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Trace Elements in Sera of Patients with Hepatitis B: Determination and Analysis

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Abstract. Chronic Hepatitis B (HBV) is the leading cause of morbidity and mortality worldwide with about 248 million people having HBV infection. Trace elements e.g. copper (Cu), zinc (Zn), selenium (Se) and iron (Fe) are constituent components of many metal proteins and metalloenzymes in human sera. Therefore, the ratios of these trace elements in human sera are often stated to be a good marker for diagnosing various diseases including HBV. The aims of this study are: to compare the level of trace elements in sera of patients infected with HBV and healthy participants, and to evaluate the efficiency of analytical techniques (e.g. Inductively Coupled Plasma- Mass spectrometry (ICP-MS), Atomic Absorption Spectroscopy (hydride generation) (AAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) that are currently used to detect Fe and Se elements in Patients' human sera. The findings of this study show that the concentration range of copper element between (132.80 ±28.64 µg/dl) to (105.66±23.20 µg/dl) was significantly higher in HBV infected patients as compared to those in healthy controls (91.27± 9.20 µg/dl). Iron concentration range between (206.64±61.60 µg/l) to (170.00±36.71 µg/l) was significantly higher in HBV infected patients as compared to those in healthy controls (158.00±15.13 µg/l). However, patients with HBV had significantly lower serum concentrations of zinc with a concentration range between (111.64±20.90 µg/dl) to (99.25± 24.06 µg/dl) as compared to those in healthy controls (113.44±16.38 µg/dl). While selenium concentration range between (64.39±7.39 µg/l) to (51.10±4.96 µg/l) was significantly lower in HBV infected patients as compared to those in healthy controls (67.68±7.60) (µg/l). Moreover, the results of this study suggest that (AAS) technique was the most accurate method to measure the concentration of selenium element, while (UV and ICP-MS) analytical techniques have the same efficiency in measuring the iron concentration.

Introduction

Chronic Hepatitis B virus (HBV) infection is a major liver disease that clinical outcome of infection is linked to immune response (1). Carriers of HBV have an increased risk of developing cirrhosis and hepatocellular carcinoma (HCC) (2). Many studies have shown that trace elements have an important role in metabolic activity and health condition (3). I t is demonstrated that trace elements have a major role in protein synthesize, pregnancy abnormalities and immune function (4) e.g copper (5), selenium (6) and iron (7). Zinc element also plays an important role in the function of the liver. There are hepatic and extrahepatic actions for Zn in the prevention of alcoholic liver injury (8). Zinc deficiency has been involved in the pathogenesis of a number of clinical findings in chronic liver disease. These include the possible role of Zn deficiency in the pathogenesis of hepatic encephalopathy (9).

Several studies have reported the important role of trace elements in liver disease and viral Hepatitis (10) (11) (12). However, these studies have been conducted by using only single analytical method for trace elements detection. Therefore, the objectives of this study are: to find and compare the level of trace elements in sera of patients infected with HBV and healthy participants and to evaluate the efficiency of analytical used for detection Fe and Se elements in Patients' human sera.

Materials and Methods

Clinical Study

One hundred sixty sera samples were collected from patients with chronic hepatitis B who were referred for advisory on hepatitis B at Fallujah teaching Hospital unit. All the patients included in this research were tested positive for HBSe Ag test and their specimens were examined by profile test which includes (HBS Ab, HBe Ag, HBe Ab, HBc Ab, (IgM-IgG), HCV and HIV) and also viral load (HBV

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PCR) with unit (copies/ml) were carried to test the patients sera. Healthy control groups (n=30) were selected based on non-alcohol drinkers, with no smoking, no history of viral hepatitis and absence of any acute or chronic pathology.

Trace Element Concentrations and Statistical Analysis

Copper, zinc, and selenium levels were determined by using AAS method. Iron was determined first by using a spectrophotometer. The concentrations of (Se and Fe) also was determined using inductively Coupled plasma- Mass spectrometry to compare with AAS techniques. Pearson correlation test was used to correlate between different variables among the studied groups.

Results and Discussions

Prevalence of Chronic Hepatitis B

The record of patients and healthy group shows a difference in disease incidence in rural and urban regions. There is an increase in disease's incidence in rural areas (114 patients) as compared to the urban regions (76 patients). This finding can be attributed to lack of the health awareness and because the patients have not been vaccinated with hepatitis B vaccine. Note: details of patients and health groups are shown in (Table 1).

Trace Elements concentrations in sera samples

Copper and Zinc

Copper levels were higher (119.36 ± 25.88 µg\dl) among patients in comparison with a healthy individual. While zinc concentration was less (104.66±20.66 µg\dl) in HBe Ag (-ve) groups in comparison to HBe Ag(+ve) (111.2 ± 20.6 µg\dl) and healthy individual(113.44±16.38 µg\dl).

Table 1. Details of patients and control groups.

Symbols	Details	Numbers
1A	Ptients HBeAg (+ve)normal liver function PCR= (>100000)	36
2A	Patients, HBeAg (+ve)abnormal liver function PCR= (>100000)	28
1B	Patients HBeAg (-ve) PCR= (>100000)	30
2B	Patients HBeAg (-ve) PCR=4000-100000	36
3B	Patients HBeAg (-ve) PCR=N0N	30
C	Healthy individuals	30

Serum concentration of copper was significantly higher (132.80 ±28.64 µg/dl) in chronic hepatitis B patients in comparison to controls (91.27± 9.20 µg/dl), as in fig.(1A). The copper increment in patient's serum may due to the release of copper from damaged necrotic hepatocytes(13).These elevated serum Cu levels indicate an alteration of Cu metabolism during the chronic phase of uncomplicated hepatitis disease(14) and (15).

The results show that serum concentration of zinc was significantly lower (99.25± 24.06 µg/dl)in chronic hepatitis B patients as compared to controls (113.44±16.38 µg/dl), fig.(1A).The effects of zinc deficiency on the liver damage is not clear but the effect of zinc deficiency on liver repair mechanism might be modified by dietary zinc consumption (16). There are some findings which suggest that zinc supplementation can cause an increment in glutamine release from skeletal muscle and also activate glutamine synthesis, which can decrease the level of ammonia and improve hepatic encephalopathy (17). The relationships between Cu and Zn in HBV e Ag (+ve), HBV e Ag (-ve) and healthy individuals were determined using Pearson product moment correlation analysis. ANOVA results show significant differences ($p<0.05$) in Zn and Cu concentrations between 2A, 2B , 3B and the healthy groups. Furthermore, there are significant differences in Zn and insignificant differences in Cu concentrations for 1A and 1B groups.

Selenium

Selenium serum concentration was significantly lower (55.4 ± 6.32 µg\l) in chronic hepatitis B patients as compared to healthy individual (67.68±7.60 µg\l) fig. (1B). The decrease in serum selenium 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 (18). The reduction in Se element significantly increases the risk of cancer mortality (19). Declining serum Se might involve reduction biosynthesis of the hepatically derived Se transport protein selenoprotein P (SePP) for its human promoter is negatively regulated by prion inflammatory cytokines *in vitro* (20). Moreover, four-year animal studies showed that dietary supplement of Se reduced the HBV infection by 77.2% (21).

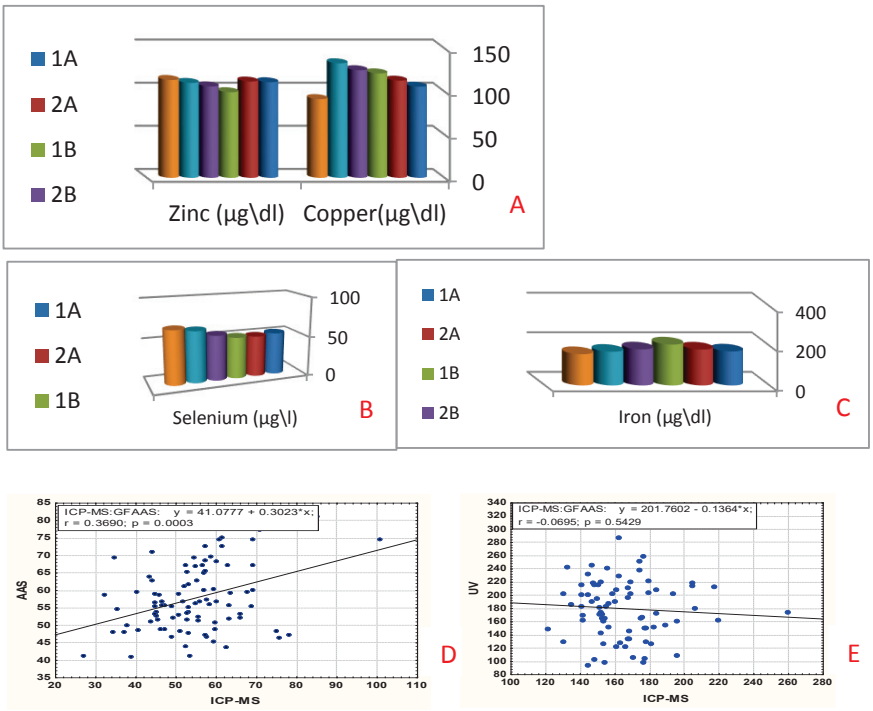

FIGURE 1. A. Serum copper and zinc concentrations in HBV patient and healthy individuals. B. Serum selenium concentration in HBV patient and healthy individuals. C. Serum iron concentration in HBV patient and healthy individuals. D. Scatter plot for selenium concentration measured by AAS and ICP-MS. E. methods. Scatter plot for Iron concentration measured by UV-Vis and ICP-MS methods.

Serum selenium concentration decreases as the disease progresses from acute to chronic stage (22). In this context, other authors have linked the reduction in serum selenium concentration with the progression of severe cirrhotic conditions and selenium concentration in patients' sera was lower as compared to control (23). In this study, analysis of t-test showed a significant difference between group B and group C ($P<0.001$) as compared to control group. Based on results of this study, it is suggested that patients should get selenium supplementation to raise serum concentration. In result of Pearson's correlation coefficients of Se, A positive significant correlation (at $p<0.05$) was observed between group 1B and group 1A (HBV e Ag (+ve), HBV e Ag (-ve) and healthy individuals.

ANOVA analysis for Se levels in patients groups and healthy individual suggested significant differences for all groups. Furthermore, significant differences were found for Se reading measured by AAS and ICP-MS methods based on scattering plotting analysis. The linear equation between y (AAS) and x (ICP-MS) was plotted in the fig (1D). The slope of the line equals to -0.1492. Statistically, the increase in slope value suggests significant differences in readings of Se level measured by AAS and ICP-MS methods. According to this result, we recommend using (AAS) method to measure Se level.

Iron

Iron serum concentration was significantly higher in chronic hepatitis B patients (179.6±41.30) as compared to healthy individuals (158.00±15.13 µg\dl). The results showed that value of iron in group HBe Ag (-ve) was higher (186.6 ± 42.3 µg\dl) as compared to HBe Ag (+ve) group (176.5 ± 33.3 µg\dl) (Fig. 1C). Iron is an essential element for cell survival; it serves as a cofactor for essential enzymes in oxidative metabolism and (in the form of heme) as the major oxygen transporter in most forms of life on earth. Both deficiency and excess of iron often lead to diseases (24) e.g anemia. The role of iron in viral hepatitis B was first emphasized by Blumberg (25), who studied 67 hemodialysed patients infected with HBV. In 33 of the patients who developed chronic hepatitis, serum iron levels were found to be significantly higher than in 34 patients who eliminated the virus. Another histopathological study suggested that iron accumulation in HBV infection was related to hepatocyte damage (26). In this

study, a positive correlation relationships were observed between Fe level in groups 1A and 1B, 1B and 2B. ANOVA analysis for Fe levels in patients and healthy individual groups showed significant differences for all groups. However, an insignificant difference was found for Fe level measured by UV-Vis and ICP-MS methods based on scattering plotting analysis. The linear equation between y (UV-Vis) and x (ICP-MS) was plotted in the fig. (1E), the slope of the line equals to -0.1364. Statistically, the decrease in slope value shows that iron can be determinate by UV-Vis or ICP-MS method with no preference for one method over another. The change in concentration of trace elements in chronic hepatitis B patients are probably caused by defense strategies of organism and induced by the hormone-like substances (27). Moreover, these trace element alterations may reflect the pathological abnormalities e.g liver dysfunction, damaged necrotic hepatocytes, cholestasis, hepatic fibrosis or liver regeneration (28).

Conclusions

This study results showed that serum, copper, and iron concentrations were higher in chronic hepatitis B patients as compared to healthy individuals. While selenium and zinc concentrations were significantly lower in patients' serum as compared to healthy individuals. AAS (hydride generation) method is more efficient in detection of selenium concentration as compared to ICP-MS method. In addition, the iron element can be determinate by both UV-Vis and ICP-MS analysis.

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