Biomedical Letters
2016| Volume 2 |Issue 2|Pages 99-102

RESEARCH ARTICLE OPEN ACCESS

Serum metal levels as diagnostic indicators of Hepatitis C Virus Infection

Saghir Ahmad Jafri[1*], Kalsoom Zaigham[2], Kamran Mukhtar[2], Muhammad Imran[3], Khawar Ali Shahzad[2,4]

[1]Nur International University, Fatima Memorial Hospital, Shadman, Lahore 54000, Pakistan.
[2]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore 54000, Pakistan.
[3]School of Botany, Minhaj University, Lahore 54000, Pakistan
[4]Department of Microbiology and Immunology, Southeast University, Nanjing 210009, China.

Abstract
The serum metal profile is a good indicator for hepatitis C virus infection as serum trace element profile reflect a significant variation in HCV patients. The present study was aimed to evaluate variation in minerals in HCV patients as compared to normal controls as indicator of HCV. HCV positive patients from different hospitals of Lahore and its adjoining areas were taken as experimental subjects. The control group was included after thorough medical and lab reports verifications of metal levels in normal range with no other disease. The serum was stored at -25^{o}C until analyzed for Na, K, Ca, Mg, Co, Ni and Pb. The estimation of different metal was performed by using Inductively Coupled plasma optical emission spectrometer (ICP-OES) in all normal controls and hepatitis C patients. It was observed that none of the metal had significant differences (P>0.05) at serum level between HCV patients and controls both in male or female groups indicating no direct effect of disease on the metals except Ni. It was observed that Ni metal was not present in the serum of HCV infected patients where as in controls normal levels of Ni were observed. No previous supportive work on the issue could be traced, which needs specific study. It may be assumed that none of these metals can be taken as indicator of HCV for early disease diagnosis.
Keywords: HCV, RNA, Virus, Hepatitis, Cirrhosis, ICP-OES.

Received September16, 2016; **Revised** October29, 2016; **Accepted** November29, 2016
[*]**Correspondence:** Saghir Ahmad Jafri, **Email:** jafri43@yahoo.com, **Contact**: +923009488693

***To cite this manuscript*:** Jafri SA, Zaigham K, Mukhtar K, Imran M, Shahzad KA. Serum metal levels as diagnostic indicators of Hepatitis C Virus Infection. Biomed Lett 2016; 2(2):99-102.

Introduction

Hepatitis is an inflammation of the liver. Viral hepatitis refers to several common infectious diseases caused by viruses that attack the liver [1]. The most important types of viral hepatitis throughout the world are hepatitis B, and C [2]. Hepatitis C is a viral infection of the liver. When the liver malfunctions or infection continues for six or more months, it is known as chronic hepatitis. Hepatitis C viral infection (HCV) is a form of hepatitis caused by an RNA virus. HCV accounts for the majority of the hepatitis cases previously referred to as non- A, non-B hepatitis. The hepatitis C virus was first identified and described in 1987. In 1990 a hepatitis C antibody test (anti-HCV) became commercially available to help identify individuals exposed to HCV. In 1995 the hepatitis C virus was seen for the first time ever by scientists with the aid of an electron microscope which was enveloped and encased with glycoprotein peplomers or "spikes" [3].

This infectious process and the ensuing host immune response lead to cell a damage that is the hallmark of liver disease. At least 70% of the individuals who contact HCV will develop chronic infection and hepatitis out of these 20% to 50% of these eventually will progress to cirrhosis and 1 to 2% will develop liver cell cancer after 10 to 20 year period. Once getting inside the cell, the virus directly damages or kills the cell [4]. Human occupational exposure to metals occurs primarily via inhalation and ingestion of metal-containing dust and is particularly high among stainless steel welders, miners, and metallurgy workers. Metals generate reactive oxygen species and other reactive intermediates or react directly with DNA [5].

In a study on hepatic encephalopathy [6] it was determined that iron Mg and Zn are significantly decreased due to hepatic function disturbances and need supplementation. In another study [7] surface enhanced laser desorption/ionization time-of-flight mass spectrophotometry (SELDI/TOF MS) was used to identify serum protein biomarkers liver disease progression by estimating metals but the results were variable.

The present study was designed to find a trend in rise or fall of the minerals in HCV patients as compared to normal controls as indicator of HCV. Main objective of the present study was to compare the

blood serum levels of Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Co^{++}, Ni^{++} and Pb^{++} metal ions between HCV patients and normal persons and to see if serum electrolytes/metals can help as indicators in hepatitis C Diagnosis.

Materials and Methods

The experimental work was carried out in chemistry department of the University of Punjab, Lahore and at Institute of Molecular Biology and Biotechnology (IMBB) of The University of the Lahore, Lahore. In all one hundred (100) individuals (50 HCV patients and 50 healthy controls) were included in the study. These two groups were further divided to two sub groups having 25 male and 25 females) in each. The HCV positive patients from different hospitals of Lahore and its adjoining areas were taken as experimental subjects. The control group was included after thorough medical and lab reports verifications of metal levels in normal range with no HCV other disease. The informed consent was obtained from all patients and control individuals. All the HCV patients of both sexes and the control individuals were between the ages of 35 to 60 years. All the HCV infected patients included in the study were in initial stages of liver damage as revealed by liver scan of their records and had Hb conc. above 9g/dl. Inductively coupled plasma optical emission spectrometer (ICP-OES) was used to estimate Co, Ni, Pb, Na, K, Ca and Mg metal Ions in the blood serum of HCV infected patients and controls. The estimation was done by standard curve drawing. Results were analyzed statistically by applying ANOVA and t test for the calculation of means/ statistical significance between metal levels of patients and controls (steel and Torrie, 1982).

Results

The results of present study are presented in Table 1 pertaining to serum metal levels for HCV patient in comparison with control group with statistical significance. Sodium (Na) metal ion concentration in 25male and 25 female HCV infected patients and 25 normal persons (male and female each). There was no significant differences (P>0.05) in serum Na concentration between HCV patients and normal person both in male and female and within the gender. Results obtained for the serum potassium (K) metal ion concentration (table 1) in the same subjects has been presented in table. It showed no significant differences (P>0.05) in serum K metal ion concentration between HCV patient and normal person both in male and female similar to Na ion results. Serum calcium (Ca) metal ion concentration in HCV infected patients and normal person (male and female) it again showed no significant differences (P>0.05) in serum Ca metal ion concentration between HCV patient and normal person both in male and female between or within the groups. Determination of serum Magnesium (Mg) metal ion concentration showed no significant effect of HCV on serum Mg metal ion concentration between HCV patient and normal person both in male and female(P>0.05)within groups or gender values for Mg. Results obtained for the serum cobalt (Co) metal ion concentration in HCV infected patients and normal persons (male and female) showed no significant effect(P>0.05) of HCV on serum metal ion concentration between infected patient and normal person both in male and female. Serum Nickel (Ni) metal ion concentration also showed non-significant changes (P>0.05) between HCV infected patient and normal person both in male and female (**Table 1**).

Table 1: Statistical Analysis of Metals ion Serum levels of HCV patients and controls

Sr. No.	Metals	Concentration in PPM of HCV Infected Patients n=25		Concentration in PPM of HCV Control Patients n=25		p Value
		Male	Female	Male	Female	
1	Na	3.578± 0.193	3.511± 0.283	3.108±0.142	3.140±0.159	8.49
2	K	0.210±0.052	0.209±0.0515	0.160±0.020	0.177±0.029	2.25
3	Ca	0.105±0.032	0.087±0.012	0.088±0.023	0.092±0.005	0.55
4	Mg	0.048±0.011	0.051±0.011	0.028±0.005	0.032±0.0004	39
5	Co	1.958±1.362	1.716±0.782	0.878±0.800	0.453±0.314	12.8
6	Pb	0.800±0.656	1.284±1.180	0.564±0.431	0.527±0.251	1.91
7	Ni	Nil	Nil	0.259±0.175	0.276±0.151	31.47

Significance: $p<0.05$

Discussion

Looking at the results somewhat higher level of sodium was found in the blood serum from male patients and controls though the differences were statistically non- significant. Serum sodium (Na) concentration in HCV patients (male and female) as

compared to normal individuals did not show any significant effect (P>0.05). These findings were in disagreement with the result of Irnius et al [8] who conducted a similar study and reported higher sodium levels in HCV patients but non- significant, which might be due to stage of the disease which may be different in all studies. The lower serum potassium levels were observed in HCV patients but the differences were non- significant (P>0.05) and level of potassium in male or female as compared to controls was also similar).

The level of K metal also compared between male and female separately which showed slightly higher K ion in females as compared to healthy control males and the difference were similar which correlated with the findings of Irnius et al [8].

The serum calcium levels of HCV patients compared with controls both in male and female groups also showed similar values. These findings are in agreement with the previous researchers' reports [9]. No relation was observed for Ca metal ion in HCV infected patients with HCV or control. There were slight variations in metal levels within and between the groups which might be due to individual disease status but no statistically significant differences were observed.

Serum Mg metal ion concentration increased with the increasing duration of the illness but insignificantly. Contrary to the findings of our study where non-significant differences existed (P>0.05) in serum Mg levels of normal and HCV patients, Versieck et al [10] reported significantly elevated serum Mg levels in HCV positive cases (P<0.05) as compared to control persons. These differences might have been due to severity of the liver damage, because the patients included in our study were in the initial stages of liver damage.

Serum magnesium (Mg) concentration in HCV patients males compared to normal individuals showed non-significant differences (P>0.05) in HCV patients as compared to controls.). In contrast to these findings Bukhari et al [11] reported significantly lower Mg level in serum of HCV patients as compared with normal persons. The results of different researches may be different due to the duration or severity of cirrhosis or other physiological factors confounding.

Interestingly, according to the previous research of Guo et al [12]. The concentration of Pb ion was found to be higher than the normal range in HCV patients, which is not in correlation with the findings of present study. However no traces of Co and Ni were found in either of the selected samples with Hepatitis C in his research. This correlated partly with the results of the present work where Ni ions were not found in HCV infected patient but was present in control group serum.

In the present study inductively coupled plasma optical emission spectrometer (ICP-OES) was used to analyze this metal ion in blood serum of HCV infected patients and control samples. According to the previous research of Steel and Torrie [13] Co metal ion was not found in the blood serum of HCV infected patient but was present in control group.

Serum cobalt (Co) concentration in our study in HCV patients (male and female) as compared to normal individual showed non-significant difference. These finding are in disagreement with the result of Irnius et al [8] who conducted a similar study. Serum cobalt (Co) concentration in HCV infected patients male and female as compared to normal same gender were also similar.

According to Thimmaraju and Biswajit [14] Pb metal ion was found in the blood serum of HCV infected patient. But there were no significant change of this metal ion with HCV infection. Metal ion was higher in male as compared to female but in control samples female had higher Pb levels though the differences were non- significant (P>0.05). The findings are in correlation with the results of our study where no significant difference was observed between serum Pb levels in HCV patients or controls in both the genders. 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.

Nickel (Ni) metal was not present in the blood serum of HCV infected patient but present in trace amount in the blood serum of normal persons which correlates with the findings of the present study. These finding were in agreement with the results of Willieet al [15] who conducted a similar study.

Conclusion

It can be concluded from our results regarding the level of these metal ions in the blood serum of HCV infected patients and their comparison with normal persons that, sodium, potassium, calcium, magnesium, cobalt, nickel being similar in both the groups may not be considered as indicators of HCV and have no correlation with the chronicity of the disease in both the genders. The absence of Ni in HCV patient's serum was only supported by only one researcher reported above and needs specific work.

The excess or deficiency of any of the metal may be taken for individual case with its correlation to specific symptoms if observed in any patient with some other disease along with HCV.

Acknowledgement

We acknowledge Ms. Rabail Alam, Lecturer, IMBB The University of Lahore, Lahore, Pakistan for providing assistance in statistical analysis of the study.

References

[1] Puro V, Girardi E, Ippolito G. Prevalence of hepatitis B and C viruses and human immunodeficiency virus infections in women of reproductive age. Br J ObstetGynaecol 2002; 99: 598-600.

[2] Nelson D, Lau J. Pathogenesis of hepatocellular damage in chronic hepatitis C virus infection. Clinics in Liver Diseases. J Infect Dis 2003;1: 515-528.

[3] Alter M, Margolis H, Krawezynski K. The natural history of community acquired hepatitis C in the United States: the Sentinel Countries Chronic non- A, non-B Hepatitis Study Team. N. Eng1. J. Med 2002; 327: 1899-1905.

[4] Schreiber GB, Busch MP, Kleinman HS. The risk of transfusion transmitted viral infections. N Eng1 J Med 2006; 334: 168-169.

[5] Kasprzak MK, Chakroborty R, Bannazaden T. Introduction to metal ions in human body. Am J Clin Path 2002; 16:250-262.

[6] Devrajani BR, Aneela AR, Shah SZA, Qureshi GA. Serum trace metals and enzyme activity in patients with Hepatitis encephalopathy. World Applied Sci J 2012; 16(8): 1053-1059.

[7] Schwegher EE, Cazares L, Steel LF, Adams BL, Johnson DA, Semmes OJ, Block TM, Marrero JA, Drake RR. SELDI-TOF MS profiling of serum for detection of progression of chronic HCV. Hepatology 2015; 61(6):22-24.

[8] Irnius A, Deleris G, Petibois C. Rapid quantitative determination of metals in blood and liver by FAAS. J At Spectrosc 2005; 16: 29-33.

[9] Diana U, Tautkus S, Kareiva A, Irnius A. Analysis of Mg, Ca, and Sr distribution between blood plasma and blood cells. Germ J Buio1 2007; 18: 40-43.

[10] Versieck J, Barbier F, Speeke A and Hoste, J. Manganese, copper and zinc concentrations in serum and packed blood cells during acute hepatitis, chronic hepatitis and post hepatitis cirrhosis. ClinChem2015; 20: 1141-1145.

[11] Bukhari IH, Hassan MN, Haleem A, Bhatti MM. Role of metals (Cobalt and lead) in patients of hepatitis and their relationship with normal persons. Res JAgrBio1Sci 2005; 2: 190-194.

[12] Chih-Hung G, Chen CP, Lin KP, Shih MY, Sheng KW. Trace Metal imbalance associated with Oxidative Stress in HCV positive subjects. Environmental ToxicolPhamacol 2002; 2:288-296.

[13] Steel RGD and Torrie JH. Principal and Procedures of Statistics Mc. Gra. Hill. Book co. inc.^{2nd}New York (USA) (1982).

[14] Thimmaraju K.V. &Biswajit D. A Comparison of study of Serum Magnesium in Cirrhosis of liver and Infective Viral Hepatitis. J AdvRes Biological Sci 2011; 3(2):7-10

[15] Willie, Y Iida and Mclaren JW. Determination of Cu, Ni, Zn, Mn, Co, Pb, Cd, and V in seawater Using Flow Injection ICP-MS. J AtSpectrosc 2004; 19: 67-73.