Estimation of blood manganese (trace element) levels in patients of hepatitis C, cirrhosis and hepatocellular carcinoma

Mohibullah Khan[1], Shabir Ahmed Orakzai[2], Walayat Shah[3], Asif Ali[3], Adnan Sarwar[1], Mehak Husnain[4], Fatima Dawood[1], Naveed Shareef[3]

[1]*Pak International Medical College, Peshawar*
[2] *Swat Medical College, Swat*
[3] *Khyber Medical University, Peshawar*
[4] *Pakistan Institute of Medical Sciences, Islamabad*

ABSTRACT

Objective

To determine the levels of manganese (Mn) trace element in patients with cirrhosis, hepatitis C, and hepatocellular carcinoma (HCC).

Methodology

Samples were collected from patients at tertiary care hospitals in Pakistan over a period of two years. In total, 90 patients who were diagnosed with hepatitis C virus infection (HCV), cirrhosis and hepatocellular carcinoma (HCC) were selected. Blood manganese (Mn) levels were quantified using atomic absorption spectrometer in the three groups and mean with standard deviation of Mn was calculated. Moreover, frequency and percentage of different variables was calculated. Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) was used for comparing manganese mean levels within the study groups. P value less than 0.05 was considered statistically significant.

Results

In cirrhotic patients there is no change in the mean concentration of manganese in the body (mean ± SD, 0.01±0.015) compared to normal reference values; while, the mean concentration of manganese is high in patients with hepatitis C (mean ± SD, 0.32±0.66) and in patients with hepatocellular carcinoma (mean ± SD, 0.36±0.65) (p=0.02, ANOVA). When Tukey's HSD is applied, a statistically significant change in the Mn level is found between cirrhosis and HCC patients (*p*-value=0.03).

Conclusion

High levels of manganese can compensate the loss of superoxide dismutase (SOD) in the patient body by protecting against oxidative stress. Present study suggests that manganese has differential levels in HCV, cirrhosis and HCC. Thus, the evaluation of blood manganese levels may be performed periodically in chronic liver diseases to assess the potential progression of liver diseases.

Key words: Hepatocellular carcinoma, cirrhosis, hepatitis C virus, manganese

*For Correspondence.

Dr. Shabbir Ahmed Orakzai

Associate professor, Swat Medical College, Swat.
Email: drshabiramc@gmail.com

INTRODUCTION

Liver disorders are the major cause for worldwide mortality and fatality.[1] The prevalence of a particular liver disease differs with topographical areas. It effects almost all the countries either they are highly developed, developing or economically underdeveloped countries. The liver diseases can be the contagious diseases of the liver or it can be due to neoplastic changes. About 29 million people were diagnosed with liver disorders in 2013 across the world.[2] This figure increased in 2017 and 30 million people were diagnosed with liver disorders in developed countries.[3] After cancer and ischemic heart diseases liver disorders are the common cause of death in United Kingdom. In India, 10 million cases of liver disorders has been reported in 2019. Among all these liver disorders, hepatitis C gains a lot of attention because it causes chronic liver inflammation and hepatocellular carcinoma (HCC) which eventually results in cirrhosis.[4] Recent research suggests that 80% of hepatocellular carcinoma patients has hepatitis C infection. The total account of

This article may be cited as: Khan *et al.* Estimation of blood manganese (trace element) levels in patients of hepatitis C, cirrhosis and hepatocellular carcinoma. Adv Basic Med Sci. 2022; 6(1): 03-07

deaths due to liver diseases per year across the globe is about 2 million of which 1 million is due to cirrhosis and 1 million is due to hepatitis and hepatocellular carcinoma. The 11th dominant cause of death across the world is cirrhosis, while cancer of liver is 16th common cause of death, totally they constitute about 3.5% of the deaths across the world.[5]

Liver, the major and most important organ in the body, has ability of regeneration.[6] But alcohol consumption and virus if in the body can damage the liver. So the liver gets injured and scared, ultimately result in losing the functions just like bile production, blood purification and formation of blood clotting proteins. If this damage prevails then it result in cirrhosis of liver.[7]

Hepatocellular carcinoma (HCC) is usually the common type of liver cancer. It mostly occurs in people having chronic liver diseases just like cirrhosis or infection of hepatitis C. Those people who have excess amount of fat on liver and those who drinks alcohol on regular basis have higher risk of developing hepatocellular carcinoma (HCC).[8] It also spreads from liver cells (hepatocytes) to other organs like pancreas, stomach and intestine.

Hepatocellular carcinoma is the sixth most prevalent cancer worldwide.[9] In 2012, 745000 deaths were reported due to hepatocellular carcinoma. Around 85% cases are found in underdeveloped countries. In Pakistan, 3.7-16% population is affected by HCC having lower chances of treatment.

The main cause of liver diseases are parasites and virus infections, alcohol consumption, accumulation of fat on liver, abnormal inherited genes which damage the liver (Wilson's disease), exposure to chemicals and drugs, and toxicity of trace elements.[10] The current paper is aimed to evaluate the estimation of blood trace elements in patients of hepatitis C, hepatocellular carcinoma (HCC) and cirrhosis.

Trace elements are defined as those elements of the human body which are present in minute quantity i.e. 0.0001g/g or 0.1g/l but play vital role in growth, oxidation-reduction reactions and metabolism.[11] Most common trace elements are zinc, copper, manganese, and iron. But recent studies shows that the trace elements are a risk factor for the cancer development. They inhibit or activate many enzymatic reactions which affect cell membrane permeability, compete with other major elements and metalloproteins.[12]

In Pakistan high prevalence of liver disorders has been observed in recent years especially in Khyber Pakhtunkhwa. Thus, we aimed to explore the relationship of blood trace elements with the hepatitis C virus, cirrhosis, and hepatocellular carcinoma (HCC). This research study is aimed to determine the levels of manganese (Mn) trace element in patients with cirrhosis, hepatitis C, and hepatocellular carcinoma (HCC).

METHODOLOGY

Samples were collected from patients at tertiary care hospitals in Peshawar, Pakistan. For this research study, 90 patients were selected whom were diagnosed with cirrhosis of liver, chronic hepatitis C and hepatocellular carcinoma. All these patients were chosen with the help of a non-probability convenient sampling technique. This research study is conducted after the approval of the ethical committee of Khyber Medical University, Peshawar (DIR/KMU-EB/BT/000024).

Those patients were selected having positive ELISA report of hepatitis C, no age restriction with the biopsy and had a radiological evaluation of new cancer with positive alpha-fetoprotein.

Patients who were diagnosed with diseases like Menkes disease, Hepatitis C patients on interferon therapy or Wilson disease were not part of this research study. Patients with history of chemotherapy and radiotherapy and those using any sort of mineral supplements for the last four months were also excluded.

After complete scrutiny of patient history, the blood collection kit (syringe, aseptic solutions, and blood collection tubes) was opened. Patient comfort was our priority and the sample was taken with biosafety measures.

Venous blood samples of all patients were taken in the morning time with a sterile syringe. Almost 3ml of blood sample was taken and kept in evacuated tubes which are heparinized. The samples were stored in the refrigerator before the process of digestion. Samples were further proceeded with the help of an atomic absorption spectrometer located in the Nuclear Institute for Food and Agriculture (NIFA) department. 10 ml Nitric acid and Perchloric acids were mixed with blood samples in a 4:1 ratio and kept overnight. Furthermore, blood samples were heated until white fumes appear and cooled down at room temperature. 50ml distilled water was used in the cooling process.

At NIFA institute, 213 wavelengths of atomic absorption spectrometer were set. Also set a 4.0 Hallow cathode (HC) lamp current along with 0.5 slit width and 2.0 fuel gas flow rate per minute.

Frequency statistics were calculated to describe the variables. Nominal variables were presented in the form of percentage; whereas, continuous variables were presented in the form of mean and standard deviations. For inferential statistics, analysis of variance (ANOVA) was used. Tukey's honestly significant difference (HSD) was used for comparing manganese mean levels within the study groups. The p-value which is less than 0.05 was considered statistically significant in all types of analyses. SPSS version 23 was used for all statistical analyses.

RESULTS

Blood Manganese distribution in the study group

Blood manganese levels were evaluated in the study groups. Mean blood manganese levels in different study groups, frequency and its association with different age groups are given below.

The value of mean blood manganese level in the study group are given as,

- Cirrhotic patient has 0.01±0.015 mg/L
- Hepatitis C patients has 0.32±0.66 mg/L
- HCC patients has 0.36±0.65 mg/L

The results shows that mean manganese level in cirrhotic patients is less than hepatitis C patients while hepatocellular carcinoma has more mean value than hepatitis C patient. The mean and standard deviation values of manganese evaluated in the study group is given in the table 1 and figure 1.

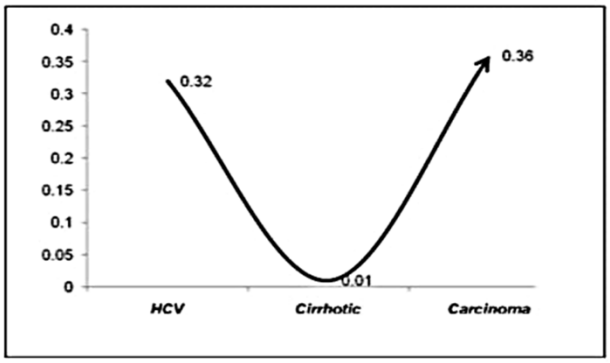

Figure 1: Differential levels of Mn in different groups of patients.

Parameter	HCC	Chronic HCV	Cirrhosis	P value
Manganese	0.36±0.65	0.32±0.66	0.01±0.015	0.025

p value calculated through ANOVA. The unit of mean value is mg/L.

Table 1: Mean values of Mn in different patients groups

Tukey honestly significant difference (HSD) comparing manganese mean levels within the study groups. When Tukey's HSD is applied in the study a statistically significant change is found between cirrhotic and HCC (p-value=0.03) (Table 2).

	Study Group	*p*-Value
HCC	Cirrhotic	0.037
	HCV	0.940
HCV	HCC	0.940
	Cirrhotic	0.081
Cirrhotic	HCC	0.037
	HCV	0.081

Based on observed means in HCV, Cirrhosis and HCC (0.32 mg/L, 0.01 mg/L, 0.36 mg/L respectively

Table 2: Tukey's HSD for subgroup analysis

Percentage and frequency wise distribution of manganese levels in the study groups

The normal range of blood manganese levels is 0.004-0.015 mg/L in healthy individuals. Out of the 30 cirrhotic patients; 15 had normal, 11 had high and 4 had low Mn levels. Moreover, Out of the 30 patients with hepatocellular carcinoma; 5 had normal, 23 had high and 2 had low Mn levels. Finally, Out of the 30 patients with chronic hepatitis C infection; 11 had normal, 14 had high and 5 had low Mn levels (Table 3).

Chemical parameter		Cirrhotic No (%)	Hepatocellular carcinoma No (%)	Chronic HCV infection No (%)
Manganese	Normal	15 (50.0)	5 (16.7)	11 (36.7)
	High value	11 (36.7)	23 (76.7)	14 (46.7)
	Low value	4 (13.3)	2 (6.7)	5 (16.7)
	Total	30 (100)	30 (100)	30 (100)

Table 3: The percentage and frequency wise distribution of manganese levels in different patients groups

Manganese levels in different age groups

To understand the relation between age groups and mean blood manganese levels in patients of cirrhosis, hepatitis C and HCC, ANOVA was applied using SPSS version 23. The results showed that there is a close relation between the blood manganese levels in age group of 31-40, 51-60 and >60 with p-value less than 0.05 (Table 4).

Age group	Number of Cirrhotic patients (mean±SD)	Number of patients with carcinoma (mean±SD)	Number of patients with Chronic HCV (mean±SD)	ANOVA
>60	(7) 0.02±0.021	(17) 0.21±0.445	(3) 1.37±1.186	**0.001***
51-60	(12) 0.01±0.011	(7) 0.81±1.022	(2) 0.54±0.729	**0.039***
41-50	(7) 0.018±0.015	(5) 0.31±0.531	(9) 0.01±0.016	0.096
31-40	(4) 0.02±0.017	0	(7) 0.01±0.005	**0.000***
20-30	0	(1) 0.02	(9) 0.46±0.734	0.583

** Age in years. SD is standard deviation. P value calculated through ANOVA. Significance at 95% confidence level.*

Table 4: Manganese levels stratified in different age groups

DISCUSSION

Manganese is a main component of various mitochondrial enzymes. It is involved in the metabolism of carbohydrates, proteins (amino acids) and lipids (cholesterol). It also scavenges the reactive oxygen species present in the body.[13]

In the present research study, there is high level of manganese in the blood of patients of viral hepatitis C which are in line with the findings of Versieck *et al.* and Rashed *et al.*[14] While according to Minhe *et al.* there is no change in the concentration of manganese in the blood of viral hepatitis C patients.[15]

In the cirrhotic patients, the mean level of manganese were present in normal amount in our study. This finding support the published literature (Moscarella *et al.)*[16] that shows that there is no noticeable change in the blood level of manganese of the cirrhotic patients.[16] However, other studies (Hamed *et al.* & Rahelic *et al*.)[17] shows that cirrhosis patients have higher level of manganese in their blood.[17]

Furthermore, in the current study, hepatocellular carcinoma patients have higher level of manganese in their blood. This was also shown by Teitz *et al.* While no notable difference in blood manganese level of hepatocellular and normal individual was found by Takikawa S.[18]

Level of manganese in blood suggests that manganese is stored in blood cells in different concentration. Red blood cells contain 65% of manganese, white blood cells and platelets contain 30% while the plasma has remaining 5% of the blood manganese in a healthy individual.

Liver is involved in the excretion of manganese so the concentration of manganese is higher in liver as compared to the manganese concentration in blood. Manganese has antioxidant and anticancerous property so it inhibits the growth of tumor or cancer in the body. Balo and Banga [19], suggested that the inhibitory effects of manganese by converting anaerobic to aerobic metabolic process[19]. Manganese is a cofactor in many enzymes and the most common example is manganese superoxide dismutase (Mn-SOD) which is an important enzyme present in mitochondria and protects mitochondria from oxidative damage.[20] If the mutation occur in the genes encoding Mn-SOD this will decrease its anti-oxidant property and may impart the carcinogenic process in HCC progression. If the concentration of manganese increases in the body then it can make up the loss of superoxide dismutase enzyme by scavenging free radicals.[21]

The inverse relationship was found in the blood manganese level and the oxidative stress. Therefore, two assumptions can be made for the increased level of manganese in the blood in hepatocellular carcinoma patients and hepatitis C patients. One assumption is that in chronic hepatitis C infection and HCC, there is damage in liver parenchyma cells which cause the release of hepatic manganese. Whereas, in the second assumption, manganese is excreted through bile whose production decreases in hepatocellular carcinoma so it go along either with intrahepatic shunting or portosystemic shunting.

CONCLUSION

Manganese is a trace element present in the human body. It acts as a cofactor in the formation of enzyme known as Manganese superoxide dismutase (MnSOD) which is present in mitochondria. Manganese super oxide dismutase prevents the cell from oxidative damage. It has been found in the current study that in case of cirrhotic patients there is no change in the concentration of manganese in the body; while, the concentration of manganese increases in hepatitis C patients and in hepatocellular carcinoma. High levels of manganese can compensate the loss of superoxide dismutase (SOD) in the patient body by protecting against oxidative stress or damage. Thus manganese may have potential anticancerous activity. Blood level of manganese is also increased in the patient suffering from hepatocellular carcinoma. Present study suggests that manganese has differential levels in HCV, cirrhosis and HCC. Thus, the evaluation of blood manganese levels may be performed periodically in chronic liver diseases to assess the potential progression of liver diseases.

REFERENCES

1. Vilar-Gomez E, Calzadilla-Bertot L, Wong VW, Castellanos M, Aller-de la Fuente R, Metwally M, Eslam M, Gonzalez-Fabian L, Sanz MA, Conde-Martin AF, De Boer B. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: a multi-national cohort study. Gastroenterology. 2018 Aug 1;155(2):443-57.
2. Blachier M, et al. The burden of liver disease in Europe: a review of available epidemiological data. Journal of Hepatology, 2013; 58(3): 593-608.
3. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, AlvisGuzman N, Amoako Y, Artaman A, Ayele TA. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. JAMA oncology. 2017 Dec 1;3(12):1683-1691
4. Yarlott L, Heald E, Forton D. Hepatitis C virus infection, and neurological and psychiatric disorders–A review. Journal of Advanced Research. 2017 Mar 1;8(2):139-48.
5. Xiao J, Wang F, Wong NK, He J, Zhang R, Sun R, Xu Y, Liu Y, Li W, Koike K, He W. Global liver disease burdens and research trends: analysis from a Chinese perspective. Journal of hepatology. 2019 Jul 1;71(1):212-21.
6. Ozougwu J. Physiology of the liver. International Journal of Research in Pharmacy and Biosciences. 2017;4(8):13-24.
7. Pimpin L, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, Sheron N, Easl Hepahealth Steering Committee. Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. Journal of Hepatology. 2018 Sep 1;69(3):718-35.
8. Ogunwobi OO, Harricharran T, Huaman J, Galuza A, Odumuwagun O, Tan Y, Ma GX, Nguyen MT. Mechanisms of hepatocellular carcinoma progression. World journal of gastroenterology. 2019 May 21;25(19):2279.
9. Beal EW, Tumin D, Kabir A, Moris D, Zhang XF, Chakedis J, Washburn K, Black S, Schmidt CM, Pawlik TM. Trends in the mortality of hepatocellular carcinoma in the United States. Journal of Gastrointestinal Surgery. 2017 Dec 1;21(12):2033-8.
10. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K,

Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease:

11. practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018 Jan;67(1):328-57.
12. Al-Fartusie FS, Mohssan SN. Essential trace elements and their vital roles in human body. Indian J Adv Chem Sci. 2017;5(3):127-36.
13. Prashanth L, Kattapagari KK, Chitturi RT, Baddam VR, Prasad LK. A review on role of essential trace elements in health and disease. Journal of dr. ntr university of health sciences. 2015 Apr 1;4(2):75.
14. Erikson KM, Aschner M. Manganese: its role in disease and health. Essential Metals in Medicine: Therapeutic Use and Toxicity of Metal Ions in the Clinic. 2019 Jan 14;19:253-66.
15. Versieck J, Barbier F, Speecke A, Hoste J. Manganese, copper, and zinc concentrations in serum and packed blood cells during acute hepatitis, chronic hepatitis, and post-hepatitic cirrhosis. Clin Chem. 1974;20(9):1141-5
16. Minhe L, J. Huimin, X. Baiquan, C. Relationship between viral heptatitis and four trace elements in sera. Varian Instruments at Work Number AA-89, China. 1989.
17. Moscarella S, Duchini A, Buzzelli G. Lipoperoxidation, trace elements and vitamin E in patients with liver cirrhosis. European Journal of Gastroenterology & Hepatology. 1994;6(7):633-6.
18. Hamed SA, Hamed EA, Farghaly MH, Ezam KA. Trace elements and flapping tremors in patients with liver cirrhosis. Is there a relationship? Saudi medical journal. 2008;29(3):345-51
19. Takikawa S. Changes in serum Zn, Cu, Se, and Mn levels in patients with chronic liver diseases and hepatocellular carcinoma. Journal of Clinical Biochemistry and Nutrition. 1990;8(2):153-64.
20. Balo J, Banga I. Effect of metal complexes upon experimental carcinoma. Acta - Unio Internationalis Contra Cancrum. 1957;13(3):463-5.
21. Bresciani G, da Cruz IB, González-Gallego J. Manganese superoxide dismutase and oxidative stress modulation. InAdvances in clinical chemistry 2015 Jan 1 (Vol. 68, pp. 87-130). Elsevier
22. Azadmanesh J, Borgstahl GE. A review of the catalytic mechanism of human manganese superoxide dismutase. Antioxidants. 2018 Feb;7(2):25.