STUDY THE CLINICAL RELATIONSHIP BETWEEN CHRONIC HEPATITIS B AND SERUM TRACE ELEMENT LEVELS

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Abstract

In the present study serum trace element including Cupper (Cu), Iron (Fe), and Zinc (Zn), where determined by using atomic absorption in sera of patient with viral hepatitis (B) cases (n=40) and statistically compared with controls (n=20). The results showed that, in viral hepatitis Cu level where found as 1.5+/−0.29 ppm and this value was significantly higher than in control group1.0+/−0.15 ppm (p<0.05), Zn level found to be significantly low in viral hepatitis patients 0.56+/−0.09 ppm where in healthy individual was 0.87+/−0.07 ppm (p<0.05). There is no significantly difference between hepatitis patients and controls in serum Fe level (1.21+/−0.2, 1.27+/−0.34) ppm (p<0.05) respectively. It was indicated that absorption disorders in gastrointestinal system, especially in chronic cases, were not main causes of decrease of trace elements by iron and several other parameters in sera of the cases. Therefore, it is suggested that the decrease in Zn level and elevation in Cu levels are probably resulted from defense strategies of organism and induced by the hormone-like substances.

INTRODUCTION

Chronic Hepatitis B virus (HBV) infection is a major liver disease that clinical outcome of infection is linked to immune response (1, 2). The disease may develop to liver carcinoma during infection. Up to now, many studies have shown that trace elements have an important role in metabolic activity and health condition (3). It is demonstrated that trace elements have a major role in protein synthesis, pregnancy abnormalities and immune function (4,5). Viral hepatitis is a major human health problem worldwide [6, 7]. More than 300 million people throughout the world are affected by hepatitis B virus [8, 9]. The change in trace elements might also be associated with various diseases [10]. The relationship between chronic hepatitis and trace elements has not been understood clearly [11]. Various trace elements are responsible for many biochemical, immunological, and physiological activities. Essential micronutrients are involved in many metabolic pathways in the liver, such as enzymatic functions, protein synthesis, oxidative damage and anti-oxidant defense, immunological competence, interferon therapy response regulations and alterations of the virus genomes [12]. Trace element measures in serum/plasma are easy and used commonly. Serum metal level has been reported to be highly sensitive in the diagnosis of liver diseases [14]. The concentration of each trace element varies with different types of liver diseases because these elements may have a direct hepatic toxicity or may be decreased as a consequence of the impaired liver function. Trace elements such as Zn, Cu and Fe are required for the immune system to function efficiently. The concentration of each trace element varies with different types of liver diseases. The present study has been undertaken to
determine the quantitative estimation of trace elements (Cu, Fe, and Zn) in hepatitis B affected human serum and compare them with normal human blood serum.

MATERIAL AND METHODS

The randomly selected study group comprised 40 patients with HBV that included 24 males and 15 females (aged 35 ± 11.3 years), ranging between 11 and 63 years. The control group comprised 24 healthy volunteers that included 15 males and 9 females aged between 5 and 61 (mean of 31 ± 8.7) years. Samples were collected from Central Health Lab and Medical City Hospital (IRAQ) and Ministry of Science and Technology Iraq (M.O.S.T.). All sera were collected in the morning after fasting 8 hours. The healthy volunteers were selected on the basis of no alcoholic, no smoking habits, and no history of viral hepatitis and absence of any acute or chronic pathology, clinically evident at the moment of examination, routine clinical check up during the entire period of research. Sera were collected from patients before drug administration. Patients with chronic hepatitis B were diagnosed based on clinical, biochemical, histological and virological evidence that included HBsAg, HBsAb, HBcAb. Marker enzymes were measured by spectrophotometric method; other parameters were determined by respective commercially available ELISA kit in Central Health Lab and Medical City Hospital (IRAQ). HBsAg positive for more than 6 months was considered as chronic hepatitis. Normal range of serum transaminases level was accepted as 40 IU/L. Samples were restricted from other diseases. Blood samples were taken from all subjects in accordance with standard procedure; six to eight milliters of blood was collected from the vein and protected in evacuated tubes without adding any anticoagulant agent. Patients were not administered antiviral treatment previously to this study. Collected blood samples were placed in sterile place and allowed to clot. The collected sera were stored in plastic vials at −20°C until further analysis.

Sample digestion

One milliliters of serum was transferred to 10 mL of de-ionized water. The sample was measured using Flam atomic absorption techniques through by preparing the suitable standard solution for each metal. The present work was performed using atomic absorption from Phoenix-986, UK. The operating conditions were:

Current lamp: (Zn 2-3 Am), (Cu 2-3 Am) and Fe (2-4 Am)
Slid: (Zn 0-4 nm), (Cu 0-4nm) and Fe (0-2 nm)
Microscopic flame: (Zn 1000 ml/min), (Cu 2000 ml/min) and (Fe1700 ml/min)
Flame height: (Zn 6 mm), (Cu 6 mm) and (Fe 8 mm)
Wave length: (Zn 213.9 nm), (Cu 224.7 nm) and (Fe 248.3 nm)
Correlation factor: standard curve for each metal
Concentration: ppm

TRACE ELEMENT ANALYSIS

Reagent and solutions
Natural element standard solutions (1000 μg/mL) of Cu, Fe, and Zn were obtained from Merck, USA. High pure nitric acid was purchased from Fisher Scientific Inc, India. The standard solutions were kept at 4 °C in dark room. Other chemicals were purchased from Fluka. Standard solutions were prepared freshly from the stocks in order to obtain appropriate atomic absorption responses, the experiments were performed using different concentration levels.

**Statistical analysis**

Statistical analysis has been performed using Statistical package for the social sciences (SPSS, version 11.5) 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.

**RESULTS**

All blood serum samples (40 patients and 20 controls) were analyzed using Flame atomic absorption. The serum Cu concentration was 1.5+/−0.29 ppm in patients with viral hepatitis B, which was higher than that of controls (1.0 ± 0.159 ppm). Serum Zn level found to be significantly low in viral hepatitis patients (0.56+/−0.09 ppm) where in healthy individual was (0.87+/−0.07 ppm). There is no significantly difference between hepatitis patients and controls in serum Fe level (1.21+/−0.2, 1.27+/−0.34) ppm. Results of this study has been summarized in table 1:

**DISCUSSION AND CONCLUSION**

Trace elements are used as a diagnosing tool during disease; it is important to know whether the balance is changed in free or bound elements. Chronic hepatitis is insidious, in that the primary clinical signs are not readily evident until a well established pathogenic process has developed. Differences in trace elements have been associated with various diseases. The above results show that serum Cu concentrations of HBV patients are higher than normal individual serum concentrations. These elevated serum Cu levels indicate an alteration of Cu metabolism during the acute phase of uncomplicated hepatitis [14]. It may be resulted from defense strategies of organism and induced by hormone like substances [15]. As the disease progresses from chronic hepatitis to liver cirrhosis, serum calcium, magnesium, phosphorus and zinc concentrations decrease while copper concentration increases [16]. It may be explained by the release of copper from damaged necrotic hepatocytes [17].

Zn is an essential element that is found in all cells. It stimulates the activity of approximately 100 enzymes [18] and binds to several viruses. The reduced zinc concentration indicates the severity
of liver damage [7]. Kalkan et al. [13] have reported Zn concentration associated with viral hepatitis decrease with the development of hepatitis patients; it reveals that Cu concentration increases statistically while Zn level decreases. Decrease in serum Zn is due to poor appetite, during the infection with the help of leukocyte endogenous mediator (LEM) and uptake of more Zn to synthesize nucleic acid, protein and enzymes by liver cells. With progression of the liver damage, due to poor appetite, impaired function of intestines and stomach and high pressure of the portal vein, the zinc intake and absorption decreases and also the low content of serum albumin results in less combination with zinc and because of the diffusion characteristic of blood zinc, it is easily lost through urine and sweat [19, 20]. Fota Markowska et al. [19] studied the serum Zn level dynamics in patients with acute hepatitis B and the early recovery periods. They observed significantly decreased serum Zn levels during hospitalization and the supplementation of Zn to HBV patients resulted in early recovery. Loguercio et al. reported that cirrhotic patients had a significant decrease of serum Zn and Fe level [22]. In the present study the slight variation of iron concentration may be due to the accumulation of iron in liver parenchyma [23]. Assessment of the disorders of trace element metabolism should be based not only on the dietary intake, but also on various factors such as absorption transportation, storage, excretion, and metalloproteinase and metallo-enzymes synthesis which are impaired when liver cell is damaged [24, 25].

In conclusion, the trace elements such as Cu, Zn 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. Change in trace elements associated with some biological reaction takes place in the liver. Further research, both basic and applied, is needed to assess properly the possible role of malnutrition in contributing to the emergence of novel viral diseases.

References


دراسة العلاقة السريرية بينالتهاب الكبد الفيروسي نوع بي ومستويات بعض العناصر الضئيلة في مصل الدم

الخلاصة:
في الدراسة الراهنة تم تقدير مستوى بعض العناصر الضئيلة وهي النحاس والحديد والزنك في مصل مرضى التهاب الكبد الفيروسي نوع ب (40 مريضاً) بجهاز طيف الامتصاص الذري. أظهرت النتائج ان تركيز النحاس في مصول المرضى يساوي (1.5±0.29 ppm) وهو أعلى من قيم مجموعة السيطرة (0.15 ppm) (p<0.05) . وجد أن تركيز الزنك (0.56±0.09 ppm) عند المرضى كان أقل من مجموعة السيطرة (0.87±0.07 ppm) (p<0.05). لم توجد فروق معنوية في مستوى الحديد في مصل الدم بين المرضى ومجموعة السيطرة (1.21±0.2 ppm) ومجموعة السيطرة (1.27±0.34 ppm).

تدل هذه النتائج على ان احتمالية وجود اضطرابات في عملية امتصاص هذه الايونات من القناة الهضمية وخصوصا في الحالات المزمنة هي بعيدة الاحتمال وان هناك عوامل اخرى قد تكون هي المؤثرة. لذلك تقترح هذه الدراسة ان الانخفاض في تركيز الزنك والارتفاع في تركيز النحاس ربما يعود الى ميكانيكية الدفاع المحتشة بسبب مواد شبيهة بالهرمونات.