



Biochemical Assessment of the Liver in SCD in a Tertiary Hospital in South-South, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KA and OE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BOB and OR managed the analyses of the study. Author OB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell disease (SCD) is often associated with liver disease. The constant state of haemolysis, multiple blood transfusion, viral hepatitis, hepatic sinusoidal congestion, haemosiderosis and cholestasis, are all conditions which may eventually evolve into liver disease. Sickle cell disease is a heterogeneous group of disorders that is usually associated with an autosomal recessive structural haemoglobin disorder. Biochemical abnormalities have been associated with SCD and it is usually more pronounced in vaso occlusive crises; an acute bone crisis and common painful complication of SCD, than in steady state.

Aim: The aim of the study was to assess some biochemical parameters in relation to SCD patients in our environment with a view to improving the monitoring and management of these patients.

Methodology: The study was a comparative hospital based research carried out at the University of Calabar Teaching Hospital (UCTH), Calabar, South-South Nigeria. Liver function tests were

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carried out on 60 SCA both in steady state and in crisis and also on 50 apparently healthy adults. The data collected were analyzed using statistical data for social sciences (SPSS) Version 22 for windows. Pearson linear correlation and simple inferential statistical methods were employed for data analysis, a $P \leq 0.05$ was considered to be statistically significant.

Result: The serum concentrations of AST, ALT, ALP, LDH, Total and conjugated bilirubin were seen to be elevated in VOC compared to in steady state and with the apparently healthy control group. The AST/ALT ratio was also observed to be elevated in VOC as compared with the steady state and the control. Significant product moment correlation was observed in the biochemical parameters both in steady state and in VOC.

Conclusion: The findings of this study revealed marked changes in the biochemical parameters of the liver in VOC than in steady state. It will be recommended that routine evaluation and proper interpretation of liver enzymes is paramount in early detection of liver pathology in SCD.

Keywords: SCD; liver function tests; haemolysis; Calabar.

1. INTRODUCTION

Assay of AST, ALT & ALP are the routine common enzymes measured. This enzymes helps in the diagnosis of viral, metabolic and autoimmune hepatic disorders and are also used as a criteria to select patient for liver transplant [1,2]. The activity of this enzyme is presumably increased following release of cytoplasmic protein from damage hepatocytes into the vascular system following tissue necrosis by drug intoxication, ischemia, reperfusion injury or rejection after liver transport [3,4].

Elevation of the liver enzyme correlates with the different categories. AST is raised in haemolysis, ALP is elevated during bone pain crises; studies suggest that bone ALP contribute to this increase while ALT level more accurately reflects hepatic injury [1]. In sickle cell disease, the liver shows some attribute of siderosis, congestion and hepatomegally [5]. Hepatic complication in SCD can be classified based on the following disorders related to increase haemolysis, the problem of anaemia and transfusion iron overload, hepatitis, the complication of sickling and repeated vaso-occlusions leading to intrahepatic sinusoidal dilation and hepatic crisis. Other sequel including intrahepatic cholestasis and ischaemic necrosis may occur [6,7,8].

Endothelia haemolytic dysfunction intensify VOC [9,10]. This is supported by an elevation of LDH, with low level of Hb and high bilirubin [10,11,12]. There is an increase correlation with AST and not ALT is consistent with higher concentration of AST than ALT in red blood cells released during intravascular haemolysis. Biochemical abnormalities have been associated with SCD. Bone disease with osteomalacia and osteoporosis are common in SCD; the level of

alkaline phosphatase indicates the severity of the bone damage and it is a utilitarian guide in the management of bone pain in SCD [13].

Biochemical changes in liver function test are common in patients with SCA, even in the absence of hepatic complication. The aim of this study is to determine the biochemical pattern of liver function test and their correlation with haemolysis both in steady state and VOC in Calabar, South-South Nigeria.

2. METHODOLOGY

A total of 110 participants comprising of 60 SCA and 50 apparently healthy adults with HbAA as controls. Sample size was calculated using the formula for comparative study:

$n = (U + V)^2 (\sigma_1^2 + \sigma_2^2) / ((\mu_1 - \mu_2)^2)$ [14]. The control patients were individuals with no liver disease and apparently healthy on physical examination and were consecutively recruited from the blood donor clinic of the department of Haematology and Blood Transfusion, UCTH. The SCA patients were recruited from SCD Clinic/Haematology Day-Care Clinic of the Department of Haematology and Blood Transfusion, UCTH, Calabar. All subjects that tested positive for hepatitis B surface antigen, HCV, and HIV/AIDS, those with documented conditions that could affect LFTs results such as; malnutrition, jaundice, and/or liver disease were excluded from the study.

Informed consent for inclusion into the study was obtained from all the participants using a standard informed consent format. Ethical approval was obtained from the Health Research and Ethical Committee of UCTH, Calabar. A comprehensive medical history was obtained

from all the participants followed by collection of 5 ml of blood samples by venipuncture into plain tubes. The blood samples in the plain tubes were allowed to stand for 30 minutes and the clotted samples were centrifuged at 4500rms. The serum was transferred into clean plain sample containers and then analyzed for LFTs and lactate dehydrogenase (LDH).

Serum aspartate and alanine transaminases (AST & ALT) were estimated using colourimetric method of Reitman and Frankel [15], alkaline phosphatase (ALP) was analyzed using King and Armstrong method [16]. Serum bilirubin was estimated using Van den Bergh diazo reaction method of Malloy and Evelyn [17]. Serum LDH was analyzed using kits manufactured by Sigma-Aldrich, Germany. The manufacturers protocol was adhered to. De Ritis ratio was calculated by dividing AST by ALT activities (AST/ALT), as described by De Ritis et al. [18].

3. RESULTS

A total of one hundred and ten participants were recruited into the study, sixty participants make up the SCA patients' group while 50 participants were the apparently health control group. The age of the participants ranges from 16-60 years. The SCA patients group comprises of 23 (38.3%) males and 37 (61.7%) females while the control group comprises of 29 (58%) males and 21 (42%) females respectively. The liver function test of the participants showed that the serum concentrations of AST, ALT, ALP, LDH, total and conjugated bilirubin were elevated in VOC compared to in steady state and with the apparently healthy control group. The AST/ALT ratio was also observed to be elevated in VOC

as compared with the steady state and the control.

Serum concentrations of AST in steady state, VOC and control group includes; 42.47 ± 10.50 , 47.95 ± 21.41 , and 21.42 ± 8.38 respectively. ALT was 37.75 ± 10.78 in steady state, 40.30 ± 18.84 in VOC and 26.86 ± 11.66 for the control group respectively. De Ritis ratio was 1.18 ± 0.32 , 1.26 ± 0.90 , 0.90 ± 0.44 for steady state, VOC and control respectively.

Concentration of ALP was 64.28 ± 17.94 , 72.63 ± 27.19 , 72.82 ± 20.10 for steady state, VOC and in control respectively. LDH concentration was 425.08 ± 215.95 , 681.90 ± 304.12 for steady state, VOC and control respectively. Conjugated bilirubin 13.23 ± 4.65 , 17.94 ± 12.99 , and 2.54 ± 0.99 ; total bilirubin 50.54 ± 17.16 , 59.21 ± 22.06 and 13.52 ± 4.65 for steady state, VOC and apparently healthy control respectively. The Pattern of liver enzymes and some biochemical parameters in SCD Patients and apparently healthy controls are presented in Table 2.

Pearson product moment correlation showed a significant moderate positive correlation between liver enzymes in steady state, VOC and a negative correlation was observed in the control group with $r = 0.460$, $r = 0.147$, and $r = -0.239$ for AST while $r = 0.460$, $r = 0.460$, $r = -0.239$ for ALT was reported in steady state, VOC and control group respectively. Serum ALP concentration showed a significant correlation in steady state, negative correlation coefficient in VOC when compared with control and steady state. A positive correlation was observed between LDH in steady state when compared with control and VOC $r = 0.064$ while a significant

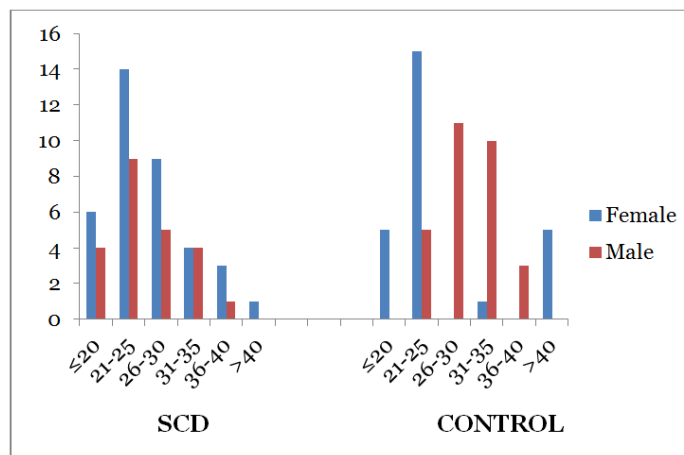


Fig. 1. Showing age and gender distribution of the participants

Table 1. Showing liver function tests values in SCA patients and controls (Mean \pm SD)

Subject	AST (IU/L)	ALT (IU/L)	AST/ALT	ALP	LDH	Conjugated bilirubin	Total bilirubin
STEADY STATE	42.47 \pm 10.50	37.75 \pm 10.78	1.18 \pm 0.32	64.28 \pm 17.94	425.08 \pm 215.95	13.23 \pm 4.65	50.54 \pm 17.16
VOC	47.95 \pm 21.41	40.30 \pm 18.84	1.26 \pm 0.90	72.63 \pm 27.19	681.90 \pm 304.12	17.94 \pm 12.99	59.21 \pm 22.06
CONTROL	21.42 \pm 8.38	26.86 \pm 11.66	0.90 \pm 0.44	72.82 \pm 20.10	67.50 \pm 38.74	2.54 \pm 0.99	13.52 \pm 4.65
ANOVA	F = 47.122, P = <0.001	F = 13.133, P = <0.001	F = 2.80, P = 0.386	F = 2.80, P = 0.064	F = 103.99, P = <0.001	F = 45.67, P = <0.001	F = 110.92 P = <0.001

Table 2. Showing the pattern of liver enzymes and some biochemical parameters in SCD patients

Liver enzymes	Steady state (n = 60)		VOC(n = 60)		Control (n = 50)	
	Normal (%)	Elevated (%)	Normal (%)	Elevated (%)	Normal (%)	Elevated (%)
AST	28 (46.70)	32 (53.3)	26 (43.3)	34 (56.7)	50 (100.00)	0 (0.00)
ALT	57 (95.00)	3 (5.00)	55 (91.70)	5 (8.30)	50 (100.00)	0 (0.00)
ALP	58 (96.70)	2 (3.3)	57 (95.00)	3 (5.00)	50 (100.00)	0 (0.00)
LDH	4 (6.70)	56 (93.30)	4 (6.70)	56 (93.30)	49 (98.00)	1 (2.00)
CONJUGATED BIL	5 (8.30)	55 (91.70)	1 (1.70)	59 (98.30)	50 (100.00)	0 (0.00)
TOTAL BIL	3 (5.00)	57 (95.00)	2 (3.3)	58 (96.70)	50 (100.00)	0 (0.00)

Table 3. Correlation between liver enzymes in steady state, VOC and control

Liver enzymes	Steady state	VOC	Control
	r (P-value)	r (P-value)	r (P-value)
AST	0.460 (<0.0001)**	0.147 (0.309)	-0.239 (0.095)
ALT	0.460 (<0.0001)**	0.460 (<0.0001)**	-0.103 (0.035)
AST/ALT	-0.089 (0.537)	0.484 (<0.001)**	-0.215 (0.134)
ALP	0.556 (<0.0001)**	-0.092 (0.526)	0.094 (0.515)
LDH	0.064 (0.515)	0.587 (<0.0001)**	0.183 (0.204)
CONJUGATED BIL	-0.253 (0.076)	0.396 (0.002)**	-0.059 (0.683)
TOTAL BIL	-0.008 (0.958)	0.466 (<0.001)**	0.131 (0.363)

correlation was seen in the VOC $r = 0.587$. Both conjugated and total bilirubin showed a negative correlation when compared with in steady state, VOC and control; $r = -0.253, 0.396, -0.059$ for conjugated bilirubin in steady, VOC and control respectively while total bilirubin have coefficients; $r = -0.008, 0.466$ and 0.131 for steady state, VOC and control respectively as reported in Table 3.

4. DISCUSSION

The index study showed a significant higher level of total bilirubin, conjugated bilirubin, AST, ALT and LDH in SCA patients in VOC compare to steady state. The high level of total and conjugated bilirubin can be attributed to the widespread ongoing haemolysis which is exacerbated in VOC, also contribution from ineffective haemolysis; which is a feature of the disease condition. AST is said to be raised during haemolysis and is more pronounced in VOC. In like manner, during VOC; the level of ALT is also raised. Similarly, the level of LDH is also raised and more pronounced in VOC. Other biochemical parameters showed no statistically significant difference. Johnson et al reported a similar finding to the above [19].

The ALP in this study showed no statistically significant difference on the mean value in both steady, VOC and control but 5% of the SCA had a higher value both in steady state and VOC. This is similar to the finding by Kotila et al. [20]; furthermore, Brody et al. also reported similar finding [21]. This study is somewhat similar to the study by Akuyam et al whom reported statistically significant elevated levels of AST, ALT and TB but at variance with their finding on ALP, the difference in study could be attributed to pattern of care, use of hydroxyurea and transfusion modalities [22]. Also, the ease of accessing treatment; such as establishment of day care facilities and competent personnel, all these help to improve the patients' health outcome.

ALP is said to be the major enzyme portion that is increased during crises and there is also a correlation between crisis severity and serum ALP level. These abnormalities could also be detected even in steady state [23].

De Ritis ratio which is used to determine hepatic necrosis was observed to be of no statistical significance; this could be due to haemolysis. This was similar to the reports from previous studies [24,25,26] and somewhat similar to the findings by Akuyam et al but was at variance with previous studies reported but which shows that

AST/ALT ratio was lower in adult compared to children [21]. However, De Ritis ratio was higher in the patients than in the control.

5. CONCLUSION

The findings of this study reveal marked changes in the biochemical parameters of the liver in VOC than in steady state. It will be recommended that proper interpretation of the biochemical parameter of the liver in SCD is essential in avoiding misdiagnosis and management of patients with SCD.

CONSENT

Informed consent for inclusion into the study was obtained from all the participants using a standard informed consent format.

ETHICAL APPROVAL

Ethical approval was obtained from the Health Research and Ethical Committee of UCTH, Calabar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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