asia and sub-Saharan Africa, where the associated complications of chronic liver disease and liver cancer are the most important health problems [(5)].
Most cases of acute hepatitis are subclinical, and less than 1% of symptomatic cases are fulminant. Worldwide, about 350 million people are estimated to be infected chronically with HBV. Persistent HBV infection is sometimes associated with histologically normal liver and normal liver function, but about one third of chronic

HBV infections are associated with cirrhosis and hepatocellular carcinoma [3].

Trace metals play an important role in liver disease particularly liver degeneration. During most viral infections the plasma levels of trace elements change, but it is not clear if this reflects changes in the infected tissues also. Influence of trace elements has been studied in a large number of viruses belonging to different groups [6].

While other trace elements act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects [7].

It is found in a wide variety of foods including beans, nuts, certain seafood, red meat, oysters, whole grains and dairy products. It is an essential mineral that is found in almost every cell. It stimulates the activity of approximately 100 enzymes. It supports a healthy immune system and is needed for wound healing, the sense of taste and smell, and for DNA synthesis [8].

Deficiency of Zinc most often occurs when zinc intake is inadequate or poorly absorbed, due to increased losses of zinc from the body, or when the body's requirement for zinc increases. Liver functions like urea formation require the presence of zinc. Severe zinc deficiency depresses immune function [9].

When zinc supplements are given to individuals with low zinc levels, the number of T-cell lymphocytes circulating in the blood increases and the ability of lymphocytes to fight infection improves. It is the structural component of a wide variety of enzymes, proteins, neuropeptides, hormone receptors and polynucleotide [7].

The iron is a mineral with important biological functions – most notably, its role in normal hemoglobin and red blood cell production. However, it is also an oxidant with free radical activity that has the ability to break down cellular membranes and other tissues (such as liver cells), Iron overload can result in damage to other biologic tissues such as the kidneys, heart and lungs [10].

Excess iron levels can potentially increase the activity of viruses by increasing their mutation rates. Elevated iron levels have been associated with raised levels of the liver enzyme ALT (alanine transaminase) in HBV infected humans. It is not perfectly clear whether the elevated iron levels are a cause or a result of elevated viral loads [11].

The oxidized form of iron (ferrous) causes lipid peroxidation - degeneration of fats that can injure the cells' mitochondria. Mitochondrial dysfunction has recently been linked to a variety of symptoms and degenerative changes found in HBV, including fatigue, liver cell damage, necrosis, inflammation, depression, gastrointestinal symptoms, jaundice, elevated liver enzymes, muscle aches, pains and others [12].

Lead has been implicated in a number of health effects, ranging from severe encephalopathy to and death to subtle effects on IQ [13].

The serum concentration of lead plays an important role in the interrelation among immunoglobulin IgA, IgM and IgG (Immunoglobulin are antibodies in immune system and some of types IgA, IgM and IgG) in subjects with various forms of liver diseases [14].

Selenium is a trace mineral that is essential to good health but required only in small amounts [15].

Selenium is a key nutrient in counteracting certain viral infections. Selenium has several public health implications particularly in relation to chronic disease prevalence. It is important for healthy human response.[16]

Decrease in serum Se might indicate the development and progression of HBV. It also links to the disease progress of some viral agents in relation to the biosynthesis of selenoproteins and decrease in serum Se significantly increases the risk of cancer mortality [17].

Aim of the study

The aim of this study is to determine the quantitative estimation of some trace elements (lead (Pb), iron (Fe), selenium (Se) and zinc (Zn)) in the serum of patients with chronic HBV infection and compare with serum of healthy group, and also to evaluate these trace elements concentrations in chronic HBV infected patients in regards to their serum transaminase levels

Material and Methods

A case control study that randomly selected group comprised 40 patients with chronic HBV infection while the control group comprised 30 healthy volunteers. Data and samples were collected from Babil GI center (Merjan Medical City) in period from February to August 2012.

The study population includes patients with chronic HBV infection attended Babil GI center of Merjan medical city.

The patients and healthy volunteers were enrolled in the study after obtaining written consent. Patients with positive HBsAg for more than six months were considered for this study including both normal and abnormal liver function test (Normal range of serum transaminase level was accepted as 40 IU/L), while the healthy volunteers were selected on the basis of no alcoholic, no smoking habits, no history of viral hepatitis and absence of any acute or chronic pathology, clinically evident at the moment of examination.

Exclusion criteria of cases include alcoholic, chronic liver disease, cirrhosis or other co- morbidities while the

exclusion criteria of healthy volunteers include alcoholic, smoking, history of viral hepatitis and any acute or chronic diseases.

Blood samples were taken from all subjects in accordance with standard
procedure; eight to ten mL of blood was collected from the vein and protected in evacuated tubes without adding any anticoagulant agent. Collected blood samples were placed in sterile place and allowed to clot. The blood samples were centrifuged at 300 g for 45 min and the serum was pipetted out and filtered through 0.45 µm membrane filter. The collected sera were stored in plastic vials at –20 C˚ until further analysis.

After complete all samples, allow samples to equilibrate at room temperature, then add 3 ml of 1.0 molary nitric acid digested to each sample , then equal all sample to volume of 10 ml by adding distal water, Allow beaker to cool then Transfer all beakers to store in refrigerator. An electrothermal atomic absorption spectrophotometer has been extensively used in the analysis of major, minor and trace elements in biological material because of its high sensitivity, accuracy, low matrix effect and simpler operation. The ultra-trace elements in such biological fluids could be determined by E.AAS.

The presence of various elements in the sample was identified by determining the wavelength of the emitted radiation (table 1) and the concentration was calculated by intensity of the radiation, which might be sufficiently low for certain applications with a simple matrix. Sample and standard were analyzed in triplicate.

Table 1 : Wave length of the emitted radiation for the trace elements

Trace element	Wave length
Iron	248.2 nm
Lead	283.3 nm
Selenium	196 nm
Zinc	213.9 nm

The special structural questionnaires were performed to the patients and control regarding their name, sex, residence, jobs, marital status, past medical history, past surgical history, drug history, history of previous liver disease, alcoholic and smoking.

Statistical analysis has been performed using Statistical package for the social sciences (SPSS, version 18) for windows. Continuous variables were expressed as mean ± standard deviation (SD). Data were analyzed using independent sample Student's t test. Significance was assigned for p values (<0.05) with 95% Confident Interval.

Results

The patients group was 40 patients with chronic HBV infections that included 31 males and 9 females (aged 41 ± 11 years), ranging between 17 and 70 years and 15 of them live in urban area while 25 live in rural areas.

The control group comprised 30 healthy volunteers that included 20 males and 10 females aged between 22 and 58 (mean of 43 ± 8.79) years, 16 live in urban area and 14 live in rural area. (Table 2)

Table 2: Age, gender and residence distribution of control and HBV patients group

Group	N	Age in years		Gender Male/Female	Residence Urban/rural
		Mean ± SD	Range of year		
Control	30	43 ± 8.79	22 – 58	20/10	15/25
Patient HBsAg +VE	40	41 ± 11.16	17 – 70	31/9	16/14

All blood samples (40 patients and 30 controls) were analyzed using E.AAS.

The serum level of Fe was high (2042 ± 582 ppb) in patients with chronic HBV infection with high liver function test and significantly differ from that of controls (1620 ± 396 ppb) (p value<0.005) as shown in table 3

The serum level of Pb was high (88 ± 24 ppb) in patients with chronic HBV infection with high liver function test, which was significantly differ from that of controls (37 ± 18 ppb) ($p < 0.01$). The serum level of Zn was low (554 ± 292 ppb) in patients with chronic HBV infection with high liver function test and significantly differ from that of controls (786 ± 333 ppb) ($p < 0.006$). The serum level of Se was low (1846 ± 345 ppb) in patients with chronic HBV infection with high liver function test, which was significantly differ from that of controls (2084 ± 155 ppb) ($p < 0.005$)

Table 3. Trace elements concentration in the chronic HBV patients with high liver function test and healthy control. Data are expressed as Mean ± SD

Elements	Control (healthy) n=30	Chronic HBV with high LFT n=20	P value
Iron	1620 ± 396	2042 ± 582	<0.005
Lead	37 ± 18	88 ± 24	<0.01
Zinc	786 ± 333	554 ± 292	<0.006
Selenium	2084 ± 155	1846 ± 345	<0.005

The serum level of Fe was high (2042 ± 582 ppb) in patients with chronic HBV infection with high liver function test and significantly differ from patients with chronic HBV infection with normal liver function test(1692 ± 466 ppb) ($p < 0.04$). Table 4

The serum level of Pb was high (88 ± 24 ppb) in patients with chronic HBV infection with high liver function test, which was significantly differ from patients with chronic HBV infection with normal liver function test(44 ± 22 ppb) ($p < 0.05$).

The serum level of Zn was low (554 ± 292 ppb) in patients with chronic HBV infection with high liver function test and significantly differ from patients with chronic HBV infection with normal liver function test (735 ± 333 ppb) ($p < 0.02$). The serum level of Se was low (1846 ± 345 ppb) in patients with chronic HBV infection with high liver function test, which was significantly differ from patients with chronic HBV infection with normal liver function test(2074 ± 302 ppb) ($p < 0.03$).

Table 4. Trace elements concentration in the chronic HBV patients with normal and abnormal AST and ALT. Data are expressed as mean ± SD

Elements	Chronic HBV with normal LFT N=20	Chronic HBV with high LFT N=20	P value
Iron	1692 ± 466	2042 ± 582	<0.04
Lead	44 ± 22	88 ± 24	<0.05
Zinc	735 ± 138	554 ± 292	<0.02
Selenium	2074 ± 302	1846 ± 345	<0.03

Table 5 show significant differences in serum iron level of chronic HBV patients between male (1819 ± 451 ppm) and female (1536 ± 560 ppm) (P value < 0.03) while there were not significant differences in serum level of zinc, selenium and lead between male and female (P value > 0.05).

Table 5. Differences in Trace elements concentration in the chronic HBV patients between male and female. Data are expressed as mean ± SD

Elements	Male mean ± SD N=31	Female mean ± SD N=9	P Value
Iron	1819 ± 451	1536 ± 560	0.035
Lead	55 ± 8	43 ± 4	0.447
Zinc	722 ± 274	666 ± 248	0.974
Selenium	2014 ± 271	2016 ± 298	0.443

While table 6 show there were no significant differences in serum level of zinc, selenium, iron and lead in chronic HBV patients regarding their residence (rural and urban areas). (P value<0.05).

Table 6. Differences in Trace elements concentration in the chronic HBV patients between rural and urban areas. Data are expressed as mean ± SD

Elements	Urban N=15	Rural N=25	P value
Iron	1685 ± 505	1795 ± 484	0.36
Lead	55 ± 14	49 ± 4	0.67
Zinc	734 ± 282	687 ± 256	0.85
Selenium	2021 ± 215	2009 ± 319	0.46

Discussion

Trace elements play an important role in liver disease particularly with paranchymal liver degeneration [18].
The most important trace elements in this concept are Zn, Se, Pb, and Fe. As the disease progresses from chronic hepatitis to liver cirrhosis, serum calcium, magnesium, phosphorus, zinc and selenium concentrations decrease, while the lead, iron and copper concentration increases [19].
These trace element abnormalities may reflect such pathological conditions as liver dysfunction, cholestasis, hepatic fibrosis or liver regeneration [20].
In this study, we found that the concentrations of zinc and selenium were low in patients with chronic HBV infection with high liver function test when it compared with patients with chronic HBV infection with normal liver function test or healthy controls.
The explanation for that change in liver cell was associated with the functional impairment, which alter the metabolism of trace elements, in particular zinc.
Selenium, zinc and other antioxidants may play a role in slowing the progression of chronic hepatitis B infection to cirrhosis [21]. Decreased plasma selenium levels in patients with chronic HBV infection increased the risk of hepatocellular carcinoma [22].
Versieck et al. reported that zinc concentration in serum of normal controls, patients with acute and chronic hepatitis and cases of postnecrotic cirrhosis was frequently decreased [23].
Rashed et al. reported that mean serum zinc level in chronic hepatitis patients was lower than in those of healthy control [24].
Pramoolsinsap et al. have compared serum zinc level in viral hepatitis and associated chronic liver disease. They reported that, serum zinc level was significantly decreased in patients with chronic active hepatitis, cirrhosis and hepatocellular carcinoma compared to healthy individuals [18].
Pramoolsinsap et al. compared serum zinc concentration in patients with acute hepatitis B and healthy groups. They found that the patients had significantly decreased serum zinc concentration than the healthy controls [19].
Teles-Martos et al. was reported that serum selenium concentration was reduced in hepatitis B patients as compared to control [25].
Serum Fe and Pb concentrations of chronic HBV patients with high liver function test are higher than patients with chronic HBV infection with normal liver function test or healthy controls.
The explanation for these differences are the iron and lead are hepatotoxic and excess iron and lead levels can potentially increase the activity of viruses by increasing their mutation rates [11].
Rashed et al. reported that serum cadmium(Cd) ,copper(Cu), Fe, manganese (Mn) and Pb in hepatitis patients were in higher levels than in healthy controls, while cobalt(Co), nickel(Ni) and Zn revels the opposite trends [24].
Khan and Qayyan determined serum concentrations of Fe and Pb in patients with chronic HBV infection which were higher than (significantly differ) healthy controls [26].

Conclusions

In conclusion, the trace elements such as Pb, Fe, Zn and Se are found to be statistically significant ($p < 0.05$); this study confirms the variation of their concentration in HBV affected patients compared with healthy volunteers. This study showed that Pb, Fe, Zn and Se might be considered as a marker of healthier liver. It is also concluded that suitable concentration of zinc and selenium are necessary for preventing the liver damage in chronic HBV patients

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