Comparison of Serum Trace Metals Concentration in Blood of Hepatitis B, C and Healthy Individuals and Their Correlation with Viral Load

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Abstract: Several trace elements are essential micronutrients and are required for various body functions. The relationship between chronic liver disease and trace heavy metal contents in blood are debatable and have not been understood clearly. The present study was carried out to determine Cu, Fe, Mn, Cd, Zn, Ni and Co concentration in blood sera of viral hepatitis patients. Sixty patients (thirty with chronic HBV and thirty with HCV) and thirty healthy individuals were selected for this study. This study showed that Zn, Cu, Fe, Ni, Co, Mn and Cd Concentration in serum of chronic HBV were 88.28µg/dL, 434.5µg/dL, 3.44µg/dL, 3.26µg/dL, 77.14µg/dL, 4.28µg/dL and 2.30µg/dL respectively whereas in patients with chronic HCV, these concentrations were 95.9µg/dL, 464.68µg/dL, 3.08µg/dL, 3.84µg/dL, 69.24µg/dL, 3.79µg/dL and 1.69µg/dL respectively. In healthy individuals these concentration were found to be 74.52µg/dL, 382.8µg/dL, 2.84µg/dL, 2.67µg/dL, 95.87µg/dL, 5µg/dL and 2.15µg/dL respectively. These results indicate that Cu, Fe, Mn and Cd were present in higher concentration in the sera of hepatitis patients as compared to healthy controls whereas Zn, Ni and Co shows opposite trend. Moreover, a good correlation was observed between copies/ml (viral load) and serum metal’s concentration.

Key words: HBV - HCV - Serum - Trace Elements - Liver Disease

INTRODUCTION

Viral hepatitis has been a major human health concern worldwide [1]. An estimated 2 billion people worldwide had been infected with the hepatitis B virus (HBV) resulting in 400 million chronic infections in 2000 [2]. Prevalence of HCV in Pakistan is among the highest in the world and estimated to be 4.8%[3]. In Pakistan 10 million people are presumed to be infected with HCV [4, 5].

Human body is composed of macromolecules such as proteins, carbohydrates, fats and nucleic acids. These molecules result from a chemical combination of C, H, N, O and P as the major elements [6]. In addition to these constituents, human body contains several trace elements such as Zn, Cu, Fe, Mn, Co, Ni and complex compounds. Serum trace elements level and their ratios are frequently reported to be a good marker for diagnosing various diseases. These parameters are not always specific to diseases; however, it is necessary to use other serum parameters for an exact diagnosis [7].

Among body parts, blood is considered as the most reliable limitation for the assessment of exposure of metal pollutants in general population [8, 9]. Serum metals level has been reported to be highly sensitive in the diagnosis of liver diseases [10].

The present study has been undertaken to determine the quantitative estimation of trace elements (Zn, Cu, Fe, Co, Ni, Mn, Cd) in hepatitis B, C affected human blood serum and compared with normal human blood serum using atomic absorption spectrophotometer.
MATERIAL AND METHODS

Description of Participants: The randomly selected study group comprised 60 patients (30 each from HBV and HCV). Patients with HBV included 18 males and 12 females aged between 12 and 62 (Mean 35±14.2) years. Similarly patients with HCV included 22 males and 8 females (Mean 32±12.9), ranging between 17 and 58 years. 30 healthy individuals including 23 males and 7 females were also included in this study (Mean 30±10.1), ranging between 20 and 59 years old (Table 1). Consent was obtained from all the patients included in this study. Patients were not administered antiviral treatment before this study. All blood samples were collected in the morning after fasting of 8 hours. Diagnosis of hepatitis was based on symptoms, physical findings as well as blood tests for liver enzymes and viral antibodies.

The healthy volunteers were selected on the basis of no smoking habits and no history of viral hepatitis. All people were in the same socio-economic status and similar diet habits. Patients with chronic hepatitis B and C were diagnosed based on clinical, biochemical, histological and virological evidences that included HbsAg, HbsAb, Anti HCV by third generation ELISA assay and HBV DNA, HCV RNA by PCR technique. Normal range of serum transaminase level was accepted, as 40 IU/L. Samples were restricted from other diseases.

Blood Sample Collection: 5 ml blood sample from each participant was collected from vein and protected in glass tubes without adding anticoagulation agent. Blood samples of healthy donors were collected from the same areas of patients. Separate and disposable sterilized plastic syringes were used for blood collection.

After coagulation, serum was separated at 2500-rpm centrifugation for 10 minutes, transferred to 5 ml polystyrene tubes and stored at 0°C until further analysis.

Reagents and Solutions: De-ionized water was obtained from Pak-Arab fertilizers Multan, Pakistan. Analytical reagent grade HClO₂ and HNO₃ were obtained from E. Merck, Germany. Standard stock solutions of Zn, Cu, Ni, Co, Fe, Mn and Cd for calibration were prepared from chemicals obtained from Fluka Riedel-de Haen Scientific Research, India and E. Merck, Germany.

Fresh standard solutions were prepared from stock solutions on each day of analysis by dilution with 1% HNO₃ in De-ionized water. Blank solution were treated and prepared exactly in the same way as the samples.

Sample Preparation for Analysis: 1 ml of each blood sample with nitric acid and perchloric acid (3:1) was taken in pre-weighed china dish and placed on heating mantle for 20-30 minutes till liquid was evaporated. The residue remained behind is cooled for 10 minutes. After cooling, nitric acid and perchloric acid was again added and residue heated on burner for 20-30 minutes until its blackish color is disappeared. Then it was again cooled for 10 minutes. This process was repeated three times. As a result, a pure white fine powder was obtained. Finally in dried sample, de-ionized water containing 1% nitric acid was added and filtered with Whatman 42 filter paper. This filtrate is made up to 25 ml with 1% nitric acid and stored in plastic bottle. Blank solution was treated and prepared in the same way as the samples.

Determination of Elements: A Hitachi Model A-1800 atomic absorption spectrometer equipped with the standard burner and air acetylene flame was used. Standard hollow cathode lamps were used as radiation source for elements reported. In calculating data, AAS (conc.) measurement mode with the peak area and peak height of absorbance signal was employed. Before the absorbance measurement, about half an hour was given for warming up instrument. Blank, standard solutions and samples were directly aspirated and absorbance measured. The output absorbance of each solution along with correlation co-efficient is reported. The presence of various elements in the sample was identified by determining the wavelength of the emitted radiation (Zn: 213.8, Cu: 324.8 nm, Fe: 248.3 nm, Ni: 232 nm, Co: 240.7 nm, Mn: 279.6 nm, Cd: 228.8 nm) and the concentration was calculated by intensity of the radiation. Samples and standards were analyzed in duplicate.

Statistical Analysis: Statistical analysis of this study was performed using statistical command line software R-3.0.2. The normality and homogeneity of variables was
evaluated using shapirowilk test and bartlett test respectively. Normally distributed variables such as zinc, copper and iron were expressed as mean±standard deviation. Non-normally distributed variables such as cobalt and nickel were presented as median and IQR (inter quartile range). The differences of level of zinc, copper and iron in the blood of the three groups (controls, HCV and HBV patients) were compared using one-way analysis of variance (ANOVA) test with scheffe test as a post hoc comparison. The differences of level of cobalt and nickel in the blood of three groups were compared using Kruskal Wallis test. A two-tailed p value less than 0.05 was considered statistically significant. Pearson's correlation or Spearman's rank correlation coefficients depend on the normality of variables were performed to identify relationships of the blood variables.

RESULTS AND DISCUSSION

Macromolecules such as proteins, carbohydrates, fats and nucleic acids are important constituents of human body [6]. It also contains several essential elements for the normal functions of the body. These elements, i.e. Zn, Cu, Fe, Ni, Fe, Co and Cd are assimilated through food, water and environment [11]. Trace metals play an important role in liver disease and are used as a diagnosing tool during disease.

Zinc: It stimulates the activity of approximately 100 enzymes [12]. Zinc supports a healthy immune system and is needed for wound healing, the sense of taste and smell and for DNA synthesis [13-15]. Our results showed that the mean serum zinc level was significantly higher in healthy volunteers (95.87µg/dL) as compared to HBV patients (77.14µg/dL) and HCV patients (69.24µg/dL). Lowest amount was observed in HCV patients. This is indicated by Fig 1.1a and 1.1. The diminution of serum zinc concentration indicates the severity of liver damage [16].

Versieck et al. [17] zinc concentration in serum of normal controls, patients with acute and chronic hepatitis and cases of post necrotic cirrhosis, was frequently decreased. Faiza et al. [32] noted considerable alternations in zinc profile of hepatitis patients and healthy volunteers with diminished levels in hepatitis patients. Mahmood et al. [33] also reported lower zinc concentrations in patients of liver cirrhosis. Cesur et al. [18] observed no significant variations in zinc profile of hepatitis patients and healthy persons. 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 [18, 19].

Our results were also confirmed by inverse relation found between log of copies/mL and Zn concentration in HCV patients as indicated by the Fig. 1.2.

Copper: Copper is an integral part of many important enzymes involved in a number of vital biological processes like hemoglobin synthesis and connective tissues metabolism. It is integral part of superoxide dismutase and ferroxidase ceruloplasmin. It also maintains the balance of other metals like zinc molybdenum. Although normally bound to proteins, copper may be released and become free to catalyze the formation of highly reactive hydroxyl radicals that have a capacity to initiate oxidative damage and interfere with important
Fig. 1: Comparison of Zn concentrations in Healthy, HCV & HBV Patients

Fig. 2: Zn concentrations (µg/dL) and viral load (log copies/mL)

Fig. 3a: Copper concentration in healthy, HCV and HBV patients.

Fig. 3: Comparison of Cu concentrations in Healthy, HCV & HBV Patients

Fig. 4: Zn concentrations (µg/dL) and viral load (log copies/mL)

cellular events [20]. Our results showed that mean Cu concentration in the blood serum samples of Healthy individuals (74.52 µg/dL) was lower than that of HBV (88.28 µg/dL) & HCV (95.9 µg/dL) patients. Lowest amount was observed in Healthy individuals as indicated in Fig 1.3. Copper accumulation in fibrotic livers, causes chronic HCV infection, may contribute to hepatic injury [21] (Fig 1.3a and 1.3). Devrajan et al. [31] found elevated copper levels in hepatitis patients as compared to healthy individuals. Kalkan et al. [10] have studied serum trace elements, including copper in sera of patients with viral hepatitis cases and controls. They have shown elevation in copper levels and suggested that this probably resulted from defense strategies of organism and induced by hormone-like substances.

Our results were also confirmed by direct correlation found between log of copies/mL and Cu concentration in HCV patients as indicated by the Fig. 1.4.

Iron: 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 [22]. The mean Fe values in the serum samples of Healthy individuals (382.8 µg/dL) was lower than that of HBV (434.5 µg/dL) and HCV (464.68 µg/dL) patients. The lowest amount was observed in healthy individuals (Fig 1.5a and 1.5). Our results were in agreement with Shan et al. [23]. They reported higher concentration of ferritin and iron in HCV infected US population. This may be due to free radical activity or defense strategies of the organism. The liver is the main iron storage organ and it plays a fundamental role in iron metabolism. The iron transport protein, transferring and the major iron storage protein, ferritin, are both
Fig. 5a: Iron concentration in healthy, HCV and HBV patients.

Fig. 5: Comparison of Fe concentrations in Healthy, HCV & HBV Patients

Fig. 6: Fe concentrations (µg/dL) and viral load (log copies/mL)

Nickel: Nickel is an essential micronutrient. The best sources of nickel include oatmeal, legumes, nuts, cocoa, whole wheat bread and some leafy vegetables such as kale and lettuce. Nickel is found in blood and tissues at consistent levels and is also associated with DNA and RNA in amounts that suggest physiological significance. Nickel is required for normal growth and reproduction in animals and presumably in human beings as well. It appears to have a role in the modulation of the immune system and in development of brain [21].

The minimal risk level of nickel and its compounds is set to 0.2 µg/m³ for inhalation during 15–364 days. Nickel sulfide fume and dust are believed carcinogenic and various other nickel compounds may be as well [27]. Nickel carbonyl, [Ni(CO)₄], is an extremely toxic gas. The toxicity of metal carbonyls is a function of both the toxicity of the metal as well as the carbonyl's ability to give off highly toxic carbon monoxide gas [28].

Concentration of Ni in the blood sera collected from Healthy, HCV & HBV patients aged between 12 to 60 years was determined by Atomic Absorption Spectrophotometer. From the Figure 1.7, it can be observed that Ni in the serum samples of Healthy individuals (5µg/dL) was greater than that of HBV (4.28µg/dL) & HCV (3.79µg/dL) patients. Lowest amount was observed in HCV patients (Fig 1.7a and 1.7). From the Fig. 1.8, it is also clear that there is inverse correlation between Ni Concentration and viral load in the blood sera of HCV patients showing that with the increase in the number of copies/mL, there is a decrease of Ni concentration gradually. It can be concluded that viral load has effect on Ni concentration.

The diminution of serum nickel in hepatitis patients may reflect diminished concentration of serum nickeloplasmin and albumin. This protein has an essential physiological role [29].

Cobalt: Cobalt is a mineral required by the body for blood formation. It is an integral part of vitamin B12 (cobalamin), a vitamin essential for producing red blood cells and maintaining the nervous system. The food sources of cobalt are meat, dairy products and green leafy vegetables. Cobalt chelates act as antiviral agents. Cobalt deficiency caused anemia deficiency [21] reported...
Fig. 7a: Nickel concentration in healthy, HCV and HBV patients.

Fig. 8: Ni concentrations (µg/dL) and viral load (log copies/mL)

Fig. 9a: Cobalt concentration in healthy, HCV and HBV patients.

Fig. 9: Comparison of Co Conc. in Healthy, HCV and HBV patients

Fig. 10: Co concentration (µg/dL) and viral load (log copies/mL)

Fig. 11: Comparison of concentration of different elements

that an excessively high intake of cobalt may damage the heart muscles and may cause an over-production of red blood cells or damage to the thyroid gland.

Bacteria in the guts of ruminant animals convert cobalt salts into vitamin B₁₂, a compound which can only be produced by bacteria. The minimum presence of cobalt in soils therefore markedly improves the health of grazing animals and an uptake of 0.20 mg/kg a day is recommended [30].

Concentration of Co in the blood sera collected from Healthy, HCV & HBV patients aged between 12 to 60 years was determined by Atomic Absorption Spectrophotometer as shown in Figure 1.9. From
Table 2: Relationship of healthy participants, HCV and HBV patients with Zinc, Copper and Iron Concentration

<table>
<thead>
<tr>
<th></th>
<th>Controls (Healthy persons) = 30</th>
<th>HCV patient = 30</th>
<th>HBV Patient = 30</th>
<th>F-test</th>
<th>P-value</th>
<th>p &lt; 0.001</th>
<th>p &lt; 0.001*</th>
<th>p &lt; 0.005*</th>
<th>p &lt; 0.001*2</th>
<th>p &lt; 0.001*3</th>
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</thead>
<tbody>
<tr>
<td><strong>Zinc</strong></td>
<td>95.58±6.71</td>
<td>68.78±9.59</td>
<td>76.65±10.15</td>
<td>71.13</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*1</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.005*2</td>
<td>p &lt; 0.001*3</td>
<td></td>
</tr>
<tr>
<td><strong>Copper</strong></td>
<td>74.52±7.35</td>
<td>97.21±8.68</td>
<td>88.28±5.65</td>
<td>72.92</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*1</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*2</td>
<td>p &lt; 0.001*3</td>
<td></td>
</tr>
<tr>
<td><strong>Iron</strong></td>
<td>383.3±83.03</td>
<td>464.7±123.8</td>
<td>452.5±137.24</td>
<td>4.22</td>
<td>p &lt; 0.05*</td>
<td>p &lt; 0.05*1</td>
<td>p = 0.078</td>
<td>p = 0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Relationship of healthy participants, HCV and HBV patients with Cobalt and Nickel Concentration**

<table>
<thead>
<tr>
<th></th>
<th>Controls (Healthy persons)</th>
<th>HCV patient</th>
<th>HBV Patient</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cobalt</strong></td>
<td>0.0±4.4</td>
<td>0.0±3.38</td>
<td>0.0±4.17</td>
<td>0.24</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Nickel</strong></td>
<td>0.0±10.97</td>
<td>0.0±8.02</td>
<td>0.0±8.92</td>
<td>0.40</td>
<td>0.82</td>
</tr>
</tbody>
</table>

The figure it can be observed that Co in the serum samples of Healthy individuals (2.15µg/dL) was greater than that of HBV (2.3µg/dL) & HCV (1.69µg/dL) patients. Lowest amount was observed in HCV patients (Fig. 1.9a and 1.9). The results found by Faiza et al. [32] were also in accordance with the current study. They found significantly high values of cobalt in healthy volunteers as compared to patients with hepatitis C.

**CONCLUSION**

Concentration of copper, iron, manganese and cadmium are found to be at higher level while zinc, nickel and cobalt at lower level in patient’s sera as compared to healthy ones. Furthermore, graphs between copies/ml (viral load) and serum concentrations of copper, manganese, iron and cadmium showed a direct relation while graph between copies/ml (viral load) and serum metal concentration of zinc, nickel and cobalt showed inverse relation.

Exact mechanism is difficult to predict. But however the results obtained from this study reveal that these serum metals have a certain role in virus activity. So in the light of present study, complementary therapy can be provided to chronic patients by giving them balanced diet and supplements of metals available in market.

**REFERENCES**


