Original Article

Assessment of Biochemical Liver Function Tests in Relation to Age among Steady State Sickle Cell Anemia Patients

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ABSTRACT

Background and Objective: Multiorgan failure including liver dysfunction is a common finding in sickle cell anemia (SCA) patients, the cause of which is multifactorial with advancing age said to be a major determinant. There is a paucity of data on liver function among SCA patients in relation to age in northern Nigerian hospitals, including Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. This study was to assess the biochemical liver function tests (LFTs) as they relate to age among SCA patients in steady state, with a view to improving the overall monitoring of these patients. **Subjects and Methods:** This study was carried out in ABUTH, Zaria, Northern Nigeria. LFTs were carried out in 100 SCA and 100 apparently healthy participants (controls). The SCA group was made up of fifty adults and fifty children diagnosed of SCA, whereas the control group was made up of fifty adults and fifty children who were apparently healthy and had hemoglobin AA. Paired two-tailed Student's *t*-test for matched samples and Pearson's linear correlation statistical methods were employed for the data analysis using Microsoft Office Excel 2007. A $P \leq 0.05$ was considered as statistically significant. **Results:** The serum concentrations of total bilirubin (TB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and AST/ALT ratio were significantly higher in SCA patients compared to the controls ($P = 0.001$, $P = 0.001$, $P = 0.05$, $P = 0.05$ and $P = 0.001$, respectively). Serum total protein (TP) and ALB were significantly lower ($P = 0.01$ and $P < 0.05$, respectively) in SCA patients compared with the controls. The levels of TB, ALT, AST, ALP, and AST/ALT were significantly lower in SCA adults compared to SCA children, whereas TP and ALB were higher in SCA adults compared to the SCA children. There were significant negative correlations between age and each of TB, ALT, AST, ALP, and AST/ALT, and significant positive correlations between age and each of TP and ALB in SCA patients. **Conclusion:** There are mild LFTs derangements in SCA patients even in steady state with the extent of the abnormalities decreasing with advancing age of the patients.

KEYWORDS: *Children, liver function tests, sickle cell anemia, sickle cell anemia adults, sickle cell anemia patients, sickle cell disease*

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INTRODUCTION

Sickle cell anemia (SCA), the prototype and severe form of sickle cell disease (SCD), results from the inheritance of two sickle genes.[1-5] It arises from a single-base change in the DNA where adenine is replaced by thymine; this leads to the substitution of glutamic acid by valine at position 6 of the ß-globin chain which leads to the production of a defective form of hemoglobin (Hb) and HbS.[5] SCD affects millions of people throughout the world, and it is a major medical

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problem in certain parts of the world, particularly in tropical Africa, the Caribbean, and the Middle East.[2] The prevalence of SCD is quite variable, but it is estimated that 8% of Black population in America and 40% of the population in certain countries of tropical Africa have the sickle cell gene.[6] In the United States, it is present in 1 in every 500 African-American births.[6] The WHO[6] has reported 2% (about 20/1000 births) as the prevalence of SCA in Nigeria.

SCD is characterized by chronic hemolytic state and vaso-occlusions arising from the rigid sickled red blood cells leading to multiple organ infarctions.[2,7] It was reported that sickling of red cells in various parts of the body causes acute and chronic ischemia leading to progressive tissue damage.[1,4] This, in turn, is associated with increased morbidity and mortality.[2]

The liver is one of the organs involved in the multiorgan infarctions that occur in SCD. It has been reported that the liver and biliary tract dysfunctions are common complications of SCA.[8-15] The liver in SCA can be affected by a number of complications due to the disease itself or as a complication of therapy, viral hepatitis, and other hepatobiliary diseases. The clinical spectrum of SCD ranges from mild liver function test (LFT) derangements in asymptomatic participants to significant hepatic abnormalities with marked hyperbilirubinemia (sickle cell hepatopathy).[14]

Most pathologic studies of liver disease in SCA and its variants were performed retrospectively on autopsy specimens.[16,17] The presence of liver disease in SCD can be determined either from abnormal biochemical or histological tests. There is a paucity of data on the pattern of LFTs in relation to age, from childhood to adulthood among SCA patients in most Nigerian hospitals, including Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. Most of the studies reported were carried out elsewhere in the world.[8,14]

The objective of the present study was to assess the biochemical LFTs as they relate to age among SCA patients in steady state, to make a case for their inclusion in the routine monitoring of the health status of individuals with this disorder.

Subjects and Methods

The sample size for the study was determined from a standard formula for the calculation of minimum sample size.[18,19] A totals of 200 participants were consecutively recruited for the study. These consisted of 100 SCA patients and 100 participants with HbAA (controls). The control participants were consecutively recruited from individuals attending Pediatric Outpatients Department and Family Medicine General Outpatients Department clinics of ABUTH, Zaria, with minor ailments and who have no liver disease, using convenience random sampling technique. This was achieved by determining the phenotype (Hb electrophoretic pattern) of the participants using Hb electrophoresis. In this case, participants with AA phenotype were consecutively recruited. The target populations (SCA patients) were participants aged 1–40 years who were attending the Hemato-Oncology Clinic of the Department of Pediatrics and SCD Clinic of the Department of Hematology of ABUTH, Zaria.

All the SCA patients were in steady state as indicated by the findings from the history and physical examination. These are SCA patients who were not in crisis, that is, without clinical features such as vaso-occlusive crisis severe bone pain, anemia among others. Similarly, 100 participants with HbAA who were age- and sex-matched with the cases with SCA were consecutively recruited for the study as controls.

All participants who tested positive for hepatitis B surface antigen, HCV, and HIV/AIDS, and those with documented conditions such as liver disease, jaundice, and malnutrition that can affect the results of LFTs, as well as those with the history of recent (that is, in the past 6 months) blood transfusion were excluded from the study. The children whose parents/guardians declined to give consent for inclusion, as well as the adults who declined to give consent were also excluded from the study.

Informed consent for inclusion into the study was obtained from the parents/guardians of the selected children, as well as from the selected adults using a standard informed consent format. Ethical approval was obtained from the Health Research and Ethical Committee of ABUTH, Zaria, in accordance with the Helsinki Declaration.

A full medical history was obtained from the parents/guardians of the selected children, as well as from the selected adults. A detailed physical examination was carried out followed by collection of 5 ml of blood samples. The findings were filled in the study pro forma. Blood specimens were collected from all participants, into plain tubes by venipuncture in children with prominent veins and adults, and femoral tap in children whose veins were tiny, most particularly under 5 years of age. The blood specimens in the plain tubes were allowed to stand for 30 min and then the clotted samples were centrifuged, and the serum was transferred into clean plain sample bottles and then analyzed for LFTs immediately. However, in a situation where it was not possible to carry out the analysis on the day of specimens' collection due to logistic problems, the sera were kept frozen at −20°C until the following day when the analysis was done.

Serum bilirubin (SB) was estimated using Van den Bergh diazo reaction method of Malloy and Evelyn.[20] Serum alanine and aspartate tranaminases (ALT and AST) were estimated using colourimetric method of Reitman and Frankel,[21] whereas alkaline phosphatase (ALP) was estimated using method of King and Armstrong.[22] Serum albumin (ALB) was estimated using method of Doumas *et al*.,[23] whereas total protein (TP) was estimated by the Biuret method, as modified by Kingley.[24] De Ritis ratio was calculated by dividing AST by ALT activities (AST/ALT), as found by De Ritis *et al*.[25]

The data obtained were checked for completeness, entered, and analyzed using Microsoft Office Excel 2007. The results of LFTs obtained from the SCA patients were compared with those of controls using the paired two-tailed Student's *t*-test for matched samples. Similarly, the LFT results obtained from the SCA children were compared with those of SCA adults using the paired two-tailed Student's *t*-test for matched samples. Correlations between age and each of the LFT parameters in SCA patients were carried out using Pearson's linear correlation analysis. A $P \leq 0.05$ was considered statistically significant.

Results

The mean ± standard deviation (SD) ages of 100 SCA adult patients and controls were 26.00 ± 6.03 (ranged 15–40) and 28.00 ± 7.19 (ranged 15–40) years, respectively ($P = 0.105$), with males and females constituting fifty each. Similarly, the mean ± SD ages of 100 SCA children and controls were 7.00 ± 5.48 (ranged 1–14) and 7.00 ± 7.11 (ranged 1–14) years, respectively ($P = 0.113$), with males and females constituting fifty each. The serum LFTs among SCA patients and age- and sex-matched controls are shown in Table 1. The results showed that concentrations of serum total bilirubin (TB), ALT, AST, ALP, and AST/ALT ratio were significantly higher in SCA patients compared to the controls ($P = 0.001$, $P = 0.001$, $P = 0.05$, $P = 0.05$ and $P = 0.001$, respectively). Serum TP and ALB concentrations were significantly lower in SCA patients compared to the controls ($P = 0.01$ and $P = 0.05$, respectively). Table 2 shows the results of serum LFTs among SCA adults and children. The results showed that the levels of TB, ALT, AST, ALP, and AST/ALT were significantly lower ($P = 0.001$, $P = 0.001$, $P = 0.001$, $P = 0.05$, and $P = 0.001$, respectively) while TP and ALB higher ($P = 0.001$) in adults compared with children.

Correlations between age and LFTs among SCA patients are presented in Table 3. The results showed that there were significant negative correlations between age and TB, ALT, AST, ALP, and AST/ALT and significant positive correlations between age and TP and ALB.

Table 1: Liver function tests profile (mean±standard deviation) among sickle cell anemia patients and controls

Participants	*n*	TB (µmol/L)	ALT (IU/L)	AST (IU/L)	ALP (KAU/L)	TP (g/L)	ALB (g/L)	AST/ALT
SCA patients	100	31.80±25.17	34.87±17.94	40.98±19.81	133.23±61.69	63.99±13.20	38.60±8.43	1.62±1.44
Controls	100	18.73±6.34	27.71±15.09	37.19±27.14	128.65±63.78	76.45±9.32	40.68±5.08	1.53±0.95
P		0.001	0.001	0.05	0.05	0.01	0.05	0.001

n=Sample size; TB=Total bilirubin; ALT=Alanine transaminase; AST=Aspartate transaminase; ALP=Alkaline phosphatase; TP=Total protein; ALB=Albumin; AST/ALT=De Ritis ratio; SCA: Sickle cell anemia

Table 2: Liver function tests profile (mean±standard deviation) among adult and children with sickle cell anemia

Participants	*n*	TB (µmol/L)	ALT (IU/L)	AST (IU/L)	ALP (KAU/L)	TP (g/L)	ALB (g/L)	AST/ALT
SCA adults	50	17.00±0.00	22.57±14.29	27.85±4.92	121.42±69.98	75.35±7.50	43.40±7.61	0.63±0.21
SCA children	50	46.60±28.85	47.17±11.71	54.10±20.43	145.05±49.93	52.63±5.70	37.80±8.33	2.61±0.76
P		0.001	0.001	0.001	0.05	0.001	0.001	0.001

n=Sample size; TB=Total bilirubin; ALT=Alanine transaminase; AST=Aspartate transaminase; ALP=Alkaline phosphatase; TP=Total protein; ALB=Albumin; AST/ALT=De Ritis ratio

Table 3: Relationship between age and liver function tests in sickle cell anemia patients

Correlation	*r*	*P*
TB (µmol/L)	0.247	0.05
ALT (IU/L)	0.246	0.05
AST (IU/L)	0.607	0.001
ALP (KAU/L)	0.637	0.001
TP (g/L)	0.226	0.01
ALB (g/L)	0.248	0.01
AST/ALT	0.686	0.001

TB=Total bilirubin; ALT=Alanine transaminase; AST=Aspartate transaminase; ALP=Alkaline phosphatase; TP=Total protein; ALB=Albumin; AST/ALT=De Ritis ratio

Discussion

This study assessed biochemical LFTs and their relationships with age among SCA patients in steady state in Zaria, North-Western Nigeria. The findings of increased levels of serum TB, ALT, AST, ALP, and AST/ALT, and reduced levels of ALB and TP in SCA patients as observed in the present study demonstrates that some hepatic functions were deranged in SCA. Furthermore, this study showed that the derangement of LFTs decreases with advancing age of the SCA patients. This is indicated by the findings of significantly higher levels of TB, ALT, AST, ALP, and AST/ALT, and lower levels of ALB and TP in SCA children compared with SCA adults. Moreover, there was a significant negative correlation between age and each of serum TB, ALT, AST, ALP, and AST/ALT, and significant positive correlation between age and each of serum ALB and TP among these patients. This, therefore, suggests that as the SCA patients ages the severity of LFTs abnormalities decreases.

The finding of mild LFTs abnormalities in SCA as compared to controls, as indicated by slight to moderate elevation/reduction of some LFTs components in the present study agreed with previous reports.[2,10,13,15,16,26,27] Kotila *et al*.[11] in Ibadan, South-Western Nigeria reported the finding of jaundice, minimal increase in liver size and slightly elevated levels of ALT, AST, and ALP activities among adult SCA patients in the steady state. Traina *et al*.[16] in the State University of Campinas, Brazil suggests variable degrees of LFTs abnormalities. The finding of minor derangement in LFTs is consistent with the report of Maher and Mansour[14] who observed that the clinical spectrum of SCD ranges from mild LFT abnormalities in asymptomatic participants to significant hepatic abnormalities with marked hyperbilirubinemia. However, many studies reported that the abnormalities in LFTs tend to be more severe during vaso-occlusive episodes,[2] fever, and leukocytosis.[28] However, these were not studied in our patients since they were all in steady state.

The elevation of SB concentrations as found in SCA patients of our study could be due to ongoing hemolysis even in the absence of crisis. The finding of significantly higher activities of ALP in SCA patients could be due to cholestasis and long-standing vaso-occlusion. A finding of a previous study has shown that bone ALP is the principal enzyme fraction that is often increases during sickle cell crises and also suggested that there is correlation between severity of crises, serum ALP activities, and isoenzyme patterns.[11] In another study, it was suggested that the elevation of ALP activities in SCA patients could be detected even when the participants are asymptomatic,[28] and this is consistent with the finding of our study of elevated ALP despite the fact that the SCA patients recruited were in steady state. Kotila *et al*.[11] reported that 74% of the SCA patients showed elevated serum ALP levels, but no significant correlation was found between it and liver size. Thus, suggesting that liver pathology may not be solely accountable for the elevation of this enzyme.

It was reported that the incidence of liver dysfunction in children with SCA is common, being a component of the multiorgan failure that occurs in this disorder.[29] However, the pathophysiology of the liver disease in SCA is not certain because of its complexities. Moreover, it has been observed that enlargement of the liver does not connote disease and a normal-sized liver may be diseased.[11,30] Therefore, the LFTs abnormalities reported in the present study could not be ascribed to a particular factor, rather it could be a combination of factors.

The occurrence of liver disease in SCA may be due to various causes such as obstruction of sinusoids by sickle cells with subsequent hepatic infarction during vaso-occlusive episodes, red cell sequestration, cholelithiasis, and cardiac failure. Other factors which may contribute to the etiology of the liver disease in SCD includes ischemia, transfusion-related viral hepatitis, iron overload (hemosiderosis), and gallstones. Coiner *et al*.[30] suggest that the most common causes of liver disease in SCD are those related to repeated blood transfusion such as hemosiderosis and viral hepatitis. These authors reported hemosiderosis and erythrophagocytosis in the liver biopsies of all sickle cell participants with chronically elevated LFTs in their study. A similar finding was reported by Traina *et al*.[16] and Mills.[17] However, our study excluded SCA patients with hepatitis and recent blood transfusion, and therefore these are unlikely to be causes of abnormal LFTs in these patients. The presence of hemosiderosis and cardiac failure was also not established in the SCA patients in this study, and therefore their role in the causation of the observed abnormal LFTs could not be ascertained. The most likely causes of altered LFTs in the SCA patients in our study could be hemolysis and red cell sequestration with resultant hyperbilirubinemia. The presence of hyperbilirubinemia might have caused bilirubin toxicity, and the excess bilirubin could lead to irritation of biliary tract and gallstones formation/cholelithiasis which then leads to the elevation of serum ALP.

It was suggested by Benerjee *et al*.[9] that the hepatic complications of the SCDs, including SCA can be separated into disorders related to hemolysis, the problem related to anemia and subsequent transfusion management, the consequences of sickling and vaso-occlusion, and

defects unrelated to SCD. Maher and Mansour[14] have also suggested a multifactorial etiology for liver disease in SCD patients, including factors associated with chronic hemolytic anemia (cholelithiasis), multiple transfusions (viral hepatitis and iron overload), vascular damage, and the sickling process itself. However, data points to the importance of vascular changes and the significant precipitation of the sickling process in most participants, as reported by Charlotte *et al*.[31] Interestingly, a distinct clinical presentation of sickle cell intrahepatic cholestasis, and a syndrome characterized by progressive cholestasis in the absence of cirrhosis has been reported in a small number of cases of SCA.[32] These cases are characterized by right upper quadrant pain, extreme elevation of bilirubin, striking elevation of ALP, and variable elevations of transaminases, as well as histological features including intracanalicular cholestasis, sinusoidal dilatation, kupffer cells hyperplasia, and erythrophagocytosis.

The decrease in the extent of liver function abnormalities with advancing age among SCA patients in this study could be due to increase hepatic maturity and reduction of the number and severity of crisis in adults compared with children. It was reported by McKerrell *et al*.[26] that the numbers of crises and admissions among SCA patients were higher in the younger group than in the older. The frequent crisis in SCA could lead to various systemic imbalances and multiorgan dysfunction. It was reported that both the autonomic nervous system activity and blood viscosity are impaired in patients with SCA exhibiting high frequency of pain crisis in comparison with those who did not experience a crisis within the previous year.[33] It has been demonstrated that patients who had suffered more frequent pain crises had lower parasympathetic activity, greater sympathovagal imbalance, and higher blood viscosity than both controls and patients with milder disease.[33] These abnormalities could be some of the mechanisms for the more severe LFTs abnormalities observed in children as compared with adults in the present study.

Conclusion

The findings of this study reveal that there are mild LFTs abnormalities in SCA patients even in the steady state, and the extent of the abnormalities decreases with advancing age of the patients. It could be recommended that routine evaluation of liver function status be considered to monitor SCA patients, especially children. It is recommended that ALP isoenzymes be evaluated, in the future studies, to ascertain the origin of its elevation in SCA patients.

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Conflicts of interest

There are no conflicts of interest.

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