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Evaluation of iron metabolic alteration in patients suffering from hepatitis B and C: a study in local population of Punjab, Pakistan

Kalsoom Zaigham[1*], Maryam Tariq[1], Rahina Ahmed[1], Khawar Ali Shahzad[1, 2], Muhammad Shahzad Iqbal[3], Muhammad Zaigham Javed[1]

[*1] Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore 54000, Pakistan
[2] Department of Microbiology and Immunology, Southeast University, Nanjing 210009, China
[3] Center of Excellence in Molecular Biology, University of the Punjab, Lahore 53700, Pakistan

Abstract

Iron overload and altered metabolism are the consequences of many disorders frequently observed in chronic liver diseases i.e., hepatitis B virus and hepatitis C virus. The present study, therefore, was aimed to evaluate the effect of HBV and HCV on iron metabolism. For this purpose blood samples of 25 patients with HCV and 25 patients with HBV were collected from Mayo hospital, Lahore, Pakistan along with 25 normal individuals. Serum was separated and processed for the estimation of markers of iron metabolism via free iron, ferritin, total Iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) by performing ELISA and flame atomic absorption spectroscopy. Ferritin levels were measured separately in male and female individuals. The outcome showed significant alteration in mean levels of free iron, TIBC and UIBC in case groups (HBV and HCV) as compared to control group while insignificant alteration was observed in mean levels of ferritin in serum of HCV and HBV male patients as compared to control group. It was concluded that the manifestation of HBV and HCV significantly altered basic parameters of iron metabolism; however, the level of alteration of ferritin depends upon the viral load of HCV and HBV resulting in significant or insignificant alteration in ferritin level.

Keywords: Hepatitis B virus, Hepatitis C virus, total iron binding capacity, unsaturated iron binding capacity.

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***Corresponding author**: KalsoomZaigham; **Email:** kalsimbb@yahoo.com

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Introduction

Persistent infection with hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million infected people worldwide [1]. It is well established that about 20% of patients with chronic hepatitis C (CHC) progress to cirrhosis within 20 years of infection [3]. The CHC patients frequently develop mild to moderate iron overload [4]. Many experimental and clinical studies [5-7], though not all [8, 9], suggested that the excessive iron in CHC is a cofactor that promotes the progression of liver damage and increases the risk of fibrosis, cirrhosis, and Hepatocellular carcinoma (HCC). Most of the HCV-infected patients develop a chronic slowly progressive liver disease that may result in cirrhosis and hepatocarcinoma. Several factors have been proposed to explain this unfavorable evolution, such as male gender, age at infection and alcohol abuse [10].

The liver is an important organ in iron homeostasis. Besides, the liver is involved in iron storage, produces transferrin and hepcidin, iron carrier proteins in plasma and a hormone regulating iron metabolism, respectively [11, 12]. Another aspect of the relationship between iron and the liver is that this organ is one of the main targets in hemochromatosis [13]. Serum iron (SI), total iron binding capacity (TIBC) and ferritin levels are the principal tests used in the evaluation of iron burden. Another frequently used parameter, transferring saturation (TS), is calculated by dividing the SI level by TIBC, and it shows the percent saturation of transferrin.

Testing and understanding of serum iron parameters in liver disorders is important for different reasons: Serum TS and ferritin levels are used for the screening of hereditary hemochromatosis [14]. It has been proposed that iron might be important for the progression of liver fibrosis in viral hepatitis, and serum iron parameters, especially ferritin level, might reflect hepatic iron accumulation [15]. Anemia is very frequent in cirrhotic patients for many different reasons, including iron deficiency [16]. Identification of iron deficiency in these patients is especially important, because it is an easily correctable cause of anemia. Total iron binding capacity (TIBC) level (i.e., transferrin activity) may change in hepatic disorders as transferrin is produced in the liver [17, 18]. Ferritin is increased in many patients with acute and chronic liver diseases (CLDs) [17, 18]. Therefore, serum iron parameters may not truly reflect iron homeostasis in hepatic disorders. It has been proposed that serum iron parameters were unreliable in CLD, and that systemic iron overload should be confirmed histologically in these patients [19, 20].

However, the effect of the severity of hepatic compromise on the test results has not been clearly defined in any study. Similarly, differences between liver disease- and iron overload-related iron parameter

changes have not been clarified. Previous studies related to iron homeostasis in CLDs might be confounded by difficulties in the diagnosis of hereditary hemochromatosis. Currently, this disease can be easily defined by genetic testing. We aimed to describe the effect of CLD severity on serum iron tests in patients with different Child-Pugh stages of liver cirrhosis unrelated to hemochromatosis and with chronic viral hepatitis and to elucidate differences in liver disease- and iron overload-related iron parameter changes.

Materials and methods

Collection of blood samples

The blood samples of 25 patients of hepatitis B and hepatitis C, along with 25 normal individuals were collected from Mayo hospital Lahore, Pakistan. The biochemical analysis was done at Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan. Blood samples serums were separated from blood samples and were subjected to biochemical analysis for iron metabolism biomarkers via estimation of free iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and ferritin level. The data were analyzed statistically by using multiple comparison and independent T test. Ferritin levels were measured separately in male and female individuals as iron accumulation and loss determine steady-state levels of body iron stores and ferritin levels remained relatively low in women until menopause. The relatively low iron store typical of premenopausal women represented by serum ferritin values is because of menstrual blood loss.

Estimation of total iron in serum

The technique used to detect iron concentrations in human blood was flame atomic absorption spectroscopy (FAAS). The iron stock solution was prepared by dissolving 10 mg of iron metal strips in a minimum volume of 1:1 HCL/HNO_3 and diluted to 1 dl of deionized water, which gave 10 mg/dl iron stock solution. The standard calibration curve was prepared by running a series of standard solutions ranging from 1.0-10 mg/dl made from iron stock solution. The instrumental parameters were adjusted to manufacturer recommendations. An iron hollow cathode lamp operating at 248.3 nm was used as the radiation source.

Estimation of TIBC

Total iron-binding capacity was estimated by kit method. The iron reagent 1.0 ml and sample 0.5 ml were mixed well. After 3-5 min, one level measuring spoonful of aluminum oxide (approximately 0.25-0.35 g) was added and mixed with a mixer for 10 minutes. Later, samples were allowed to stand for 3 min upright or centrifuged for 1 minute at 5,000 rpm. The cap was taken off prior to centrifugation.

Estimation of ferritin

The ferritin quantitative test was based on a solid phase enzyme linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 min incubation at room temperature, the wells were washed with water to remove unbound-labeled antibodies. The TMB reagent was added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition of stop solution. The color was changed to yellow, which was measured by spectrophotometer at 450 nm. The concentration of ferritin was directly proportional to the color intensity of the test sample.

Estimation of unsaturated iron binding capacity

For the calculation of latent or unsaturated iron-binding capacity (UIBC) the serum iron was subtracted from the TIBC (UIBC=TIBC- iron).

Statistical analysis

The data was analyzed statistically by using multiple comparison and independent T test. In addition, standard deviations were calculated.

Results

The level of alteration of iron metabolism was accessed by the estimation of iron, estimation of TIBC, estimation of UIBC and estimation of ferritin. A significant ($p<0.05$) increase in serum iron levels was observed in a group of individuals suffering from hepatitis B and C as compared to control group individuals.

Table 1: Estimation of iron (mg/dl)

Groups	Mean ± SD	Significance
Control (Normal)	2.625 ± 0.418	
Hepatitis B	3.505 ± 0.760	0.000*
Hepatitis C	3.808 ± 0.634	

"*" Significant as $p < 0.05$; SD = Standard deviation

Table 2: Multiple comparison test of iron (mg/dl).

Group		Mean Difference	Std Error	Sig.	95% confidence interval	
					Lower Bound	Upper Bound
Hepatitis C	Hepatitis B	0.304	0.212	0.158	-0.1223	0.7302
Control	Hepatitis B	1.183*	0.267	0.000	0.6450	1.7218
Hepatitis B	Hepatitis C	-0.304	0.211	0.158	-0.7302	0.1223
Control	Hepatitis C	0.879*	0.265	0.002	0.3454	1.4135
Control	Hepatitis C	-1.183*	0.267	0.000	-1.7218	-0.6450
Hepatitis B	Hepatitis C	-0.879*	0.265	0.002	-1.414	-0.345

*The mean difference is significant at the 0.05 level.

Analysis of serum ferritin level separately in male and female individuals showed overall increase in serum ferritin levels in both male and female individuals suffering from hepatitis B and hepatitis C, however, in males (suffering from hepatitis B and hepatitis C) serum ferritin level was increased insignificantly ($p>0.05$) as compared to control male individuals, while in females, highly significant ($p<0.05$) increase in serum ferritin level was observed as compared to control female individuals. Significantly ($p<0.05$) high TIBC and UIBC were observed in group of individuals suffering from hepatitis B and C as compared to control group individuals.

Discussion

The present research work was aimed to study the level of alteration in iron metabolism in HCV and HBV. Elevated iron parameters and mild iron overload are common in the liver of patients with chronic hepatitis C. It has been suggested that ferritin and serum iron might be correlated with liver inflammation and serum markers of fibrogenesis [21, 22]. Iron overload is associated with higher ALT levels and more severe histological findings in HCV-infected chimpanzees [23]. Increased iron stores may stimulate hepatic fibrogenesis, by leading to oxygen free radical injury and/or by inducing the production of profibrogenic cytokines such as tumor growth factor ß (TGF-ß). However, there is a discrepancy between the frequency of altered iron parameters in serum and in liver tissues. In a study, 40% of patients were observed to have increased iron in serum compared to 10% in tissue [24].

Table 3: Estimation of TIBC

Groups	Mean ± SD	Sig. value	
Control	0.33±0.002		
Hepatitis B	10.52±2.28	0.000[a]	0.186[b]
Hepatitis C	11.42±1.90	0.000[a]	

a = Significant value at p<0.05 as compared to control. b = Non significant value at p>0.05 in comparison between hepatitis B and hepatitis C; SD = Standard deviation.

The presence of iron overload has been reported to possibly be involved in fibrosis progression and in the development of hepatocellular carcinoma, although the studies are controversial [25, 26]. Over the last few years, there has also been much interest in the role of iron in the outcome of antiviral therapy in patients with chronic HCV infection [27, 28]. Several studies have demonstrated that iron overload is associated with lower response rates to interferon-α (IFN-α) monotherapy. Little is known about whether iron overload also has an impact on the response rate to combination therapy with interferon and ribavirin.

In the present study, the insignificant alteration in ferritin level was observed in male individuals suffering from HCV and HBV as compared to control. The outcomes were in contrary with the result of Vaguet et al. [29] as they observed significant elevation in ferritin and low level of TIBC. The level of alteration in ferritin and TIBC depends on the viral load of HCV and HBV. Higher the viral load more will be alteration in ferritin. Insignificant alteration in ferritin and TIBC observed in the present study could be due to a low viral load of patients with HCV and HBV.

Table 4: Estimation of UIBC

Groups	Mean ± SD	Sig value	
Control	0.22±0.001		
Hepatitis B	7.01±1.52	0.000[a]	0.185[b]
Hepatitis C	7.62 ± 1.27	0.000[a]	

a = Significant value at p<0.05 as compared to control. b = Non significant value at p>0.05 in comparison between hepatitis B and hepatitis C; SD = Standard deviation.

Table 5: Estimation of ferritin level in male individuals

Groups	Mean ± SD	Sig value	
Control	135 ± 10.80		
Hepatitis B	228 ± 443.91	0.479[a]	0.828[b]
Hepatitis C	191 ± 226.65	0.506[a]	

a = Significant value at p<0.05 as compared to control. b = Non significant value at p>0.05 in comparison between hepatitis B and hepatitis C; SD = Standard deviation.

Outcomes of iron estimation showed significant alteration in iron level in HCV and HBV as compared to control group. The results were in line with the work of Vagu et al. [29]. In the present study, elevated level of iron could be due to liver damage. Because liver is the main organ for iron metabolism and liver damage strongly affect the iron metabolism.

Estimation of TIBC and UIBC showed a significant alteration in HCV and HBV patients. The results were correlated with the work of Vagu et al. [29]. In the present study, total iron binding capacity with

transferrin elevation could be due to tissue damage of liver imparted by HCV and HBV due to which apotransferrine could not move inside hepatocytes. It will leads to the inactivation of transferring and iron metabolism altered as iron could not bind to transferrin.

Table 6: Estimation of ferritin level in female individual

Groups	Mean ± SD	P value	
Control	65 ± 10.11		
Hepatitis B	140 ± 207.8	0.339[a]	0.185[b]
Hepatitis C	90 ± 91.56	0.355[a]	

a = Significant value at p<0.05 as compared to control. b = Non significant value at p>0.05 in comparison between hepatitis B and hepatitis C; SD = Standard deviation.

Conclusion

The present study showed that iron overload was generated in the conditions of hepatitis C and B by overall elevation of serum iron, serum ferritin levels with high TIBC and UIBC. The observations of pathogenesis of hepatitis C and B in relation to altered iron metabolism confirms the role of iron overload as a non-overrated factor in the clinical course of disease, however, there are still many questions about the molecular mechanisms of the accumulation of iron in individuals suffering from hepatitis C and B. A better understanding of the interplay between HCV, HBV and iron may help to develop effective strategies for the treatment of HCV and HBV.

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Referrences

[1] Bialek SR, Terrault NA. The changing epidemiology and natural history of hepatitis C virus infection. Clin Liver Dis 2006;10:697-715.

[2] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;9:558-567.

[3] Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterol 2004;127:S35-S50

[4] Bonkovsky HL, Troy N, McNeal K, Banner BF, Sharma A, Obando J, Mehta S, Koff RS, Liu Q, Hsieh CC. Iron and HFE or TfR1 mutations as comorbid factors for development and progression of chronic hepatitis C. J Hepatol 2002;37:848-854.

[5] Angelucci E, Muretto P, Nicolucci A, Baronciani D, Erer B, Gaziev J, Ripalti M, Sodani P, Tomassoni S, Visani G, Lucarelli G. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100:17-21.

[6] Beinker NK, Voigt MD, Arendse M, Smit J, Stander IA, Kirsch RE. Threshold effect of liver iron content on hepatic inflammation and fibrosis in hepatitis B and C. J Hepatol 1996;25:633-638.

[7] Chapoutot C, Esslimani M, Joomaye Z, Ramos J, Perney P, Laurent C, Fabbro-Peray P, Larrey D, Domergue J, Blanc F. Liver iron excess in patients with hepatocellular carcinoma developed on viral C cirrhosis. Gut 2000;46:711-714.

[8] Nahon P, Sutton A, Rufat P, Ziol M, Thabut G, Schischmanoff PO, Vidaud D. Liver iron, HFE genemutations, and hepatocellular carcinoma occurrence in patients with cirrhosis. Gastroenterol 2008;134:102-110.

[9] Guyader D, Thirouard AS, Erdtmann L, Rakba N, Jacquelinet S,Danielou H Perrin M, Jouanolle AM, Brissot P, Deugnier Y. Liver iron is asurrogate marker of severe fibrosis in chronic hepatitis C. J Hepato 2007;146:587-595.

[10] Poynard T, Ratziu V, Charlote F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol 2001;34:730-9.

[11] Beaumont C, Beris P, Beuzard Y, Brugnara C. Disorders of iron homeostasis, erythrocytes, erythropoiesis. Eur J Haematol 2006;11(3): 392-406.

[12] Fleming RE, Sly WS. Hepcidin a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. Proc Natl Acad Sci USA 2001;98:8160-2.

[13] Batts KP. Iron overloads syndromes and the liver. Mod Pathol 2007;20:S31-S39.

[14] Phatak PD, Sham RL, Raubertas RF. Prevalence of hereditary hemochromatosis in 16031 primary care patients. Ann Intern Med 1998;129:954-61.

[15] Metwally MA, Zein CO, Zein NN. Clinical significance of hepatic iron deposition and serum iron values in patients with chronic hepatitis C infection. Am J Gastroenterol 2004;99:286-291.

[16] Ozatli D, Koksal AS, Haznedaroglu IC.Erythrocytes: anemias in chronic liver diseases. Hematol 2000;5:69-76.

[17] Jurczyk K, Wawrzynowicz-Syczewska M, Boron-Kaczmarska A, Sych Z. Serum iron parameters in patients with alcoholic and chronic cirrhosis and hepatitis. Med Sci Monit 2001;2108:2113.

[18] Wallach J. Interpretation of diagnostic tests. 6th ed. Boston: Little, Brown and Company, 1996.

[19] Di-Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterol 1992;102:2108–2113.

[20] Valberg LS, Ghent CN, Lloyd DA. Diagnostic efficacy of tests for the detection of iron overload in chronic liver disease. Can Med Assoc 1978;119:229-36.

[21] Pietrangelo A. Iron, oxidative stress and liver fibrogenesis. J Hepatol 1998;28:8-13.

[22] Casaril M, Stanzial AM, Tognella P. Role of iron load on fibrogenesis in chronic hepatitis C. Hepato-Gastroenterol 2000;47:220-225.

[23] Bassett SE, Di Bisceglie AM, Bacon BR. Effects of iron loading on pathogenicity in hepatitis C virus-infected chimpanzees. Hepatol 1999;29:1884-1892.

[24] Riggio O, Montagnese F, Fiore P. Iron overload in patients with chronic viral hepatitis: how common is it? Am J Gastroenterol 1997;92:1298-1301.

[25] Chapoutot C, Esslimani M, Joomaye Z. Liver iron excess in patients with hepatocellular carcinoma developed on viral C cirrhosis. Gut 2000;46:711-714.

[26] Ganne-Carrie N, Christidis C, Chastang C. Liver iron is predictive of death in alcoholic cirrhosis: a multivariate study of 229 consecutive patients with alcoholic and/or hepatitis C virus cirrhosis: a prospective follow up study. Gut 2000;46:277-282.

[27] Olynyk J, Reddy K, Di Bisceglie A. Hepatic iron concentration as a predictor of response to interferon alpha therapy in chronic HCV. Gastroenterol 1995;108:1104-1109.

[28] Hayashi H, Takikawa T, Nishimura N. Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. Am J Gastroenterol 1994;89:986-988.

[29] Vagu C, Sultana C, Ruta S. Serum iron markers in patients with chronic hepatitis C infection. Hep month 2013;13(10):e13136.