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Trace Elements Profile of Hepatitis C Patients in Faisalabad, Pakistan

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Abstract: This paper describes the alterations in serum trace elements including cobalt (Co), iron (Fe), manganese (Mn) and zinc (Zn) in confirmed chronic hepatitis C (HCV) patients and clinically healthy persons. Blood samples of confirmed HCV patients and clinically healthy persons were collected from a private hospital located at Faisalabad city. The samples were wet digested and subjected to atomic absorption spectrophotometry. The results indicated that mean values of Co, Fe, Mn and Zn were significantly higher ($P<0.05$) in clinically healthy persons than HCV infected patients. The mean values of Co, Fe and Zn showed no significant difference ($P>0.05$) between healthy and HCV male and female patients. Manganese concentration varied significantly ($P<0.05$) in HCV infected males compared to females. In conclusion, serum trace element concentrations reflected significant statistical alterations in HCV patients compared to healthy volunteers. There was no significant gender-wise difference in serum Co, Fe and Zn concentrations in healthy and HCV infected except Mn.

Key words: Trace Elements · Serum · Hepatitis C · Faisalabad

INTRODUCTION

In diagnosing hepatic disorders, the sensitivity of serum metal profile has been well recognized. Trace elements play an important role in liver diseases particularly liver degeneration. During oxidative stress, oxygen free radicals are produced that are harmful for the body and some trace elements act as cofactors of antioxidant enzymes to protect the body from these free radicals. There should be a balance between harmful pro-oxidant and antioxidant compounds to counter these effects [1]. It is obvious that there happened alteration in plasma trace metal profile during most viral infections, but it is uncertain that this alteration may cause changes in the infected tissues [2].

Human body needs certain trace metals like Co, Cu, Fe, Mn, Ni and Zn for proper functioning, but a deficiency or excess may occur in case of certain chronic metabolic disturbances. In general, assessment of metal pollutants can be carried out by testing blood, as metals reach to different body parts through blood circulation and cause health related problems. Martz [3] mentioned that serum trace elements profile is a good indicator to diagnose various diseases. Impaired metabolism of trace elements in hepatitis C infected individuals may happen at various stages of liver damage process as indicated during clinical trials. Rashid [4] documented that profile of metals like Cu, Se and Zn for the diagnosis of liver disease as well as other diseases like cancer is highly sensitive. The aim of this investigation is to evaluate the variations in the serum trace elements (Co, Fe, Mn, Zn) profile in chronic HCV patients and compared them with the healthy and HCV infected males and females residing at Faisalabad city.

MATERIALS AND METHODS

Reagents and Standard Solutions: Calibrated standards were prepared by using the commercially available stock solutions (Applichem®). All the glass apparatus used throughout the process of analytical work were immersed in 8N HNO_3 overnight and finally rinsed with several changes of de-ionized water, air dried and stored.

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Table 1: Instrumental conditions for metal determination by Flame Atomic Spectrophotometer

Parameters	Co	Fe	Mn	Zn
Wavelength (nm)	240.7	248.3	279.6	213.9
Slit Width (nm)	0.2	0.2	0.4	1.3
Lamp Current (mA)	12.5	7.5	7.5	7.5
Burner Head	Standard type	Standard type	Standard type	Standard type
Flame	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2
Burner Height (mm)	7.5	7.5	7.5	7.5
Oxidant gas pressure (Flow rate) (kpa)	160	160	160	160
Fuel gas pressure (Flow rate) (kpa)	7	6	7	6

Collection of Blood Samples: Blood samples of hospitalized HCV confirmed patients (n=40) and clinically healthy volunteers (n=15) of different gender and age groups were obtained from Aziz Fatima Hospital, Faisalabad. Post-fasting (12 hrs) venous blood samples 5ml were collected in vacutainer blood collecting tubes. The collected samples were left standing (1 hr.), sera were separated by centrifugation (2500 rmp) and preserved at -20°C until analysis.

Pre-Treatment of Serum Samples: Serum samples (500 µl) were wet digested on hot plate containing 5 ml HNO_3/$HClO_4$ (1:2) acid mixture. After complete digestion, 5 ml nitric acid (0.1 Ml) was added into the flasks and filtered, diluted with de-ionized H_2O up to 25 ml in volumetric flasks. The blank solution was also prepared in the similar acid matrix.

Analytical Procedure: Trace elements viz. Co, Fe, Mn and Zn were analyzed in the prepared serum samples. Analytical analysis was performed with Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC [5]. The instrumental operating conditions for the said elements are summarized in Table 1.

Statistical Analysis: The collected data was presented as mean ± standard deviation. Student t-test was applied for the statistical analysis. In all cases probability level of 95% was taken as significant.

RESULTS AND DISCUSSION

The findings of the current investigation indicated higher serum concentrations of Co, Fe, Mn and Zn in healthy volunteers as compared to HCV patients. Mean values (±SE, µg/ml) of Co (0.23 ± 0.002; 0.02 ± 0.001), Fe (1.32 ± 0.02; 1.39 ± 0.02), Mn (0.19 ± 0.08; 0.09 ± 0.008) and Zn (0.71 ± 0.02; 0.26 ± 0.01) varied significantly (P< 0.05) in healthy individuals and HCV patients (Fig. I). However,

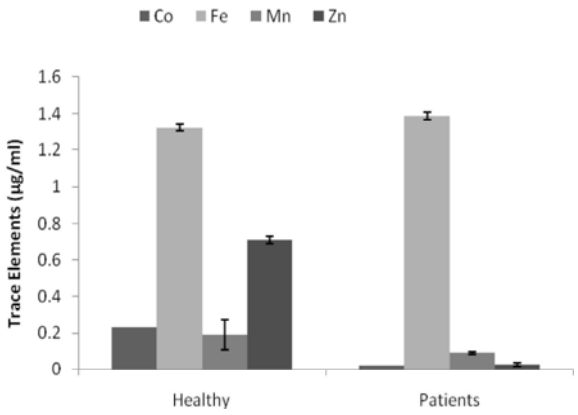

Fig. 1: Serum concentrations of Co, Fe, Mn and Zn (µg/ml) in healthy persons and HCV infected patients

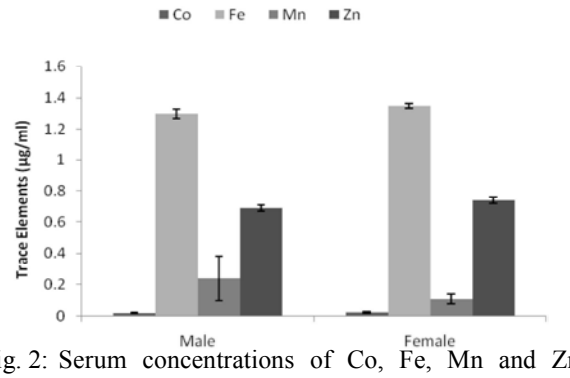

Fig. 2: Serum concentrations of Co, Fe, Mn and Zn (µg/ml) in healthy males and females

no significant difference (P>0.05) was observed in the levels of serum Co (0.022 ± 0.003; 0.025 ± 0.005), Fe (1.30 ± 0.03; 1.35 ± 0.014) and Zn (0.69 ± 0.02; 0.74 ± 0.02) in healthy males and females (Fig. 2). HCV infected males and females (Fig. 3) reflected non-significant (P>0.05) variation in the mean values of Co (0.017±0.003; 0.016±0.003), Fe (1.38±0.02; 1.40±0.03) and Zn (0.26±0.01, 0.26±0.01). Concentration of Mn varied significantly (P<0.05) in HCV infected males (0.076±0.004) compared to females (0.107±0.0135).

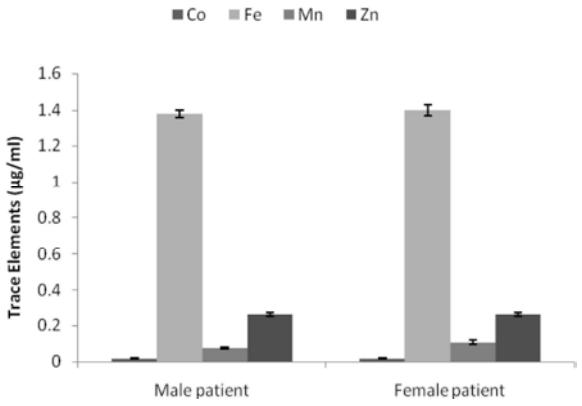

Fig. 3: Serum concentrations of Co, Fe, Mn and Zn (µg/ml) in male and female patients

The importance of trace elements cannot be ignored in liver diseases predominantly with parenchymal liver degeneration. The results of the present investigation showed higher serum Co level in HCV patients compared to healthy individuals. Scanty literature has been published regarding the level of Co in various liver diseases. Low level of Co has been reported [6] among patients having cirrhosis. Some other studies [7, 8] also reported lower or non-detectable levels of Co in hepatitis patients.

These results in the present study revealed higher concentration of Fe in HCV patients compared to healthy individuals. In 1992, role of Fe was recognized in the pathogenesis of HCV infection and higher concentration of this metal encountered in patients with HCV [9]. Liver is involved in iron metabolism, absorption and storage. During viral hepatitis, Fe metabolism may be interrupted resulting into increased accumulation in liver [10]. Deposition of hepatic Fe depends on the extent of liver inflammation and damage of infected liver tissues in HCV infection [11]. Nevertheless, exact mechanisms during Fe deposition still need a crystal clear understanding [12]. Elevated Fe concentration can increase viral activity due to rise in mutation rates. Elevated Fe concentration in chronic HCV individuals (40-46 %) has been observed by Di Bisceglie *et al*. [9] and Riggio *et al*. [13]. It was observed that liver of chronic HCV patients have increased Fe concentration and ultimately this load may reflects severe impact on HCV infection. Clinical trials in chimpanzees indicated that HCV infected group provided with Fe rich diet had more liver cell damage susceptibility than those group received diet contained low Fe contents [14]. In HCV patients, high Fe concentration is related to increase level of an enzyme, alanine transaminase. The unusual saturation of transferring and/or ferritin in hepatitis individual (50%) with slight hepatic Fe increase has been observed [15]. Afridi *et al*. [16] reported significantly higher concentration of Fe in sera of HBV (1.96, 2.35 mg/l) and HCV (2.12, 2.56 mg/l). Lower levels of Fe in patients have been observed by Devrajani & Rahman [17].

The results of the present investigation showed higher serum Mn level in healthy individuals compared to HCV patients. Our results are not coinciding with the findings of Versieck *et al*. [18] that serum Mn level increased in patients during the active phase of acute hepatitis, chronic hepatitis and post hepatitis cirrhosis. Similar increasing trend of serum Mn concentration in patients (1.803 ppm) having hepatitis than clinically healthy persons (1.27 ppm) has been reported by Rashed *et al*. [8]. On the other hand, Minhe *et al*. [19] documented that serum Mn in chronic hepatitis and post hepatitis cirrhosis has no significant alteration excluding acute hepatitis patients that showed a little decrease.

Zn concentration varied significantly in healthy individuals and HCV patients with higher levels in healthy individuals. Zinc is categorized as an essential mineral and occurs in every cell of the human body. Its deficiency occurs when Zn requirements increaseased or poorly absorbed in case of its losses from the body [20-22] and its severe deficiency depresses immune function [23]. Cellular and immunological functions are regulated by Zn and also it acts as cofactor in numerous enzymes. Some liver functions need the existence of Zn [24]. It was observed that Zn profile often decreased in patients having chronic hepatitis, post-necrotic cirrhosis and normal controls [18]. Zn concentration was lower in patients suffering from chronic active hepatitis, cirrhosis and hepato-cellular carcinoma [25]. Similarly, lower values of serum Zn in patients suffering from hepatic cirrhosis has been reported by Mezey [26], Solis-Herruzo *et al*. [27], Barry *et al*. [28], Capocaccia *et al*. [29], King, & Keen [30], Terres-Martos *et al*. [31], Rashed *et al*. [8], Devrajani & Rahman [17] and Mahmood *et al*. [32]. Cesur *et al*. [2] noted no considerable alteration in serum Zn and Cu profile of chronic hepatitis C patients and healthy persons. In contrast, Rahopoto *et al*. [33] documented that serum Cu and Zn levels were increased in patients with chronic hepatitis C in a case control comparative study at Hyderabad, Pakistan.

Factors like sex, age, nutritional condition and exposure to contaminated environment of a specific population may influence the trace elements profile

[34-36]. Gender-related metal variation in chronic hepatitis needs better explanation after exploring the above mentioned factors under local conditions.

CONCLUSIONS

In conclusion, serum trace elements profile reflected a significant statistical variation in HCV patients as compared to healthy individuals. Alterations in serum Co, Fe and Zn of healthy and HCV males and females were statistically non-significant except Mn. It could be suggested that a series of controlled studies should be executed for the determination of different trace elements level in patients suffering from chronic hepatitis before and after treatment in order to ascertain their probable role under the local environmental conditions.

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