LIVER FUNCTION TESTS PROFILE OF SICKLE CELL ANAEMIA PATIENTS IN STEADY STATE OF HEALTH: ZARIA EXPERIENCE

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ABSTRACT

Background: *Several reports across the world suggest that liver and biliary tract dysfunctions are common complications of sickle cell anaemia (SCA). However, there is paucity of data on the pattern of liver function tests profile in SCA patients in Zaria. Most of the reported studies were carried out elsewhere.*

Objective: *The overall objective of this study was therefore to evaluate the liver function status in patients with SCA in Zaria, northern Nigeria with a view to recommend or otherwise the inclusion of liver function tests in the routine investigation of SCA in Nigerian hospitals.*

Subjects and methods: *The study was conducted in the Departments of Chemical Pathology and Haematology of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, northern Nigeria. Serum levels of total bilirubin (TB), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB) and AST/ALT (De Ritis) ratio were determined in sixty (60) each of SCA patients and age- and sex-matched control individuals. These consisted of thirty (30) each of male and female patients and controls, respectively. The mean age of these patients was 21 years (ranged 13-40 years). Both the patients and controls were partitioned according to age groups, as groups I (13-20 years), II (21-30 years) and III (31-40 years) with thirty three (33), fifteen (15) and twelve (12) subjects, respectively. The data obtained were analysed using Microsoft Office Excel 2003. Two-tailed student's t- test for matched samples and one way analysis of variance (ANOVA) statistical methods were employed for the analyses. A p-value of equal to or less than 0.05 ($p = 0.05$) was considered as statistically significant.*

Results: *The results of serum TB, ALT, AST, ALP, TP, ALB and AST/ALT ratio in SCA patients were 24.33±0.21 µmol/L, 47.17±1.51, 27.85±0.63, 145.05±6.45 IU/L, 75.35±0.97, 43.40±0.98 g/L and 0.7±0.06 respectively, while those in controls were respectively 16.09±0.79 µmol/L, 27.78±1.48, 15.78±0.90, 72.32±3.57 IU/L, 72.58±0.87, 50.48±2.26 g/L and 0.6±0.03. These results show that the levels of serum TB, ALT, AST and ALP were significantly higher in patients than in controls ($p < 0.001$), while serum ALB concentrations were significantly lower ($p < 0.002$) in patients than in control individuals. The results of serum TP and AST/ALT ratio in patients and controls were not statistically different ($p>0.05$). These results therefore, demonstrate that*

INTRODUCTION

Sickle cell disease (SCD) is an autosomal recessive (inherited) blood disorder characterized by defective (sickle) haemoglobin (HbS) in the red cells when deoxygenated [1-5]. It describes a group of complex, chronic disorders characterized by haemolysis, unpredictable acute complications that can rapidly become life-threatening, and the development of variable chronic organs damage [4, 5]. Sickle cell anaemia (SCA), occurring in homozygote state (haemoglobin-S gene) results from substitution of a single amino acid, namely valine for glutamic acid in the beta globin gene at chromosome number 6, and it is the most severe SCD [1]. Sickle cell anaemia is a common genetic disorder [1] and the most prominent member of a group of inherited disorders of the oxygen carrying cells of blood [2].

Sickle cell anaemia presents a major medical problem in certain parts of the world, particularly in tropical Africa, the Caribbean and the Middle East [1]. The prevalence of SCD is quite variable, but it is estimated that 8 % of black people in America and 40 % of the population in certain countries of tropical Africa have the sickle cell trait [6]. In Jamaica, 8 % of black people carry the sickle gene [7]. It has been reported that one in eight black Americans harbours the trait, while approximately one in 400 has the disease [2]. In fact, SCD affects millions of people throughout the world . It is present in one in every 500 African-American births [3].

Sickle cell anaemia is characterized by chronic haemolytic anaemia and vaso-occlusive crises, which can lead to widespread vascular occlusion by sickled red blood cells leading to multiple organ infarctions [1]. Sickling of red cells in various parts of the body causes acute and chronic ischemia leading

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LFTs are mildly deranged in SCA patients. There were no gender variations in LFTs profile in both patients and controls. Serum TB and ALP in patients decrease significantly with advancing age, while serum ALT, AST, TP, ALB and AST/ALT ratio levels at different age groups were statistically similar. All the components of LFTs at different age groups in controls were statistically similar.

***Conclusion**: It can be concluded from the findings of this study that there is a minor derangement in LFTs profile in SCA patients and that the extent of the abnormalities decreases with advancing age. This therefore, suggests that liver functions are impaired in SCA patients, most especially below twenty years of age. Therefore, it can be recommended from this study that routine evaluation of liver function status be considered in the management of SCA patients in Nigerian hospitals. This could assist in early detection of liver dysfunction and hence reduce morbidity and mortality from SCA in Nigeria.*

to progressive tissue damage [1, 4, 5]. The rate of development of various complications is variable but many patients develop organ damage. This, in turn, is associated with increased morbidity and mortality. Sickle cell anaemia patients are at increased risk of serious morbidity and mortality [1].

The liver is one of the organs involved in the multiorgan failure that occurs in SCD [8]. Liver and biliary tract dysfunctions are common complications of SCA [8]. Early reports described jaundice, hepatic infarcts, acute and chronic hepatitis, choledocholithiasis, and cirrhosis [9]. The hepatic complications of SCA can be classified into the following: disorders related to haemolysis, the problems of anaemia and transfusion management, the consequences of sickling and vaso-occlusions, and defects unrelated to sickle cell disorder [8].

Early detection of complications of SCA such as liver and renal diseases is very essential in reducing the morbidity and mortality in SCA patients. This could be achieved by including various investigations, including liver and renal functions tests (LFTs and RFTs). However, there is paucity of data in the liver function status of SCA patients in most of the hospitals, including Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. Therefore, in view of its clinical importance, there is the need to evaluate the liver function status in SCA patients in ABUTH, Zaria with a view to recommending the inclusion or otherwise of liver function tests in the routine investigation of SCA in Nigerian hospitals. This could improve the management of this group of patients in Nigerian hospitals and hence reduce morbidity and mortality among patients with these disorders.

Subjects and Methods

The study was conducted in the Departments of Chemical Pathology and Haematology of ABUTH, Zaria, northern Nigeria. A total of sixty (60) SCA patients were studied. These consisted of thirty (30) each of male and female patients. The mean age of these patients was 21 (ranged 13-40) years.

All the SCA patients were recruited from Sickle Cell (SS) Clinic of ABUTH, Zaria. At the clinic, arrangements were made with the haematologists whereby consecutive patients who satisfied the study inclusion criteria were selected. These include patients who were confirmed to have a typical laboratory finding of SS haemoglobin electrophoretic pattern and accompanying clinical features of SCA, and who have not had crises in the last three weeks. Informed consent for inclusion into the study was obtained from the patients. All patients who declined to give consent for inclusion and those that tested positive for HB_sAg were excluded from the study. The nature of the study was explained to the patients in the appropriate languages best understood by them. A full medical history was obtained from these patients. This was followed by a detailed physical examination and collection of blood specimens. Similarly, sixty (60) age- and sex-matched apparently healthy individuals, who had AA haemoglobin electrophoretic patterns were recruited as controls. These were selected from the population of staff and students of ABUTH, Zaria and demonstration secondary school of Ahmadu Bello University (ABU), Zaria.

Blood specimens (about 10 ml each) were taken into plain tubes by venepuncture, using syringe and needle after sterilising the site with methylated spirit. The blood specimens were centrifuged and the sera were carefully drawn into sample bottles and then analysed immediately or stored frozen at -20°C until the following day, in a situation whereby the analysis is not possible because of logistic problem. The samples were analysed for total bilirubin (TB), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein (TP) and albumin (ALB). Concentrations of serum TB were measured using diazo method of Malloy and Evelyn [10], while those of TP and ALB were measured using method of Doumas *et al* [11]. Serum ALT and AST activities were determined by method of Reitman and Frankel [12], while those of ALP were determined by method of King and Armstrong [13]. De Ritis ratios (AST/ALT ratios) were determined by AST activities divided by ALT activities, as found by De Ritis *et al*[14]. Both the patients and controls were partitioned according to age groups, as groups I (13-20), II (21-30) and III (31-40) years with thirty three (33), fifteen (15) and twelve (12) subjects respectively.

Statistical Analysis

The data obtained were analysed using Microsoft Office Excel 2003. The results of LFTs obtained from SCA patients were compared with those of controls using the two-tailed student's t- test for matched samples. Similarly, comparison of the LFTs results between male and female SCA patients, as well as controls were carried out using the two-tailed student's t- test for matched samples.

The LFTs results obtained from SCA patients, as well as controls at different age groups were compared using one way analysis of variance (ANOVA). A p-value of equal to or less than 0.05 ($p = 0.05$) was considered as statistically significant.

RESULTS

The results of liver function tests (LFTs) profile in SCA patients and controls are presented in Table 1 and also illustrated in Fig. 1. These results show that the levels of serum TB, ALT, AST and ALP were significantly higher in SCA patients than in controls ($p < 0.001$), while serum ALB concentrations were significantly lower ($p < 0.002$) in the patients than in control individuals. Serum TP concentrations and AST/ALT ratio in patients and controls were not statistically different ($p>0.05$). These results therefore, demonstrate that there is a derranged liver function in SCA patients. However, the levels of serum ALB and TP were within the reference range. Table 2 shows the LFTs profiles according to gender in patients and controls. The results in these tables indicate that there were no gender variation in all the LFTs components in both SCA patients and controls. The LFTs profiles according to different age groups in patients and controls are shown in Tables 3 and 4 respectively. The results in Table 4 show that serum TB and ALP in patients decrease significantly with advancing age, while serum ALT, AST, TP, ALB and AST/ALT ratio levels at different age groups were statistically similar. Though, the activities of ALP were apparently higher in group I (13-20 years) control individuals, all the components of LFTs at different age groups were statistically similar ($p>0.05$) as shown in Table 4.

Table 1: Liver function tests profile (Mean ± SEM) among SCA patients and controls

Subjects	n	TB (µMol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	ALB (g/L)	AST/ALT
Patients	60	24.33±0.21	47.17±1.51	27.85±0.63	145.05±6.45	74.35±0.97	43.40±0.98	0.7±0.06
Controls	60	16.09±0.79	27.78±1.48	15.78±0.90	72.32±3.57	73.58±0.87	50.48±2.26	0.6±0.03
P-value		<0.001	<0.001	<0.001	<0.001	>0.05	<0.002	>0.05

n=sample size, TB=total bilirubin, ALT=alanine amino transferase, AST=aspartate amino transferase, ALP=alkaline phosphatase, TP=total protein, ALB=albumin and AST/ALT=De Ritis ratio.

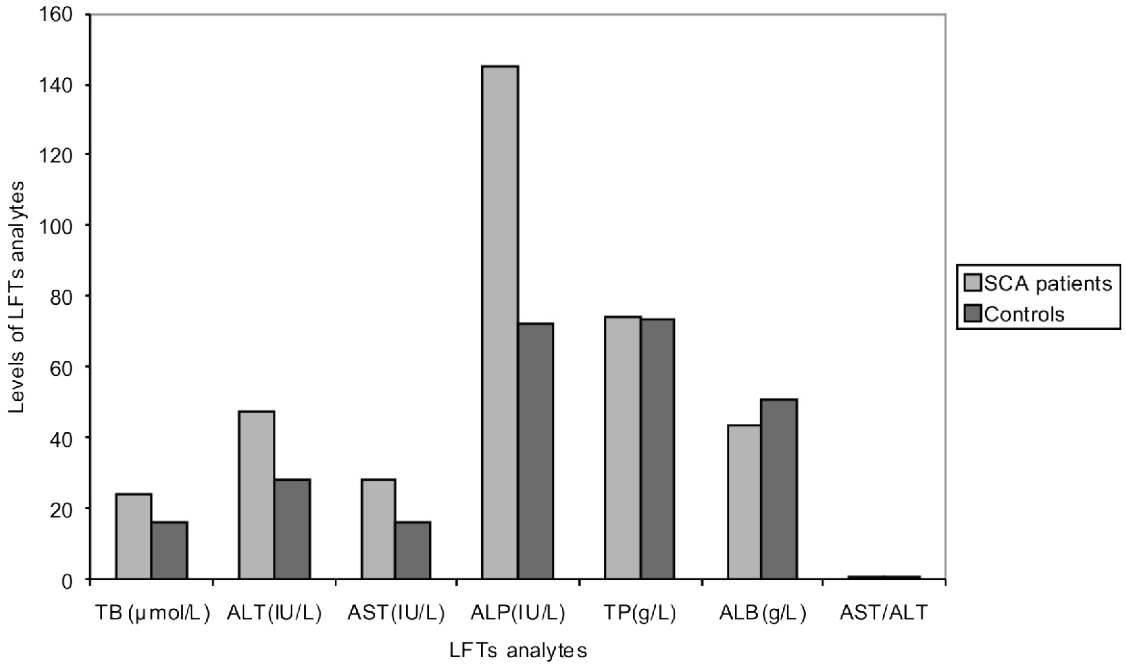

Fig. 1: Liver function tests profile among SCA patients and controls

TB=total bilirubin, ALT=alanine transaminases, AST=aspartate transaminases, ALP=alkaline phosphatase, TP=total protein, ALB=albumin and AST/ALT=De Ritis ratio.

Table 2: Liver function tests profile (Mean ± SEM) among SCA patients and controls according to gender

Gender	n	TB (μMol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	ALB (g/L)	AST/ALT
Male patients	30	24.44±0.16	46.00±2.47	29.40±1.01	145.17±8.70	75.13±1.47	43.27±1.41	0.7±0.03
(Male controls)	(30)	(16.06±0.83)	(29.56±2.98)	(14.88±1.77)	(83.75±6.52)	(72.88±1.51)	(39.31±1.05)	(0.6±0.08)
Female patients	30	24.19±0.22	48.33±1.76	26.30±0.67	144.93±9.66	75.5±1.29	43.53±1.40	0.6±0.03
(Female controls)	(30)	(16.03±0.56)	(28.29±2.02)	(14.07±1.16)	(61.20±6.26)	(74.07±1.64)	(40.47±1.40)	(0.6±0.07)
p-value		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
(p-value)		(>0.05)	(>0.05)	(>0.05)	(>0.05)	(>0.05)	(>0.05)	(>0.05)

n=sample size, TB=total bilirubin, ALT=alanine amino transferase, AST=aspartate amino transferase, ALP=alkaline phosphatase, TP=total protein, ALB=albumin and AST/ALT=De Ritis ratio.

Table 3: Liver function tests profile (Mean ± SEM) among SCA patients according to age

Age group	n	TB (μMol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	ALB (g/L)	AST/ALT
I	33	27.11±0.23	49.75±2.68	28.81±0.93	159.30±9.16	76.08±1.41	43.27±1.34	0.7±0.04
II	15	21.07±0.07	45.97±2.10	27.25±1.18	121.81±6.29	74.69±1.27	44.21±1.67	0.6±0.04
III	12	20.01±0.06	43.50±1.44	26.00±0.64	113.25±9.75	74.50±3.88	40.75±1.44	0.6±0.05
P-value		<0.05	>0.05	>0.05	<0.02	>0.05	>0.05	>0.05

Groups I=13-20 years, II=21-30 years and III=31-40 years, n=sample size, TB=total bilirubin, ALT=alanine amino transferase, AST=aspartate amino transferase, ALP=alkaline phosphatase, TP=total protein and ALB=albumin and AST/ALT=De Ritis ratio.

Table 4: Liver function tests profile (Mean ± SEM) among controls according to age

Age group	n	TB (μMol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	ALB (g/L)	AST/ALT
I	33	16.01±0.06	28.33±3.91	13.83±1.30	73.00±14.10	73.83±2.37	39.67±1.43	0.5±0.03
II	15	15.93±0.22	28.11±2.38	14.39±1.44	71.22±6.85	72.72±1.35	38.89±1.05	0.6±0.07
III	12	16.34±0,05	28.22±3.69	14.89±1.93	66.00±6.71	73.89±2.43	39.56±1.64	0.6±0.08
P-value		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Groups I=13-20 years, II=21-30 years and III=31-40 years, n=sample size, TB=total bilirubin, ALT=alanine amino transferase, AST=aspartate amino transferase, ALP=alkaline phosphatase, TP=total protein, ALB=albumin and AST/ALT=De Ritis ratio.

DISCUSSION

The liver function status (LFS) in SCA patients has been evaluated with the available facilities in ABUTH, Zaria. The findings of this study showed that serum TB, ALT, AST, ALP levels were significantly higher, while serum ALB concentrations were lower in SCA patients than in controls. These findings therefore demonstrate that LFTs were derranged in SCA. However, with the exception of ALP activities which were markedly elevated, the levels of TB, ALT and AST were moderately raised, while those of serum TP and ALB were within the reference ranges. The results suggest that gender of both SCA patients and controls did not significantly affect the levels of the LFTs components and that there was close association between age and serum levels of TB and ALP in SCA patients only. It demonstrates that the levels of TB and ALP decrease with advancing age in sickle cell patients. This therefore suggests that the severity of liver pathology is higher in patients at below twenty years of age than in those at higher age. The finding of apparently higher activities of ALP in control individuals at between 13 and 20 years of age than in older ones could be attributed to the increased production of this enzyme during the pubertal stage.

The finding of abnormal LFTs revealed by the present study agreed with previous reports [1, 8-9, 15 -18]. Kotila *et al* [8] reported the occurrence of jaundice and minimal increase in liver size and the activities of ALT, AST and ALP in steady state SCD in Ibadan, Nigeria. Isichei [16] studied serum protein profile in SCD in Enugu, Nigeria and found that there was significant decrease in serum TP concentrations. Some of the other studies conducted by Hargrove [15], Johnson [9], Traina *et al* [18] in western world suggest variable degrees of LFTs abnormalities. However, many of the studies reported that the abnormalities in LFTs tend to be more severe during vaso-occulusive episodes [1], fever and leukocytosis [17]. It has been estimated by Diggs [19] that 10 % of SCA patients admitted to hospital developed hepatic crisis and this was confirmed by Sheehy in a study of 378 admissions over a 10 year period [20].

The incidence of liver dysfunction in SCA patients is common, being a component of the multiorgan failure that occurs in this disorder [21]. However, the pathophysiology of the liver disease in SCA is not certain because of its complexities. The occurrence of liver disease in SCA is multifactorial [18, 22] and therefore there are no definite diagnostic criteria [23]. The clinical manifestation of the different causes of liver failure is also similar and interrelated, thus making the pathophysiology complex [8, 24]. Moreover, it is well known that enlargement of the liver does not connote disease and a normal size liver may be diseased. The occurrence of liver disease in SCA may be due to variety of causes such as obstruction of sinusoids by sickled cells with subsequent hepatic infarction during vaso-occulusive episodes, cholelithiasis and cardiac failure. But by far the most common causes are those related to repeated blood transfusion such as haemosiderosis and viral hepatitis which lead to chronic liver disease as suggested by Coiner *et al* [25]. They reported haemosiderosis and erythrophagocytosis in the liver biopsies of all sickle cell patients with chronically elevated LFTs in their study. This was supported by Johnson *et al* [9], Traina *et al* [18] and Mills [26]. It was suggested by Benerjee *et al* [22] that the hepatic complications of the SCDs, including SCA can be separated into the following categories: (1) disorders related to haemolysis, (2) the problem related to anaemia and subsequent transfusion management, (3) the consequences of sickling and vaso-occulusin and (4) defects unrelated to SCD.

The elevation of serum TB concentrations as found in our SCA patients could be due to haemolysis, while the increased serum ALT and AST activities reflect hepato-cellular injury, which are known to be common in this group of patients [22]. High levels of ALP found in the patients of the present study may be because of either cholestasis or bone lesion being some of the common complications of SCA [8, 27]. Acute intra-hepatic cholestasis may be a consequence of widespread sickling within the sinusoid or extreme haemolysis with resultant hyperbilirubinaemia, which is often accompanied by elevated ALP and variable levels of ALT and AST [22]. Kotila *et al* [8] in a study reported that 74 % of the sickle cell 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. A previous study has identified bone ALP as the principal enzyme fraction that increases during sickle cell crises and also appeared that there is correlation between severity of crises, serum ALP activities and isoenzyme patterns [8]. These abnormalities could also be detected even when the patients are asymptomatic [26]. This infers that the elevated levels of ALP may be more to the recurrent vaso-occulusive crises involving the bones in this group of patients.

Conclusion

It was concluded from the findings of this study that there is a minor derangement in LFTs profile in SCA patients and that the extent of the abnormalities decreases with advancing age of the patients. This implies that impairment of liver functions is more pronounced in SCA patients at below 20 years of age than in those at above 20 years bracket. Therefore, we recommend that routine evaluation of liver function status be considered in the management of SCA patients in Nigerian hospitals. This could assist in early detection of liver dysfunction in this group of patients and hence reduce morbidity and mortality from SCD in Nigeria.

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REFERENCES

1. Hofmeyr GJ : induction and augmentation of labour. In Dewhurt's Textbook of Obstetrics and Gynaecology, 7th edition. Edited by DK Edmonds. Blackwell publishers Ltd(London). 2007:pp205-212.

2. Margulies M, Perez GC, Voto LS: Misoprostol to Induce labour. Lancet, 1992; 339:64.

3. Goldberg AB and Wing DA: Induction of Labour: The Misoprostol Controversy. J Midwifery Womens Health, 2003; 48 (4):244-248.

4. Orhue AAE : Induction of labour. Trop J Obstet Gynaecol, 1997;14(1):1-15.

5. Bique C.Bugalho A, Bergstrom S.Labour induction by Vaginal Misoprostol in Grand Multiparous Women. Obstetrical & Gynaecological Survey.1999;54(10): 623-625

6. De Aquino MM, Cecatti JG.Misoprostol versus oxytocin for labour induction in term and post term pregnancy:randomized controlled trial.Sao Paulo Med J.2003;121: 102-106

7. Akhan SE, Yidirim A, Lyibozkurt AC, Turfanda A.The evaluation of misoprostol-related tachysystole in normal and high risk pregnancies. Clin Exp Obstet Gynecol.2000;27:207-211

8. Rockhead C, Fletcher H, Reid M, Morgan O: A comparison of two methods of labour induction with vaginal misoprostol. Int J Gynaecol Obstet, 2003:80(3);271-277.

9. Wing DA, Paul RH.Misoprostol for cervical ripening and labour induction:the clinician's perspective and guide to success. Contemp Ob/Gyn.1999; 44: 46-61.

10. Lee HY: A randomised double-blind study of vaginal misoprostol vs dinoprostone for cervical ripening and labour induction in prolonged pregnancy. Singapore medical journal, 1997; 38 (7):292-294.