Contents lists available at http://jmcrr.in/index.php/jmcrr

Journal of Medical Care Research and Review

Volume 2| Issue 3| 2019

Assessment of iron Parameters and Transient Elastography (FibroScan) Pattern amongPatients with Chronic Viral Hepatitis Infection in Jos, Nigeria

[1]AkinolaOyekemi I, [2]Salawu Lateef, [1]EgesieOchaka J, [3]Okeke Edith N, [1]Damulak Obadiah D, [4]Imoh Lucius C, [1]Akor Emmanuel A

[1]Department of Haematology and Blood Transfusion, Jos University Teaching Hospital, Jos, Plateau State, Nigeria
[2]Department of Haematology and Blood Transfusion, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria
[3]Department of Internal Medicine, Jos University Teaching Hospital, Jos, Plateau State, Nigeria
[4]Department of Chemical Pathology, Jos University Teaching Hospital, Jos, Plateau State, Nigeria

Abstract: ***Background:***The long-termeffect of excess iron deposition in the liver include fibrosis and cirrhosis which may progress to hepatocellular carcinoma. We assessed iron parameters among patients with chronic viral hepatitis B and C infection (CVHBI; CVHCI) to determineif any correlation existed with the degree of fibrosis in the liver.
Methods*:* A cross-sectional descriptive study was carried out on 186 patients, made up of 132 patients withCVHBI and 54 patients with CVHCI. Serum ferritin and C-reactive protein were done by ELISA, serum iron and total iron binding capacity (TIBC) by colorimetric technique while transferrin saturation (Tsat) was calculated using serum iron and TIBC values. Liver fibrosis was assessed using fibroscan.Obtained data wereanalysed using SPSS version 20 and p values < 0.05 were considered statistically significant.
Results*:* The mean values for serum ferritin, iron, TIBC and Tsat were 218.1±325.6µg/L, 25.1±22.8µmol/L, 71.13 ± 35.92µmol/L and 45.2 ± 49.9% respectively. There were no significant differences in iron parameters between patients with CVHBI and CVHCI. Elevated serum ferritin was found in 15.2% and 20.4% of CHBVI and CHCVI patients respectively; while an elevated Tsat was seen in 22.7% and 24.1% of CHBVI and CHCVI patients respectively. Using a combination of elevated serum ferritin and Tsat, the prevalence of iron overload was found to be1.6%. Fibroscan scores did not differ significantly between patients with orwithout elevated iron parameters.
Conclusion*:*Chronic viral hepatitis infection is associated elevated iron parameters though withminimal effect on liver fibrosis.
Conflict of interest*:* Nil

Key words*:* Chronic Viral Hepatitis Infection, Iron Overload, Fibroscan

INTRODUCTION

Chronic viral hepatitis is the main cause of liver cirrhosis and hepatocellular carcinoma (HCC) world over.[1]It is defined as persistent hepatic inflammation with or without periportal necrosis, for over six months from a viral aetiology.[2] Implicated viruses include Hepatitis B virus (HBV) and Hepatitis C virus (HCV). In Jos, Nigeria, the sero-prevalencerates of Hepatitis B surface antigen (HBsAg) and anti-HCV are as high as 15.1% and 4.3% respectively.[3]Replication of these viruses occurs mainly in the liver and also in lymphocytes, spleen and kidneys.[4]

Iron is an important factor in the replication of all organisms including virulent micro-organisms. The role of the liver is central to iron homeostasis as it serves both storage and synthetic functions. Within the hepatocytes, sinusoidal mesenchymal cells and reticuloendothelial cells of the liver are about one-third of the total body iron.[5] Transferrin, ferritin and hepcidin which are important in iron transport and regulation are synthesized in the liver.[5] Any form of liver dysfunction will disturb its functions as well iron homeostasis. This is even more pronounced when excess iron accumulates in the liver.[6]Elevated serum iron, transferrin saturation and ferritin levels have been noted in different studies on patients with chronic viral hepatitis infection. These findings have been linked to an up-regulation of ferroportin due to low levels of hepcidin, with increased export of iron from enterocytes and macrophages.[7] Some studies have associated hyperferritinaemia with poor response to antiviral therapy such as interferon alpha; viral persistence and progression to HCC in chronic carriers of HBV and HCV.[8-10] The role of venesection in lowering serum iron with resultant improvement in liver function test in chronic viral hepatitis has also been documented.[11] At large, excess iron portends a poor prognosis in chronic viral hepatitis. However, most of these studies have been carried out in the western countries, hence this study in a Nigerian population.

MATERIALS AND METHODS

Patients were consecutively enrolled from the Gastroenterology clinic in Jos University Teaching Hospital (JUTH) after ethical clearance was granted. Chronic viral hepatitis infection was confirmed by positive Hepatitis B surface antigen (HBsAg), positive antibody to Hepatitis B core antigen (anti-HBc) and negative Immunoglobulin M anti-HBc (IgM anti-HBc) for chronic HBV infection by immuno-chromatography (LumiQuick diagnostics, California, USA).Patients who tested positive for antibody to Hepatitis C virus (anti-HCV) were considered has having chronic HCV infection. Patients on antiviral therapy, who had been transfused or had a high serum C-reactive protein value were excluded from the study. Venous blood specimen was collected (in fasting state) into a sterile iron-free plain bottle and allowed to clot within 1-2 hours at room temperature. This was then centrifuged to obtain serum and separated into a fresh iron-free plain bottle for analysis(stored at -20^{O}C until required for use).Serum iron indices (iron, total iron-binding capacity (TIBC), and

ferritin) were measuredfor each patient. Serum iron and TIBC were measured by immuno-turbidimetric method (Teco diagnostics, California, USA). Serum ferritin was done using ELISA technique (MonobindInc, Carlifornia USA). Transferrin saturation was calculated and expressed as a percentage of the totaliron-binding capacity (serum iron/TIBC x 100%). The transient elastography (fibroscan) pattern was determined on the Echosens 402 machine and values expressed in kilopascals (kPa). All patients were then graded as no/minimal fibrosis, moderate fibrosis, severe fibrosis, and advanced fibrosis/cirrhosis using the machine score card. The data obtained was analyzed using SPSS version 20.0 statistical package.

RESULTS

We evaluated a total of 186 patients, comprising 132 with CVHBI and 54 with CVHCI between January and October 2017. The demographic and laboratory data are presented in Table 1.The mean (± SD) age of the study population was 43 ± 13years with M:F ratio of 1.2:1. Mean values for serum ferritin, iron, TIBC and Tsat were 218.1±325.6µg/L, 25.1±22.8µmol/L, 71.13± 35.92µmol/L and 45.2 ± 49.9% respectively. Iron parameters did not differ significantly between males and females, as well as between the young (≤ 45years) and old (≥46years) age groups (p>0.05).There were no statistically significant differences in iron parameters between patients with CVHBI and CVHCI (p>0.05). Elevated serum ferritin was found in 15.2% and 20.4% of CVHBI and CVHCI patients respectively; while an elevated Tsat was seen in 22.7% and 24.1% of CVHBI and CVHCI patients respectively. Using a combination of elevated serum ferritin and Tsat, the prevalence of iron overload was 1.6%. No statistically significant difference in the prevalence of iron overload between patients with CVHBI and CVHCI (1.5% versus 1.9%) was noted (t = 0.17, p = 0.87). Figure 1a shows the comparison of the degree of fibrosis among patients with CVHBI and CVHCI while figures 1b to 1e show the correlation between serum ferritin, serum iron, Tsat, TIBC and fibroscan scores in the studied population. No significant correlation was found between the parameters and fibroscan score.Mean fibroscan score was 11.3 ± 13.3kPa with no significant difference in both populations (p>0.05). Fibroscan scores corresponding to no/minimal fibrosis was found in 58.1%, moderate fibrosis in 7%, severe fibrosis in 12.9% and advanced fibrosis/cirrhosis in 22% of patients investigated. Fibroscan scores did not differ significantly between patients with/without elevated iron parameters (p > 0.05).A weak and non-significant correlation was noted between fibroscan scores and serum ferritin (r = -0.11; p = 0.12) as well as Tsat (r = -0.11; p = 0.13), serum iron (r = -0.06; p = 0.38) and TIBC (r = 0.02; p = 0.74).

Table 1: Demographic and laboratory data of patients

Parameter (Mean ± SD)	Total	CVHBI	CVHCI		T					P				
Age(yrs)	43±13	40±13	51±11		-					-				
M:F ratio	1.2:1	1.4:1	1:1.3		-					-				
Serum iron (µmol/L)	25.1±22.8	25.4±22.8	24.3±22.9		0.29					0.77				
SerumTIBC (µmol/L)	71.1±35.9	72.58±36.54	67.55±34.40		0.87					0.39				
Tsat (%)	45.2±49.9	44.3±47.7	47.5±55.3		0.40					0.69				
Serum Ferritin (µg/L)	218±325.6	206.1±308.4	247.5±365.7		0.79					0.43				
Fibroscan score(kPa)	11.3±13.3	10.5±12.6	13.2±14.9		1.25					0.36				

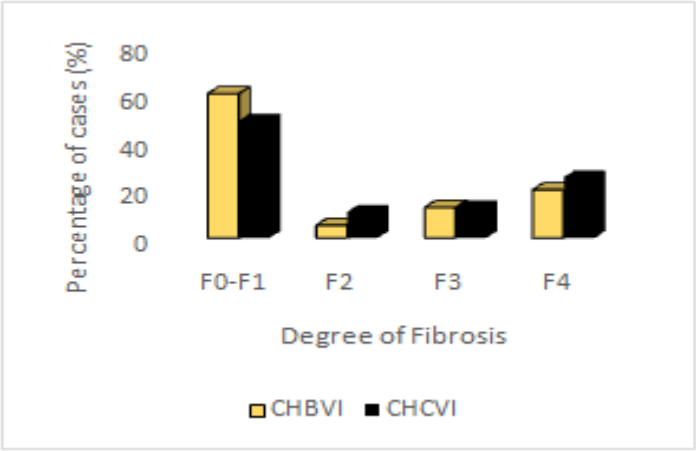


Figure 1a: Comparison of the degree of fibrosis among patients with CVHBI and CVHCI

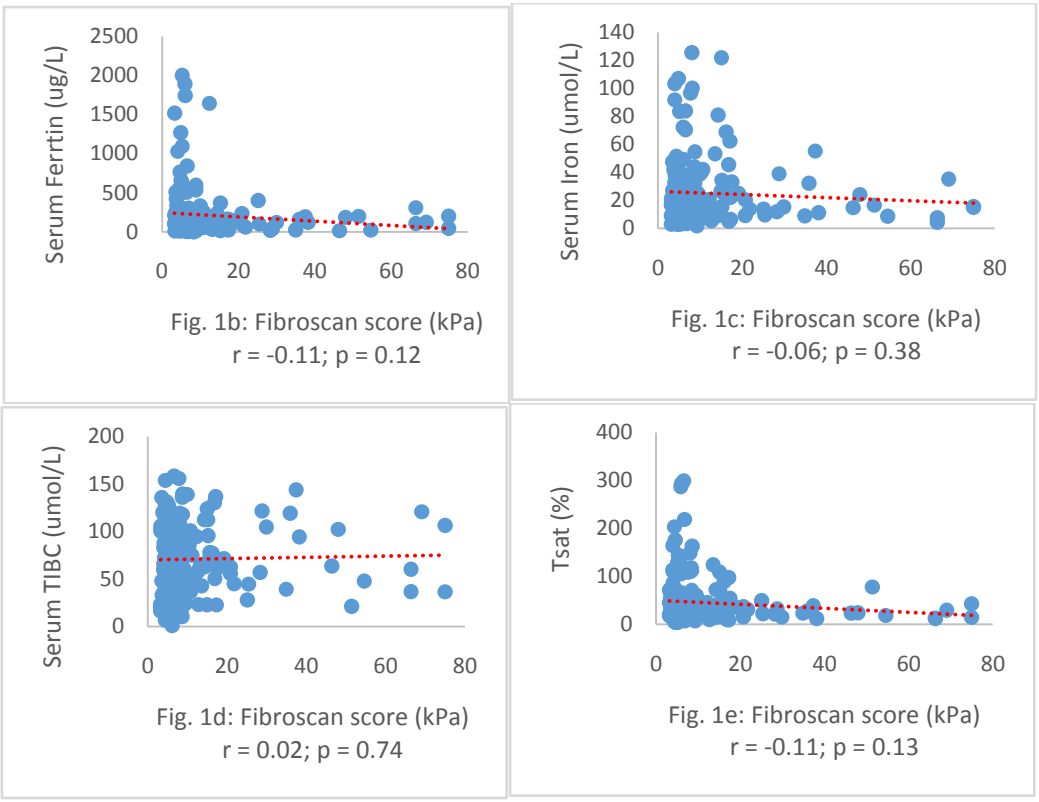
Key:

F0-F1: No/Minimal fibrosis (HBV 0-6.9kPa; HCV 0-7.9kPa)

F2: Moderate fibrosis (HBV 7-7.9kPa; HCV 8-8.9kPa)

F3: Severe fibrosis (HBV 8-11kPa; HCV 9-14.5kPa)

F4: Advanced fibrosis/Cirrhosis (HBV ≥11kPa; HCV ≥14.6kPa)



Figures1b – 1e: Correlation between fibroscan scores and ferritin, iron, TIBC, Tsat

DISCUSSION

The mean ferritin values found for patients with CVHBI and CVHCI were similar to what Olmez *et al* and Vague*t al* independently reported while studying CVHBI and CVHCI patients respectively.[12, 13] The mean ferritin value for black patients with CVHCI who participated in the National Health and Nutritional Examination Survey (NHANES) in USA was also comparable to the finding in this study.[14] Serum iron values found in patients with CHBVI in this study was at variance with what Mao *et al* reported for cirrhotic and non-cirrhotic patients with CVHBI in China.[6] Won and his colleagues in Seongnam, Korea found CVHCI patients with higher values of serum iron than what was observed in this study.[15] The works of Vague*t al* in Romania, and Lin *et al* in China also yielded higher iron values than in this study.[13, 16] No local data was found for comparison. The study by Won *et al* appeared to rule out the role of HFE gene mutation as a contributory factor to iron loading. They found no difference between carriers of the H63D mutation and those without in terms of serum and hepatic iron parameters.[15]From the aforementioned, an explanation on why these Caucasian populations have higher iron than Africans may then be linked to other unidentified genetic characteristics they bear or their dietary preferences. A difference in assay methods employed by these studies may also be implicated.Buyukasik*et al* studied 38 patients chronically infected with HBV or HCV alongside cirrhotic patients and healthy controls. The reported values for TIBC in those with chronic viral hepatitis were higher than the findings in this study but Tsat values were similar in both studies.[17] The findings of this study for Tsat were also in agreement with that of Di-Biscegli*et al* in 1992 when an equal proportion of patients with CVHBI and CVHCI were evaluated for iron status.[18]

The finding of more elevated iron indices in CVHCI than CVHBI patients in this study was in keeping withthe reports of Arber *et al*, Prieto *et al* and Sajeevan*et al*.[19-21] However, Buyukasik *et al* had a different report from that of this study. There was no difference in iron parameters among the patients they studied, perhaps due to their smaller study population.[17]

A disparity in the criteria for iron overload assessment exists. The prevalence of iron overload in this study was determined by simultaneously high serum ferritin and Tsat levels in patients. Although this is the ideal in iron overload assessment, most authors defined iron overload by elevation of either parameter.[22, 23]In this study, the prevalence of iron overload in patients with chronic viral hepatitis was 1.6%. This prevalence is low when compared to the report of Di-Biscelgie*et al* (39%) who used similar criteria for iron overload assessment.[18]This may be due to the higher prevalence of the haemochromatosis gene among whites than blacks. Fasola*et al* in identifying healthy Nigerian women with iron overload used only an elevated Tsat level above 50% and reported a prevalence of 8.6%.[24] When compared to the aforementioned, the prevalence of iron overload (using a high Tsat level only) was higher in both CVHBI (22.7%) and CVHCI (24.1%) patients in this study. Buyukasik*et al* also used a Tsat cut off of 50% for iron overload assessment.[17] They however found no one with iron overload among their subjects with chronic viral hepatitis infection.[17] Though some authors have also considered hyperferritinaemia as being diagnostic of iron overload, a major drawback that must be checked is the role of ferritin as an acute phase reactant.[25] An elevated ferritin level may then be observed in acute inflammatory states making it unreliable.[25]Okeke reported the prevalence of hyperferritinaemia to be 50% among intending blood donors

with HCV infection in Enugu, Southeast Nigeria.[26] This was higher than the finding in this study (15.2% among CVHBI and 20.4% among CVHCI patients). Okeke used fewer subjects for his study. This may account for the higher value he reported. Also, ferritin values were not controlled with another acute phase reactant such as was done in this study.[26] CVHCI patients in this study had a higher prevalence of iron overload whether high levels of ferritin and Tsat are employed singly or combined. There are limited reports on the comparison of iron overload occurrence in CVHBI and CVHCI. There is therefore room for further research on this subject matter.

There were weak negative correlations between fibroscan scores and serum ferritin, iron, and Tsat. A weak positive correlation was noted between fibroscan scores and TIBC. None of these associations were statistically significant. Previous studies comparing iron status with degree of liver fibrosis have used liver histology, liver ultrasound scan and/or biochemical plus clinical parameters in assessing the presence of liver fibrosis/cirrhosis.[6, 27]This is because fibroscan is a relatively new technique for this purpose. Some authors have assessed the reliability of fibroscan scores and found it to be accurate when compared to liver histology reports.[28,29]Verveer*et al* shared the same opinion but noted that it was suboptimal in assessing fibrosis for stages ≤ F2.[30] Liang *et al* opined that a combination of fibroscan scores with other biochemical parameters as well as liver ultrasound scan improves accuracy in liver cirrhosis confirmation among CVHBI carriers.[31] It may be inferred that the findings of this study were similar to the reports of Mao *etal* and Lin *et al* in which they used liver histology and a combination of radio-biochemical parameters respectively to assess liver fibrosis.[6, 16] The finding in this study was, however, different from the report of Vagu*et al.*[13] They employed only biochemical parameters in determining severity of liver disease and only CVHCI patients were studied.[13] Most researchers have however observed a positive association between iron status and biochemical markers of liver disease such as alanine transaminase (ALT) as well as aspartate transaminase (AST) in both CVHBI and CVHCI patients.[32, 33] This was however not assessed in this study because of the non-specificity of the elevation of these enzymes to liver disease.[34] Besides, some patients with chronic viral hepatitis infection may not have elevated liver enzymes.[35]

This study found minimal effect of iron overload on the degree of liver fibrosis. The few patients with iron overload all had no/minimal fibrosis from their fibroscan score assessment. Most patients with either a high Tsat or ferritin also had no/minimal liver fibrosis. There was no difference in the severity of liver fibrosis between patients with and without iron overload. Most researchers that investigated the influence of iron overload on liver fibrosis severity relied on the pattern and extent of iron stain as well as the degree of fibrosis from liver biopsy specimens for their judgement.[36, 37] However, Metwally*et al* compared both serum ferritin levels and hepatic iron deposition in assessing an influence on the degree of liver fibrosis among only CVHCI patients.[38] They concluded that there was a strong correlation between serum ferritin levels and hepatic iron content. They also opined that serum ferritin was an independent predictor of severe hepatic fibrosis.[38]It therefore brings to bare the disparity in the finding of this study in relation to others. Even though they used a histological assessment, it is expected either way that iron overload should worsen liver fibrosis due to the hepatotoxic nature of excess iron on the liver.[39] The difference in findings may be linked to the sensitivity of the method employed to assess liver fibrosis in this study. Further studies to assess the sensitivity of liver transient elastographyin comparison to liver histologic findings in chronic viral hepatitis especially among blacks are suggested.

CONCLUSION

It may be concluded from this study that chronic viral hepatitis infection is associated with elevated iron parameters especially in Hepatitis C infection. However, the prevalence of iron overload is low in this population. The study also showed that elevated iron parameters had minimal effect on degree of liver fibrosis. Clinicians should therefore not rely on laboratory parameters alone in the true evaluation of iron overload, these should be combined with clinical findings.

REFERENCES

[1]. Olokoba AB, Salawu FK, Danburam A, *et al*. Viral Hepatitides in voluntary blood donors in Yola, Nigeria. *European J Sci Res.* 2009; 31: 329-334.

[2]. World Health Organization. *Hepatitis B and C, Immunization, Vaccines and Biologicals.* 2013. Available at: http://www.who.int/immunization/topics/hepatitis/en/ (Accessed 18/04/2016).

[3]. Egah DZ, Banwat EB, AuduES,*et al*. Hepatitis B surface antigen, hepatitis C and HIV antibodies in a low-risk blood donor group, Nigeria. *East Mediterr Health J.* 2007; 13: 961-966.

[4]. Emechebe GO, Emodi IJ, Ikefuna AN, *et al*. Hepatitis B virus infection in Nigeria – A review. *Niger Med J.* 2009; 50: 18-22.

[5]. Franchini M, Targher G, Capra F, Montagnana M, Lippi G. The effect of iron depletion on chronic hepatitis C virus infection. *Hepatol Int*. 2008; 2: 335-340.

[6]. Mao W, Hu Y, Lou Y, Chen Y, Zhang J. Abnormal serum iron markers in chronic hepatitis B virus infection may be because of liver injury. *European J GastrenterolHepatol.* 2015; 27: 130-136.

[7]. Ma L, Zou T, Yuan Y, *et al*. Duodenal Ferroportin Is Up-Regulated in Patients with Chronic Hepatitis C. *PLoS ONE*. 2014; 9: 1-10.

[8]. Bomfim EA, Pereirade de Oliveira AC, Paraná R. Schinoni MI. A study on hepatic iron overload in chronic hepatitis C patients. *ActaGastroenterolLatinoam*. 2013; 43: 212-217.

[9]. Patil PS, Mohandas KM, Bhatia SJ, Mehta SA. Serum ferritin and the risk of hepatocellular carcinoma in chronic liver disease of viral aetiology: a case-control study. *Indian J Gastroenterol.* 2014; 33: 12-18.

[10]. Foka P, Dimitriadis A, Karamichali E, *et al*. Alterations in the Iron Homeostasis Network: A Driving Force for

Macrophage-mediated Hepatitis C virus Persistency. *Virulence.* 2016; 10: 1-39.

[11]. Kawamura Y, Akuta N, Sezaki H, *et al*. Determinants of serum ALT normalization after phlebotomy in patients with chronic hepatitis C. *J Gastroenterol.* 2005; 40: 901-906.

[12]. Olmez S, Aslan M, Saritas B, Yalcin MS, Tas A, Balahroglu R. Serum ferritin levels in patients with HBV and HDV-related cirrhosis. *ActaMedicaMediterr*. 2016; 32: 1813-1819.

[13]. Vagu C, Sultana C, Ruta S. Serum iron markers in patients with Chronic Hepatitis C infection. *Hepat Mon*. 2013; 13:1-5.

[14]. Shan Y, Lambrecht RW, Bonkovsky HL. Association of Hepatitis C Virus Infection with Serum Iron Status: Analysis of Data from the Third National Health and Nutrition Examination Survey (NHANES). *Clin Infect Dis*. 2005; 40: 834–841.

[15]. Won J, Jeong S, Chung JI, Lee JH, Hwang SH, Kim J. Hepatic Iron, Serum Ferritin, HFE Mutation, and Hepatic Fibrosis in Chronic Hepatitis C. *Intervirology*. 2009; 52: 239–246.

[16]. Lin TJ, Liao LY, Lin SY, Lin CL, Chang TA. Influence of iron on the severity of hepatic fibrosis in patients with chronic hepatitis C. *W J Gastroenterol*. 2006; 12: 4897-4901.

[17]. Büyükaşik NS, Nadir I, Akin FE, *et al*. Serum iron parameters in cirrhosis and chronic hepatitis: detailed description. *Turk J Gastroenterol.* 2011; 22: 606-611.

[18]. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of Iron Status in Patients with Chronic Hepatitis. *Gastroenterology.* 1992; 102:2108-2113.

[19]. Arber N, Konikoff FM, Moshkowitz M, *et al*. Increased serum iron and iron saturation without liver iron accumulation distinguish chronic hepatitis C from other chronic liver diseases. *Digest Dis Sci.* 1994; 39: 2656-2659.

[20]. Prieto J, Barry M, Sherlock S. Serum ferritin in patients with iron overload and with acute and chronic liver diseases. *Gastroenterology* 1975; 68: 525-533.

[21]. Sajeevan KC, Lyson L, Biju G, Parvathi KW. Serum iron parameters in alcoholic cirrhosis, cryptogenic cirrhosis, chronic hepatitis B and chronic hepatitis C. *J EvidBased Med Healthc.* 2016; 3: 5157-5161.

[22]. Feifel D, Young WY. Iron Overload among a Psychiatric Outpatient Population. *J Clin Psychiatry.* 1997; 58: 74-78.

[23]. Milman N, Byg KE, Ovesen L, Kirchhoff M, Jürgensen KS. Iron status in Danish women, 1984-1994: a cohort comparison of changes in iron stores and the prevalence of iron deficiency and iron overload. *Eur J Haematol.* 2003; 71: 51-61.

[24]. Fasola FA, Anetor JI, Ilesanmi OS. An investigation of iron overload in Nigerian women. *AfrJ MedSci.* 2013; 42:231-237.

[25]. Camaschella C, Poggiali E. Towards explaining "unexplained hyperferritinaemia". *Haematologica*. 2009; 94: 307-309.

[26]. Okeke IK. Prevalence and physiological impact of hepatitis C virus among blood donors, diabetics, pregnant women, sickle cell and HIV patients in Enugu, Nigeria. A dissertation work submitted to Department of Microbiology University of Nigeria, Nsukka. 2008; 1-198.

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

[28]. Friedrich-Rust M, Ong MF, Martens S, *et al*. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology*. 2008; 134: 960-974.

[29]. Sporea I, Sirli R, Deleanu A, *et al.* Comparison of the liver stiffness measurement by transient elastography with the liver biopsy. *W JGastroenterol*. 2008; 14: 6513-6517.

[30]. Verveer C, De-Knegt. Non-invasive measurement of liver fibrosis: Application of the FibroScan® in hepatology. *Scand J Gastroenterol* 2006; 45: 85-88.

[31]. Liang XE, Dai L, Yang SL, *et al*. Combining routine markers improves the accuracy of transient elastography for hepatitis B cirrhosis detection. *Digest Liver Dis*. 2016; 48: 512-518.

[32]. Girelli CM, Mirata C, Casiraghi A. Effect of blood-letting on serum aminotransferase levels of patients with chronic hepatitis C and iron overload. *RecentiProg Med.* 1998; 89: 241-244.

[33]. Takikawa T, Hayashi H, Nishimura N, Yano M, Isomura T, Sakamoto N. Correlation between serum levels of alanine aminotransferase and ferritin in male blood donors with antibody to hepatitis C virus. *JGastroenterol*. 1994; 29: 593-597.

[34]. Oh RC, Hustead TR. Causes and Evaluation of Mildly Elevated Liver Transaminase Levels. *Am Fam Phys*. 2011; 84: 1003-1008.

[35]. Mbaye PS, Sarr A, Sire JM, *et al*. Liver Stiffness Measurement and Biochemical Markers in Senegalese Chronic Hepatitis B Patients with Normal ALT and High Viral Load. *PLoS One*. 2011; 6: 1-7.

[36]. Correngia C, Galimberti S, Bovo G, *et al*. Iron Accumulation in Chronic Hepatitis C Relation of Hepatic Iron Distribution, HFE Genotype, and Disease Course. *Am JClin Path*. 2005; 124: 846-853.

[37]. Angelucci E, Muretto P, Nicolucci A, *et al*. 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.

[38]. 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.

[39]. Arezzini B, Lunghi B, Lungarella G, Gardi C. Iron overload enhances the development of experimental liver cirrhosis in mice. *Int J Biochem Cell Biol*. 2003; 35: 486–495.