International Journal of Research in Medical Sciences
Tripathi P et al. Int J Res Med Sci. 2016 Jan;4(1):57-60
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20151539

Research Article

Biochemical assessment of liver in sickle cell disease patients at a tertiary care hospital of north India

Pankaj Tripathi[1]*, Manju Tripathi[2]

[1]Department of Pathology, TS Mishra Medical College, Lucknow, Uttar Pradesh, India
[2]Department of Pediatrics, TS Mishra Medical College, Lucknow, Uttar Pradesh, India

Received: 13 December 2015
Accepted: 26 December 2015

***Correspondence:**
Dr. Pankaj Tripathi,
E-mail: drpankajpathologist@yahoo.com

ABSTRACT

Background: Sickle cell disease (SCD) is frequently associated with liver disease. The constant state of hemolysis due to reduced red cell survival; and multiple blood transfusions will lead to hepatic sinusoidal congestion, viral hepatitis, cholestasis, and haemosiderosis, all of these may contribute to the development of liver disease. The aim of the study was to assess the biochemical liver function tests in steady state adult SCD patients in a tertiary care hospital
Methods: Fifty seven adult SCD patients in steady state and sixty hemoglobin AA controls were enrolled in the study. Liver function tests were carried out in all subjects.
Results: The serum total bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT), alkaline phosphatase (ALP), direct and indirect bilirubin and total protein were significantly higher in SCD patients ($P < 0.001$) than in controls. Serum iron and ferritin were higher in SCD patients with hepatic disease ($p<0.01$), which could be due to iron overload, whereas serum transferrin and total iron binding capacity were decreased in these patients.
Conclusions: Steady state SCD patients had elevation of Transaminases and remaining liver function parameters compared to age matched controls. It is advisable that liver function tests be interpreted with caution in these patients.

Keywords: Liver function tests, Sickle cell anaemia, Serum iron, Ferritin

INTRODUCTION

Sickle cell disease is a common hereditary hemoglobinpathy that occurs primarily in individuals of African Americans, Arabian and Indian descent. Particularly, in India hemoglobinopathies are responsible for the largest number of genetic disorders and hence are of great public importance.[1,2]

Sickle cell disorder denotes all genotypes that contain at least one sickle gene in which hemoglobin S makes up at least half of the hemoglobin present. Sickle cell disease (HbSS) and Sickle cell Trait (HbSA) is the major in this group globally and in India. According to ICMR survey Sickle Cell gene is found amongst different tribal groups of India, which varies from 5 to 34 %.[1,3] India has also a very huge populations of tribal community about 18 crore and expected to have 1.80 crore sickle cell trait and 14 lakhs of sickle cell disease.[2]

Hepatic dysfunction is a commonly recognized complication of sickle cell disease (SCD) due to multiple factors such as intrahepatic sinusoidal sickling, bilirubin gallstones, transfusion-related hepatitis infections or excess iron deposition. There are studies suggesting that the main causes of liver injury in SCD patients are due to factors other than intrahepatic sickling, which was considered to be reversible, such as viral hepatitis or transfusional iron overload.[4-7] Therefore, present study is aimed to assess the liver function tests in sickle cell disease in population of North India.

METHODS

The cross sectional study entitled biochemical study of liver was done in Department of Pathology at Hind Institute of Medical Sciences, Lucknow, India. The study was approved by the Ethical and Scientific Committee of the Institute and was carried out between January - June 2011. The study included 57 subjects with Sickle cell Disease and 60 age matched healthy controls (Hb AA). Informed consent was taken from all the subjects.

A general examination was done on all the subjects before blood samples were taken for haematological and biochemical analysis. The general examination included assessing conjunctiva for jaundice, which was classified subjectively as mild, moderate and severe. Enlargement of the liver below the costal margin was classified as mild if it is more than 5cm, moderate if 5-10 cm below the costal margin and massive if more than 10 cm.

Sickle cell anaemia patient was diagnosed by high performance liquid chromatography (HPLC-Bio-Rad-Variant TM from Bio Rad, CA, USA) using Bio-Rad diagnostic kit. Complete blood count and red cell indices were measured by automated analyser (SYSMEX K-4500, Kobe Japan) using Trans Asia diagnostic kit. Biochemical parameters (AST, ALT and ALP), AST/ALT ratio, total bilirubin, total protein and albumin) were determined by Beckman-CX-4 and CX-9 auto analyser using Randox diagnostic kit in batches. Serum ferritin was estimated by Sandwich Elisa Method, serum iron by Ferene Colorimetric Method, Total Iron Binding Capacity by Magnesium Carbonate And Ferene Method, transferrin and percentage of transferring saturation were measured by calculation from its associated parameters and in whom the ferritin and serum iron levels are high, liver biopsy and abdominal ultrasonography were performed and subjected quantification of hepatic iron content by atomic absorption spectroscopy.[8-10] Hepatitis B antigen, hepatitis B and C surface antibody serological tests have conducted to evaluate the viral hepatitis. Each assay was validated using commercial quality control samples and standards. Data obtained were analysed using Statistical Package for Social Sciences (SPSS Inc., Chicago, USA) 15.0 for windows. Student's t-test was used to compare means of variables between patients and controls. P ≤0.05 was considered as statistically significant.

RESULTS

The mean age of the SCA patients was 25.22 ± 5.35 years, while that of the control group was 26.70 ± 1.22 years. Of the 57 sickle cell subjects, 39 were females and 16 remaining were males. None of the study subjects had a previous history suggestive of liver diseases. All the study subjects were hemoglobin SS homozygotes, while all the controls were hemoglobin AA homozygotes. There was no history of previous blood transfusion in the control subjects.

Table 1: Comparison of liver function tests between sickle cell disease patients and controls.

Parameter	SCD patient (mean ± SD)	Control (mean ± SD)
AST	51.20±72.34	24.35 ± 4.55***
ALT	45.2 ±64.99	26.47 ± 3.98 ***
ALP	210.88±29.8	134.5 ± 46.07***
GGT	64.31±7.3	28.04± 6.7 ***
Total bilirubin	5.24±10.01	0.75 ± 1.2 ***
Direct bilirubin	2.29 ±5.00	0.29 ± 0.13 ***
Indirect bilirubin	2.95±5.02	0.54 ± 0.18 ***
AST/ALT ratio	1.08±1.13	1.03 ± 0.29
Plasma total protein	5.65±5.37	6.84 ± 0.24 **
Serum albumin	4.07±3.41	4.59 ± 0.22

Group SCD V/S Group C: *** P < 0.001; **P< 0.01
SCA: Sickle cell anaemia; SD: Standard deviation; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; AST/ALT: De Ritis ratio, GGT: Gamma Glutamyl Transferase

There was a significant (P<0.001) difference in the mean values of all parameters except AST/ALT ratio in between the SCD group and controls. Statistical significance is there for ALT (P<0.001), GGT (P<0.001), Total, Direct and indirect Bilirubin values (P<0.001) in between both groups. Even total protein was lower in SCD group when compared to controls (p>0.01) (table 1).

Regarding sickle cell disease therapy, who had transfusions of more than an average of 10-12 packed RBC units /year showed a higher levels of serum ferritin and serum iron when compared with other sickle cell disease population ,which was statistically significant (table 2).

Table 2: Comparison of serum iron, serum ferritin and Transferrin in sickle cell disease (SCD).

Parameters	SCD with Transfusion Less Than 10 Packed RBC Units/Year	SCD with Transfusion More Than 10-12 Packed RBC Units/Year
Serum Ferritin	186.7±73.37	2080±589.5***
Transferrin	2.01±0.32	1.52±0.177
TIBC	302.1±45.8	189.6±25.36***
Serum Iron	122.8±25.06	636.9±168.5***
% Transferrin Saturation	41.89±12.42	339.1±94.79***

*** Group SCD (1) V/S Group SCD (2): P < 0.001

DISCUSSION

A number of seminal developments marked the pathway from the early clinical description of sickle cell anaemia in 1910 to the unparalleled advances of the past three decades. The molecular genetics of sickle cell disease has studied in the understanding for the cause of sickle cell disease.

The clinical manifestation of sickle cell anaemia in India seems to be milder than in Africa and Jamaica.[11] The clinical spectrum of SCD ranges from mild to severe liver function and clinical crises with marked hyperbilirubinemia and liver failure. Multiple factors may contribute to the etiology of the liver disease, including ischemia, transfusion related viral hepatitis, iron overload, and gallstones.[12] Delayed growth and bone destruction may contribute to the elevated levels of alkaline phosphatase

Hepatic injury can be directly related to the sickling process, acute hepatic crisis and hepatic sequestration crisis combinely causes sickle cell hepatopathy.

Transaminases are significantly elevated in sickle cell hepatopathy but AST/ALT ratio was not altered significantly when comparison with controls. ALP and GGT enzymes are markedly elevated in this abnormality, even though these two are biliary canalicualry enzymes, chronic impairment of hepatic cells may have affect in their increment. Total protein values were altered significantly and serum albumin to some extinct. No alteration was observed in iron and associated markers in comparison with sickle cell disease. The findings in this study is in concurrence with that of Akuyam et al., who in a study in 2007 found statistically significant higher values of AST, ALT, ALP, total bilirubin and protein in the SCA patients studied.[13]

De Ritis ratio, which is used to determine hepatic necrosis, was slightly higher in the patients than controls, but not statistically significant. This is most likely as a result of elevated level of AST found in majority of the patients which may be due to excessive haemolysis as reported in previous studies.[14,15]

Extremely significantly high bilirubin levels may be due to combination of on-going haemolysis, intra hepatic cholestasis and renal impairment encountered in sickle cell hepatopathy in comparison with remaining disease groups as shown by Stephan et al.[16] Shao and Orringer et al have described two cases of intra hepatic cholestasis with similar presentation, but in which very different therapeutic approaches led to very different clinical outcomes.[17] The higher serum total bilirubin could be due to chronic haemolysis and/or ineffective erythropoiesis, which characterize the disease.

Moreover, haemolysis raises serum AST level, and thus the increased level observed in SCD patients. The above findings are in agreement with previous finding by Johnson et al.[18]

While Transfusion therapy of packed RBC are proved to improve and essential in the STOP(Stroke Prevention In Sickle cell disease) of sickle cell disease and its complications, iron over load is a dreaded and inevitable consequence of on-going transfusion therapy.

Hepatic injury of sickle cell disease is usually secondary to chronic hepatitis infection or to iron over load with Ferritin levels are correlated significantly with number of transfusions.[19] Saturation of transferrin by excess circulating iron results in increased non transferring bound iron (NTBI), it tends to enter tissues more readily and results in formation of reactive oxygen species (ROS) such as the hydroxyl radical by Haber Weiss reaction.[20] Excess iron tends to deposit in the hepatic parenchyma causing end organ damage by ROS mediated lipid peroxidation.

Transaminases and remaining liver function parameters are elevated significantly but naturally there is a striking significant elevation in serum iron and ferritin concentrations with elevated % transferrin-iron saturation levels, at the same time there is a significant fall in TIBC and serum transferrin levels are observed.

Portal fibrosis caused due to iron over load tissues are taken for biopsy and after wards finding the histological features quantified for the finding of hepatic iron content, gold standard parameter in hemochrmatosis. This group of Patients is taken repeated packed RBC transfusions with more than 12 units per year (220ml per unit accumulating 1mg/ml iron).

CONCLUSION

Taken together, our findings and data in the literature indicate that liver injury in patients with SCD seems to be a multifactorial phenomenon depending mostly on overlapping factors such as iron overload and viral damage rather than the primary disease itself. The finding of studies state the biochemical abnormality play a significant role in sickle cell patients physiopathology and can be used to management, of the disease. However, further follow-up study with other investigations like non-invasive techniques and liver biopsy are warranted which could provide a valuable tool for the identification of underlying etiology and correct diagnosis in SCD patients.

ACKNOWLEDGEMENTS

I wish to thank all the staff members of Department of Medicine and Pathology, HIMS, Lucknow for their kind co-operation during the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Kar BC. Sickle cell disease in India. J Assoc Physicians India. 1991;39:954-60.
2. Mohanty D, Mukherjee MB. Sickle cell disease in India. Curr. Opin Hematol. 2002;9:117-22.
3. Rosatelli MC, Altay C, Oner R, Leoni GB, Moi B, Atzori G. Globin haplotype and XmnI polymorphism at position Gy-158 and HbF production in Fanconi's anemia. Haematologica. 1992;77:106.
4. Kakarala S, Lindberg M. Safety of liver biopsy in acute sickle hepatic crisis. Conn Med. 2004;68(5):277-9.
5. Beutler E. The sickle cell diseases and related disorders. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U, eds. Williams Hematology. New York: McGraw Hill. 1999;581-605.
6. Rosenblate HJ, Eisenstein R, Holmes AW. The liver in sickle cell anemia. A clinical-pathologic study. Arch Pathol. 1970;90(3):235-45.
7. Omata M, Johnson CS, Tong M, et al. Pathological spectrum of liver diseases in sickle cell disease. Dig Dis Sci 1986; 31(3): 247-56.
8. Polson RJ, Kenna JG, Shears IP, Bomford A, Williams R. Measurement of ferritin in serum by an indirect competitive enzyme-linked Immunosorbant Assay. Clin Chem. 1988;34(4):66-4.
9. Stookey LL. A New Spectrophotometric Reagent for Iron. Anal. Chem. 1970;42:779.
10. Tietz NW. Text book of clinical chemistry, 3rd C.A.Burtis, E.R.Ashwood, W.B.Saunders. 1999:1669-1703.
11. Mohanty D, Mukheriee M. Sickle cell disease in India. Haematology. 2002;9(2):117-22.
12. Banerjee S, Owen C, Chopra S. Sickle cell hepatopathy. Hepatology. 2001;33(5):1021-8.
13. Akuyam AS, Bamidele AS, Aminu SM, Aliyu IS, Muktar HM, Mamman AI. Liver function tests profile of sickle cell anaemia patients in steady state of health: Zaria experience. Borno Med J. 2007;4:1-6.
14. Sheehy TW. Sickle cell hepatopathy. South Med J. 1977;70:533-8.
15. Schubert TT. Hepatobiliary system in sickle cell disease. Gastroenterology. 1986;90:2013-21.
16. Stephan JL, Merpit-Gonon E, Richard O, Raynaud-Ravni C, Freycon F.Fulminant live failure in a 12-year-old girl with sickle cell anaemia:favourable outcome after exchange transfusions. Eur J Pediatr. 1995;154:469-71.
17. Shao SH, Orringer EP. Sickle cell intrahepatic cholestasis: approach to a difficult problem. Am J Gastroenterol. 1995;90:2048-50.
18. Johnson CS, Omata M, Tong MJ, Simmons JF Jr, Weiner J, Tatter D. Liver involvement in sickle cell disease. Medicine (Baltimore). 1985;64:349-56.
19. Porter JB, Huchas ER. Transfusion and exchange transfusion in sickle cell anaemias, with particular reference to iron metabolism. Acta Haematol. 1987;78:198-205.
20. Link G, Pinson A, Hershko C. Heart cells in culture: a model of myocardial iron overload andchelation. The Journal of Laboratory and Clinical Medicine. 1985;106:147-53.

Cite this article as: Tripathi P, Tripathi M. Biochemical assessment of liver in sickle cell disease patients at a tertiary care hospital of north India. Int J Res Med Sci 2016;4:57-60.