Original Research Article

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BIOCHEMICAL ASSESSMENT OF THE LIVER IN SCD IN A TERTIARY HOSPITAL IN SOUTH-SOUTH, NIGERIA

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, all of which may eventually evolve into liver disease. Biochemical abnormalities have been associated with SCD and it is usually more pronounced in vaso occlusive crises than in steady state.

Aim: The aim of the study is 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 is a comparative hospital based research carried out at the University of Calabar Teaching Hospital (UCTH), Calabar, South-South Nigeria. Liver function tests were 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 reveal marked changes in the biochemical parameters of the liver in VOC than in steady state. It is~~will be~~ recommended that routine evaluation and proper interpretation of liver enzymes is paramount in early detection of liver pathology in SCD.

KEY WORDS: SCD, Liver Function Tests, Haemolysis, Calabar

INTRODUCTION

The liver is one of the most important organ in the body. It integrity, synthetic, storage, transport and secretory function can be accessed through liver function test[1]. 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[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 overlaod, 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.

METHODOLOGY

A total of 110 participants comprising of 60 SCA and 50 apparently healthy adults with HbAA as controls. 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.

Comment [R1]: How many patients are enrolled in the clinic? How did you select the 60 SCA for this study? How did you arrive at the sample size?

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 5ml of blood samples by venipuncture into plain tubes. The blood samples in the plain tubes were allowed to stand for 30minutes 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 [14], alkaline phosphatase (ALP) was analyzed using King and Armstrong method [15]. Serum bilirubin was estimated using Van den Bergh diazo reaction method of Malloy and Evelyn [16]. Serum LDH was analyzed using ...(r). De Ritis ratio was calculated by dividing AST by ALT activities (AST/ALT), as described by De Ritis *et al* [17].

Comment [R2]: What is the meaning of this?

RESULT

A total of one hundred and ten participants were recruited into the study, sixty participants made 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 at± 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 below.

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 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 below.

Comment [R3]: The comparisons need to be accompanied by p-values and Pearson correlation coefficient to show whether the difference was significant

Comment [R4]: The comparisons need to be accompanied by p-values and Pearson correlation coefficient to show whether the difference was significant

Table 1: Showing Liver Function Tests Values in SCA Patients and Controls (Mean ± SD)

SUBJECT	AST (IU/L)	ALT (IU/L)	AST/ALT	ALP	LDH	CONJUGATED BILIRUBIN	TOTAL BILIRUBIN
STEADY STATE	42.47 ± 10.50	37.75 ± 10.78	1.18 ± 0.32	64.28 ± 17.94	425.08 ± 215.95	13.23 ± 4.65	50.54 ± 17.16
VOC	47.95 ± 21.41	40.30 ± 18.84	1.26 ± 0.90	72.63 ± 27.19	681.90 ± 304.12	17.94 ± 12.99	59.21 ± 22.06
CONTROL	21.42 ± 8.38	26.86 ± 11.66	0.90 ± 0.44	72.82 ± 20.10	67.50 ± 38.74	2.54 ± 0.99	13.52 ± 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

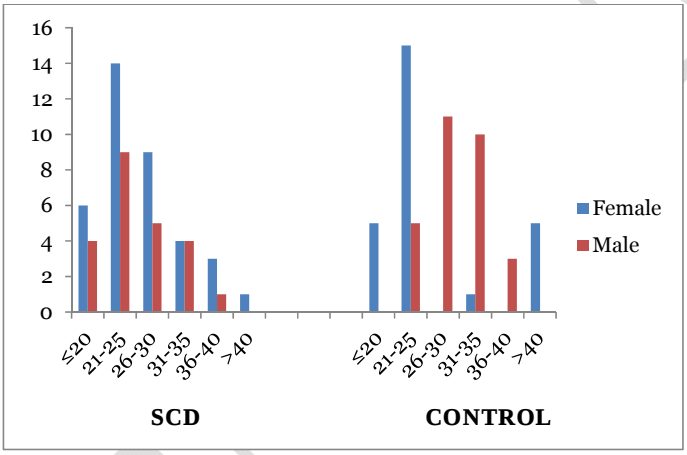

Figure 1: Showing age and gender distribution of the participants

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 (0683)
TOTAL BIL	-0.008 (0.958)	0.466 (<0.001)**	0.131 (0.363)

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 [18].

Comment [R5]: Given the nature of SCD, these findings were expected. Authors have to discuss what value do results add to the care of SCA patients

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* [19]; furthermore, Brody et al also reported similar finding [20]. 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 [21]. 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 [22].

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 [23,24,25] 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.

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 routine evaluation and proper interpretation of liver enzymes is paramount in early detection of liver pathology in SCD.

Comment [R6]: This study was not intended to show association between liver enzymes and pathology; hence it is difficult to recommend such

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