PREVALENCE OF RAISED TRANSAMINASES AND ASSOCIATED RISK FACTORS IN STABLE SICKLERS IN LAGOS, NIGERIA

Odeghe EA[1], Lesi OA[1] and Otegbayo JE[2]

Department of Medicine, Lagos University Teaching Hospital, Lagos, Nigeria[1], Department of Medicine, College of Medicine, University of Ibadan, Ibadan, Nigeria[2].

Correspondence Address: Dr Emuobor Odeghe, Department of Medicine, Lagos University Teaching Hospital, PMB 12003, Idi-Araba, Lagos, Nigeria. E-Mail: eaodeghe@yahoo.com

ABSTRACT

Background: Elevation of serum transaminases is common in sickle cell anaemia (SCA) patients and the causes are multiple and may be due to the underlying sickling state or complications of multiple blood transfusions.

Objectives: To determine the prevalence of raised serum transaminases in stable patients with SCA, and to evaluate the relationship between raised serum transaminase and common risk factors for such elevations in stable patients with SCA.

Methods: One hundred and eighty five stable SCA patients attending the Lagos University Teaching Hospital (LUTH) SCA clinic, and one hundred and eighty three age- and sex- matched healthy controls were studied between May and November 2011. They were administered a questionnaire, and blood samples were collected for the transaminases (Alanine transaminase, ALT and Aspartate transaminase, AST) and for serology for hepatitis B surface antigen and antibodies to hepatitis C virus.

Results: Female: male ratio was 1.3: 1 in the cases and 1.2: 1 in the controls. Mean age of the cases was 26.9 ± 8.1 years and 26.2 ± 6.9 years for the controls. The serum ALT was elevated in 10.8% of cases and in 6% of the controls (p=0.07). Serum AST was elevated in 88% of the cases and in 53% of the controls (p=0.001). The mean serum ALT values for the cases and the controls were 12.8 ± 8.8 U/L and 8.6 ± 4.8 U/L respectively (p=0.001), while for AST they were 35.2 ± 20.1 U/L and 21.5 ± 10.9 U/L respectively (p=0.001). There was no significant association between elevations in the transaminases and the common risk factors.

Conclusion: Patients with SCA are significantly more likely than controls to have elevation of their serum transaminases levels. They also have significantly higher values than controls. There was no significant association between elevations in the transaminases and the common risk factors.

Keywords: Sickle Cell Anaemia, Transaminase, Risk Factors, Prevalence, Stable.

INTRODUCTION

Sickle cell disease (SCD) is an autosomal recessive condition that is characterized by the presence of abnormal haemoglobin, the sickle haemoglobin or HbS.[1] The abnormal haemoglobin is caused by a point mutation in which a single DNA base change leads to the substitution of valine for glutamic acid in the 6th position on the beta-globin chain.[1] The spectrum of sickle cell disease includes sickle-cell anaemia (SCA), which is the homozygous HbSS state, Haemoglobin SC disease, HbS/â-thalassaemia and other rarer forms.[2] In populations of African ethnic origin, sickle cell anaemia typically accounts for 70% of cases of sickle cell disease, with most of the remainder being haemoglobin SC disease (HbSC disease).[3] SCA is particularly common among people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and the Mediterranean countries.[4] The prevalence of SCA among 644 newborns studied by Odunvbun *et al* in Nigeria was 2.4%,[5] while it was found to be 1.5% in a retrospective study by Umoh *et al* in Uyo, Nigeria, of all haemoglobin genotype tests carried out over a period of 5 years.[6]

The homozygote state, HbSS, is characterised by chronic haemolysis, and a lifelong, often severe, anaemia due mainly to extravascular haemolysis.[1] This chronic haemolytic state is often punctuated by episodes of crises, such as aplastic, hyperhaemolytic and megaloblastic crises and hepatic and splenic sequestration, and these conditions often require blood transfusion.[1] Other complications, such as acute chest syndrome, stroke and chronic kidney disease may also develop[1].

The liver is one of the organs affected by this disorder, with evidence of hepatobiliary disease in many patients in both antemortem[7] and post-mortem series.[8] The causes of elevated transaminases in SCA patients are multiple, complex and often inter-related. Liver disease may occur as a result of the underlying sickling disorder, or as a complication of treatment for the sickle cell anaemia.[9]

The transaminases, alanine transaminase (ALT) and aspartate transaminase (AST), are enzymes found in the hepatocytes and are released into the blood stream whenever there is hepatocellular damage.[10] ALT is found almost exclusively in the liver, while AST also occurs in a host of other organs, thus ALT is more specific for hepatocellular damage than AST.[10] The magnitude of elevation of the transaminases can be classified as "mild" (less than 5 times the upper reference limit); "moderate" (5–10 times the upper reference limit); or "marked" (greater than 10 times the upper reference limit)[10].

The causes of raised transaminases in SCA may include the acute syndromes (such as acute sickle hepatic crisis, acute hepatic sequestration crisis, sickle cell intrahepatic cholestasis).[11] These acute syndromes are due to the occlusion of blood vessels by sickled intrahepatic cells. Raised transaminases may also result from the complications of chronic haemolysis and multiple blood transfusions (such as iron overload, viral hepatitis infections, gallstone disease).[11]

This study aimed to evaluate the prevalence of raised serum transaminases in stable patients with SCA in Lagos University Teaching Hospital (LUTH) and to evaluate the relationship between raised serum transaminases and risk factors such as alcohol intake, blood transfusion and seropositivity for hepatitis B surface antigen and anti-hepatitis C seropositivity in stable patients with SCA.

PATIENTS AND METHODS

The study was carried out at the Sickle Cell clinic of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Surulere in Lagos state. Lagos state is the commercial capital of Nigeria and has an estimated population of 9 million people.[12] It

was a prospective, case-control study in which serum transaminases (ALT and AST) levels were determined among 185 cases with sickle cell anaemia, and 183 controls without sickle cell anaemia. The study was carried out from May to November 2011. Consenting patients with SCA aged at least 16 years were recruited, those in crisis were excluded.

Ethical approval was sought and obtained from the Ethics and Research Committee of the Lagos University Teaching Hospital and a signed consent was obtained from each enrolled participant.

Consecutive cases were enrolled from the Sickle Cell clinic of LUTH, while age and sex matched controls were recruited from relations and friends of patients admitted in the wards. A structured questionnaire was administered to each subject by the investigator. History obtained from the subjects included a past history of blood transfusion, indications for and total number of units of blood received; alcohol use, amount and duration; use of intravenous fluids and parenteral drugs; intravenous drug abuse; frequency and type of crises experienced and number of lifetime sexual partners.

Sample size was determined using the Fisher formula which gave a minimum sample size of 173 using a prevalence rate of 13% [13]. A total of 368 patients were recruited for the study, comprising 185 cases and 183 controls.

Each subject was examined by the investigator, noting the presence or absence of respiratory distress, facial/tribal marks, tattoos, pallor, fever, jaundice and intravenous access marks/scars. Abdominal examination was done noting: the presence of scarification marks and tattoos, measurement of liver span, with determination of the character of the liver in patients with hepatomegaly, and the presence or absence of splenomegaly. Weight and height were determined in each subject using a weighing scale and stadiometer respectively.

Blood samples were collected into appropriate bottles and sent to the research laboratory for analysis of plasma levels of the transaminases using a spectrophotometer and serology for hepatitis B surface antigen and antibodies to hepatitis C virus.

- A sickle cell anaemia patient was defined as a person homozygous for the sickle cell haemoglobin based on an acid based haemoglobin electrophoresis.
- Stable sickle cell anaemia patients were patients who had no clinical evidence of ongoing sickle cell crisis or other acute illness.
- Significant alcohol consumption was defined as alcohol consumption greater than 168 g/week in males and 84 g/week in females for at least 5 years. [14]
- According to BMI, participants were classified as underweight (<18.5 kg/m^2); ideal weight (18.5-24.99 kg/m^2); overweight (25-29.99 kg/m^2) or obese (≥ 30 kg/m^2) [15].
- Reference plasma levels of ALT and AST were 0-12U/L and 0-12U/L respectively (as per the manufacturer's instruction). Elevated transaminase level (for both ALT and AST) was defined as a level greater than 12U/L. The magnitude of elevation of the transaminases was classified as "mild" ($<$ 5 times the upper reference limit); "moderate" (5–10 times the upper reference limit); or "marked" ($>$ 10 times the upper reference limit).

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16. Continuous variables were expressed as means $\pm$ standard deviation using the student's t test, while categorical variables were expressed as frequencies. Level of statistical significance was set at p$\leq$ 0.05.

RESULTS

The study population comprised 185 cases (106 females, 57.3%) and 183 age and sex matched controls (101 females, 55.2%). (Table 1). The mean BMI for the cases was 20.6 ± 2.8 kg/m^2, and for the controls was 23.5 ± 3.3kg/m^2 (p=0.001). The SCA cases were significantly more underweight than the controls (p=0.001). In contrast, control subjects were significantly more likely to be overweight or obese than the cases (p<0.001 and p<0.001 respectively), (Table 1).

Serum aspartate transaminase was elevated in 88% of cases (163/185) and 53% of controls (97/183); (p=0.001). The mean serum AST levels in the cases was 35.2 U/L ± 20.2, while in the controls it was 21.5 U/L ± 11.0; (p=0.001).(Table 2). Serum alanine transaminase was elevated in 10.8% of cases (20/185), and in 6% of controls

Table 1: Socio-demographic characteristics of the study participants

Characteristics	Cases, n=185	Controls, n=183	P value
Males	79 (42.7%)	82 (44.8%)	0.381
Females	106 (57.3%)	101 (55.2%)	
M:F ratio	1.3:1	1.2:1	
Mean age (years) ± SD	26.9 ± 8.1	26.2 ± 6.9	0.42
Mean BMI (kg/m2) ± SD	20.6 ± 2.8	23.5 ± 3.3	0.001
Underweight	81 (43.8%)	21 (11.5%)	0.001
Normal weight	95 (51.4%)	113 (61.7%)	0.1
Overweight	7 (3.8%)	39 (21.3%)	0.001
Obese	2 (1.1%)	10 (5.5%)	0.038

Table 2: Serum transaminase parameters of the study participants

Characteristic	Cases, n=185	Controls, n=183	P value
Alanine transaminase			
Mean levels (U/L) ± sd	12.8 ± 8.8	8.6 ± 4.8	0.001
Abnormal levels > ULN	20 (10.8%)	11 (6%)	0.07
Aspartate transaminase			
Mean levels (U/L) ± sd	35.2± 20.2	21.5 ± 1	0.001
Abnormal levels > ULN	163 (88%)	97 (53%)	0.001

(11/183); (p=0.07). The mean serum ALT levels in the cases was 12.8 U/L ± 8.8, while in the controls it was 8.6U/L ± 4.8; (p=0.001). (Table 2).

multiple injections and intravenous infusions, and hepatitis B and C viruses, was determined among the participants. Exposure was significantly higher among the cases than the controls to blood transfusion (and mean units of blood transfused),

Table 3: Exposure to risk factors of the study participants

Characteristic	Cases (n=185)	Controls (n=183)	P value
Previous blood transfusion	119 (64.3%)	8 (4.4%)	0.001
Mean units of blood transfused ± SD	3.1 ± 2.4	2.1± 0.6	0.005
Use of alcohol	27 (14.6%)	26 (14.2%)	0.45
Past surgery	83 (44.9%)	42 (23.0%)	0.001
Past IVI	171 (94.4%)	70 (38.3%)	0.001
Multiple injections	154 (83.2%)	11 (6.0%)	0.001
HBsAg positive	6 (3.2%)	1 (0.6%)	0.062
Anti-HCV positive	3 (1.6%)	1 (0.6%)	0.316

Exposure to risk factors, such as blood transfusion, alcohol use, history of surgery, past surgery, multiple injections and intravenous infusions, while there was no significant

Table 4: Characteristics of the cases according to raised transaminases levels.

Risk factors	Raised transaminases, n=163	Normal transaminases, n=22	P value
Blood transfusion	105 (64.4%)	14 (63.6%)	0.559
Surgery	75(46%)	8 (36.4%)	0.267
Multiple injections	134 (82.2%)	20 (90.9%)	0.244
IVI	150 (92%)	21 (95.5%)	0.484
Alcohol	26 (16%)	1(4.5%)	0.131
BMI e" 25kg/m^2	8 (4.9%)	1 (4.5%)	0.707
Hepatomegaly	23 (14.1%)	1 (4.5%)	0.183
HBV positivity	6 (3.7%)	0	0.463
Anti-HCV positivity	2 (1.23%)	1 (4.5%)	0.318

difference between the cases and controls in terms of exposure to alcohol and the hepatitis B and C viruses, though the cases were 6 times more likely to test positive to the hepatitis B surface antigen; (Table 3).

Among the cases, exposure to the risk factors was determined between those with raised versus normal transaminases levels. Analysis showed that there was no statistical difference between those with raised transaminases levels versus normal transaminases levels in terms of exposure to the risk factors (Table 4).

DISCUSSION

In this case-control study, more than half of the participants were females. Most of the subjects were young with a mean age of 26.9 years. This is consistent with the findings from other studies, like Maher *et al* [16] (mean age 26.3 years), Kotila *et al* [13] (mean age 24 years) and Richard *et al*[17] (mean age 32.9 years). It is known that patients with SCA have a reduced life expectancy because of the underlying disease and the complications that develop.[18]

Many of the SCA patients were either underweight or of normal weight. They were significantly more likely to be underweight than the controls. This is because SCA is associated with malnutrition, poor growth and development compared with controls of similar age and sex.[19]

In terms of exposure to the risk factors, the cases were more likely to have received blood transfusion, had previous surgery (including local surgery such as scarification), and received multiple injections and intravenous fluids than the controls. This is similar to findings in other studies [16, 20]. This is because blood transfusions, intravenous fluids and injections (for example analgesia) are an important part of the management of these patients when they develop such conditions as worsening anaemia from hyperhaemolytic, sequestration and vaso-occlusive crises. There was no significant statistical difference between the cases and the controls in terms of seropositivity to HBsAg and anti-HCV as was found by other investigators [13, 21]. These findings may be attributed to the fact that in Nigeria and other parts of sub-Saharan Africa, hepatitis B infection is endemic. In these areas, it is now well established that HBV is transmitted mainly horizontally from child to child in early childhood [22]. Thus, both the SCA and control groups of patients may have been equally exposed to the virus early in life. Therefore, the transmission of the virus was not influenced by risks such as multiple injections and cultural practices such as scarification.

In this study, the prevalence of raised transaminases was significantly higher in the patients with sickle cell anaemia than in the controls. Similar findings have been noted in other studies, [13, 16, 20, 23]. Various reasons may explain why levels of transaminases are raised in SCA patients. These include acute processes such as acute hepatic crises, hepatic sequestration and intrahepatic cholestasis, as well as chronic processes such as chronic viral hepatitis and iron overload [3, 24, 25, 26]. Histological examination of the livers of many of these patients (both living and autopsy) frequently reveals the presence of vascular lesions such as intrahepatic sickling, hyperplasia of the Kupffer cells, erythrophagocytosis, sinusoidal dilation and obstruction, and focal parenchymal necrosis[27, 28].

While these histological findings have been attributed to the underlying sickling process with the resulting microvascular stasis and ischaemia[4], other findings such as haemosiderosis, portal fibrosis, chronic hepatitis and cirrhosis may occur from concomitant infection with HBV or HCV, or from secondary haemochromatosis. These latter diseases may be a consequence of multiple blood transfusions and chronic haemolysis as already pointed out. In the current

study, cases with any of the acute processes were excluded. Raised AST levels may also occur from background chronic haemolysis in the cases, as AST is also present in the red blood cells.

From this study, the seroprevalence of anti-HCV antibodies was low; there was no significant statistical difference in the seroprevalence between the cases and controls. Similar low prevalence was also reported by Lesi et al [29] and Ocak et al [30]. However, some other studies reported higher seroprevalence, for example in the study by Maher et al [16], where the high prevalence was attributed to the higher number of units of transfused blood. Thus, the greater the number of units of blood received, the greater the risk of HCV seropositivity [17, 31]. Higher levels of seropositivity for anti-HCV were also found among those who were transfused before routine screening of blood for anti-HCV became available than in those who were transfused after.[31] This demonstrates the important association of HCV transmission with blood transfusion and screening of donor blood. The low HCV seroprevalence in this present study may be due to the low background prevalence of infection with HCV in our environment, [32] the low rates of blood transfusion among the study participants and the adoption of routine screening of blood before transfusion.

HCV infection is an important cause of liver disease in developed nations, Egypt and Asia. The infection often causes liver inflammation with elevated transaminases, and progression to liver fibrosis and cirrhosis. In the current study, seropositivity for HCV was not significantly associated with raised transaminases. Richard et al[17] also reported that there was no relationship between steady state transaminases and anti-HCV seropositivity in their study. Various reasons for this lack of association may be adduced. Firstly, the presence of anti-HCV antibodies may occur in the absence of active HCV infection because

the finding of HCV RNA in serum is needed to evaluate activity of HCV virus. In resolved infection, anti-HCV in serum is associated with absent HCV RNA.[33] Other reasons include the current evidence that suggest that significant necroinflammation and/or fibrosis may occur in the presence of persistently normal ALT in some subjects with active HCV infection.[34] Liver biopsy remains an important tool to evaluate HCV infected subjects especially in highly viraemic subjects with persistently normal ALT.[35] HCV viral load and liver biopsy were beyond the scope of this study. Lastly, it is plausible that the infected SCA patients had not yet developed significant liver disease because it takes about thirty years to develop significant liver disease/cirrhosis in chronic HCV infections; most of the patients with sickle cell anaemia in this study were younger than forty years.

In this study the prevalence of seropositivity to the hepatitis B surface antigen was low in both cases and controls. Similar low prevalence has been reported in other studies[36,37]. Higher prevalence, however, has been reported in some other studies [13, 16, 38]. The low prevalence in this study may be due to the low rates of blood transfusion among participants and the routine screening of blood before transfusion. The finding of similar prevalence of hepatitis B surface antigenaemia among the cases and the controls may be because both groups have been exposed equally to the virus in childhood, as reported in some other studies.[21, 38] This study also found that there was no significant association between HBsAg seropositivity and raised transaminases among the SCA cases. This is consistent with the findings in another study [17]. This may be because the infected patients had not yet developed significant liver disease from the virus, or that they may be inactive carriers of the virus.

There was no difference in alcohol use between the cases with elevated transaminases

versus those with normal transaminases. This may be because few of the cases took alcohol, while none took alcohol in significant quantity.[14] It was therefore not likely that any of them had developed alcoholic liver disease.

Multiple blood transfusions may raise the risk of developing iron overload and hepatitis C virus infection, as already mentioned elsewhere, and these conditions may lead to elevated transaminase levels. A history of blood transfusion was not significantly associated with elevated transaminases levels in this study. This may be because many of the patients had not received blood transfusion, and among those who had received blood transfusions, the mean number of units received was less than five.

Exposure to other risk factors such as multiple injections, IVI use, and local and orthodox surgeries was not associated with the presence of raised transaminases in the patients with sickle cell anaemia. This is similar to the finding in other studies by Angyo *et al.* [21] and Emechebe *et al.* [38] where exposure to socio-cultural practices like circumcision, ear piercing and scarifications did not increase the risk of HBV infection and, possibly, derangement of transaminases from liver disease. The reason for this may be a greater use of sterilized equipment. It is also possible that single use syringes/needles are utilized to perform such practices because the practitioners are more aware of the dangers of reuse of syringes/needles due to public health campaigns. It may also be that the patients are immune to the virus due to exposure in early childhood, such that exposure to these procedures in later life will not increase the risk of acquiring the virus.

In conclusion, from the findings of this study, the SCA patients were more significantly underweight, and had higher exposures to multiple injections, intravenous infusions, surgery and blood transfusion than the controls. The patients with SCA were significantly more likely than the controls to have elevation of their serum transaminase levels. The prevalence of both hepatitis B and C infections were similar in subjects with SCA and controls, and it was unrelated to the presence of raised serum transaminase. Many of the common factors known to increase the risk of liver disease were not associated with elevated transaminase levels.

While transaminase elevations may signify the presence of liver disease, normal values do not necessarily exclude the presence of liver disease. Knowledge of the common causes of liver diseases in these patients will be necessary to guide the clinician to adopt cost effective management strategies whenever elevated serum transaminases are detected. It is possible that other factors may explain raised transaminase levels in the patients in this study, such as intrahepatic sickling and hepatic iron load. There is need for further larger studies that would include liver biopsy, iron studies and molecular testing for HCV RNA and HBV DNA. This will enable more definitive conclusions as to the association of elevated transaminases with liver disease and viral infections.

REFERENCES

1. Hassan M, Hasan S, Castro O, Giday S, Banks A and Smoot D. Hepatitis C virus in sickle cell disease. J Natl Med Assoc 2003; 95(9): 864-874.
2. Rees DC, Williams TN and Gladwin MT. Sickle-cell disease. Lancet 2010; 376 (9757): 2018 – 2031.
3. Nagel RL, Fabry ME and Steinberg MH. Blood Rev.. The paradox of hemoglobin SC disease. 2003;17 (3):167-178.
4. World Health Organisation. Fifty-ninth World Health Assembly A59/9.

Provisional agenda item 11.4 24 April 2006.

5. Odunvbun ME, Okolo AA and Rahimy CM. Newborn screening for sickle cell disease in a Nigerian hospital. Public Health. 2008; 122 (10):1111-1116.

6. Umoh AV, Abah GM, Ekanem TI and Essien EM. Haemoglobin genotypes: a prevalence study and implications for reproductive health in Uyo, Nigeria. Niger J Med. 2010; 19 (1):36-41.

7. Charlotte F, Bachir D, Nénert M, Mavier P, Galactéros F, Dhumeaux D *et al*. Vascular lesions of the liver in sickle cell disease. A clinicopathological study in 26 living patients. Arch Pathol Lab Med. 1995; 119 (1):46-52.

8. Bauer TW, Moore GW and Hutchins GM. The liver in sickle cell disease. A clinicopathologic study of 70 patients. Am J Med. 1980; 69 (6):833-837.

9. Stanca CM, Fiel MI and Schiano TD. Exacerbation of sickle cell disease itself as a cause of abnormal liver chemistry tests. Dig Dis Sci 2007; 52 (1):176-178.

10. Giannini EG, Testa R and Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ. 2005; 172 (3): 367–379.

11. Banerjee S, Owen C and Chopra S. Sickle Cell Hepatopathy. Hepatology 2001; 33 (5):1021-1028.

12. Federal Republic of Nigeria Official Gazette (15 May 2007) FGP 71/52007/2,500(OL24). Legal Notice on Publication of the Details of the Breakdown of the National and State Provisional Totals 2006 Census.

13. Kotila T, Adedapo K, Adedapo A, Oluwasola O, Fakunle E and Brown B. Liver dysfunction in steady state sickle cell disease. Ann Hepatol. 2005; 4 (4): 261-263.

14. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, physical activity and the prevention of cancer. A global perspective, 2007:157-171.

15. World Health Organization. Global Database on Body Mass Index, 2004.

16. Maher MM and Mansour AH. Study of chronic hepatopathy in patients with sickle cell disease. Gastroenterology Research, 2009; 2 (6): 338-343.

17. Richard S and Billett HH. Liver function tests in sickle cell disease. Clin Lab Haematol. 2002; 24(1):21-27.

18. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH *et al*. Mortality in Sickle Cell Disease — Life Expectancy and Risk Factors for Early Death. N Engl J Med 1994; 330:1639-1644.

19. Modebe O and Ifenu SA. Growth retardation in homozygous sickle cell disease: Role of calorie intake and possible gender-related differences. Am. J. Hematol 1993; 44: 149–154.

20. Gürkan E, Ergun Y, Zorludemir S, Balamili F and Koçak R. Liver involvement in sickle cell disease. Turk J Gastroenterol. 2005; 16(4):194-198.

21. Angyo IA and Yakubu AM. Lack of association between some risk factors and hepatitis B surface antigenaemia in children with sickle cell anaemia. West Afr J Med. 2001; 20 (3):214-218.

22. Amazigo UO and Chime AB. Hepatitis-B virus infection in rural and urban populations of eastern Nigeria: prevalence of serological markers. East Afr Med J. 1990; 67(8):539-544.

23. Isichei UP. Liver function and the diagnostic significance of biochemical changes in the blood of African children with sickle cell disease. J Clin Pathol 1980; 33: 626-630.

24. Hatton CS, Bunch C and Weatherall DJ. Hepatic sequestration in sickle cell anaemia. Br Med J 1985; 290 (6470):744-745.

25. Sheehy TW, Law DE, and Wade BH. Exchange transfusion for sickle cell intrahepatic cholestasis. Arch Intern Med 1980; 140 (10):1364-1366.

26. Hasan MF, Marsh F, Posner G, Bellevue R, Dosik H, Suatengco R *et al.* Chronic hepatitis C in patients with sickle cell disease. Am J Gastro. 1996; 91(6):1204-1206.

27. Rosenblate HJ, Eisenstein R and Holmes AW. The liver in sickle cell anemia. Arch Pathol Lab Med. 1970; 90:235–245.

28. Comer GM, Ozick LA, Sachdev RK, Kumar S, Taunk JL, Smith JA *et al.* Transfusion-related chronic liver disease in sickle cell anemia. Am J Gastroenterol. 1991; 86 (9):1232-1234.

29. Lesi OA and Kehinde MO. Hepatitis C virus infection in patients with sickle cell anaemia at the Lagos University Hospital. Niger Postgrad Med J 2003;10(2):79-83.

30. Ocak S, Kaya H, Cetin M, Gali E and Ozturk M. Seroprevalence of hepatitis B and hepatitis C in patients with thalassaemia and sickle cell anaemia in a long term follow up. Arch Med Res 2006; 37 (7):895-898.

31. Hassan M, Hasan S, Giday S, Alamgir L, Banks A, Frederick W *et al.* Hepatitis C virus in sickle cell disease. J Natl Med Assoc 2003; 95(10):939-942.

32. Madhava V, Burgess C and Drucker E. Epidemiology of chronic hepatitis C virus infection in sub Saharan Africa. The Lancet Infect Dis 2002; 2 (5):293-302.

33. Kyrlagkitsis I, Portmann B, Smith H, O'Grady J and Cramp ME. Liver histology and progression of fibrosis in individuals with chronic hepatitis C and persistently normal ALT. Am J Gastroenterol. 2003; 98(7):1588-1593.

34. Carreño V. Occult hepatitis C virus infection: A new form of hepatitis C. World J Gastroenterol 2006; 12(43): 6922-6925.

35. Batina AS, Dupont E, Kayembe T, Molima P, Malengela R, Kabemba S *et al.* Multiple transfusions for sickle cell disease in the Democratic Republic of Congo: the importance of the hepatitis C virus. Transfus Clin Biol. 2010; 17(4): 254-259.

36. Neto JP, Lyra IM, Reis MG and Goncalves MS. The association of infection and clinical severity in sickle cell anaemia patients. Trans R Soc Trop Med Hyg. 2011; 105 (3):121-126.

37. Ségbéna AY, Prince-David M, Kagoné TS and Dagnra AY. Human immuno-deficiency virus, hepatitis C virus and hepatitis B viruses in patients with sickle-cell disease in Togo. Transfus Clin Biol. 2005; 12 (6): 423-426.

38. Emechebe GO, Emodi IJ, Ikefuna AN, Ilechukwu GC, Igwe WC, Ejiofor OS *et al.* Demographic and sociocultural characteristics of sickle anaemia children with positive hepatitis B surface antigenaemia in a tertiary health facility in Enugu. Niger J Clin Pract. 2010; 13(3): 317-320