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ORIGINAL ARTICLE

Liver Enzymes and Trace Elements in the Acute Phase of Sickle Cell Anaemia

Enzymes hépatiques et des éléments traces dans la phase aiguë de l'anémie à hématies falciformes

M. O. Kehinde*, S. I. Jaja†, O. M. Adewumi†, A. I. Adeniyi†, M. O. Nezianya†, Esther O. Ayinla†

ABSTRACT

BACKGROUND: **Trace elements are required for the performance of numerous functions of immune cells. It is not clear whether levels of trace elements are elevated and whether there is a relationship between the levels of liver enzymes and trace elements in patients with sickle cell anaemia in crisis.**

OBJECTIVE: **To compare the plasma levels of liver enzymes and trace elements in non sickle cell anaemia (NSCA), sickle cell anaemia subjects in the steady state (SCASS) and sickle cell anaemia patients in crisis (SCAC).**

METHODS: **Haematological parameters, liver enzymes and trace elements were determined in 20 NSCA subjects, 20 SCASS subjects and 18 SCAC subjects. Variables studied included aspartate aminotransferase (AST) alanine aminotransaminase (ALT), alkaline phosphatase (ALP), and the trace elements copper, zinc, and manganese.**

RESULTS: **Levels of liver enzymes were higher in the SCAC subjects than in the NSCA or SCASS subjects (p<0.001). Plasma Cu^{++}, Zn^{++} and Mn^{++} were also higher in the SCAC subjects than in the NSCA or SCASS subjects (p<0.001). Correlationships were high and strong between AST and ALT (r = +0.7; p = 0.03), AST and ALP (r = +0.9; p = 0.001), Zn^{++} and Fe^{++} (r = +0.9; p = 0.001) in SCAC.**

CONCLUSION: **During crisis in sickle cell anaemia, liver enzymes, as well as the trace elements of Cu^{++}, Zn^{++} and Mn^{++} are increased; levels of aspartate aminotransaminase are strongly correlated with those of ALT and ALP. Levels of liver enzymes do not appear to be related to those of the trace elements in painful sickle cell crisis. WAJM 2010; 29(4): 244–248.**

Keywords: **Sickle cell anaemia crisis, irreversibly sickle cells, liver enzymes, platelets, trace elements.**

RÉSUMÉ

CONTEXTE: Les oligo-éléments sont nécessaires à l'exercice de nombreuses fonctions des cellules immunitaires. Il n'est pas clair si les niveaux d'oligo-éléments sont élevés et s'il existe une relation entre les niveaux des enzymes hépatiques et des oligo-éléments chez les patients souffrant d'anémie falciforme en crise.

OBJECTIF: Pour comparer les taux plasmatiques des enzymes hépatiques et des oligo-éléments dans l'anémie à hématies falciformes non (LSRN), la faucille sujets anémie à cellules à l'état stationnaire (SCASS) et la faucille anémie de cellules de patients en crise (SCAC). enzymes hépatiques METHODES: Les paramètres hématologiques et oligo-éléments ont été déterminées chez des sujets NSCA 20, 20 et 18 sujets SCASS sujets SCAC. Variables étudiées comprenaient l'aspartate aminotransférase (AST) alanine taux d'aminotransférase (ALT), la phosphatase alcaline (ALP), et les oligo-éléments cuivre, le zinc et le manganèse.

Résultats: Les niveaux d'enzymes hépatiques étaient plus élevés chez les sujets SCAC que dans la LSRN ou SCASS sujets (p <0,001). Plasma

Cu^{++}, Zn^{++}et Mn^{++} étaient également plus élevés chez les sujets SCAC que dans la LSRN ou SCASS sujets (p <0,001). Correlationships étaient élevés et forte entre AST et d'ALT (r 0,7 = p = 0,03), AST et ALP (r 0,9 = p = 0,001), Zn^{++} et Fe^{++} (r 0,9 = p = 0,001) du SCAC.

CONCLUSION: Au cours de la crise dans la drépanocytose, les enzymes hépatiques, ainsi que les oligo-éléments de Cu^{++}, Zn^{++} et Mn^{++} sont augmentées, les niveaux des taux d'aminotransférase aspartate sont fortement corrélées à celles de l'ALT et l'ALP. Les niveaux d'enzymes hépatiques ne semblent pas être liées à celles des oligo-éléments en crise douloureuse cellules de faucille. WAJM 2010; 29 (4): 244–248.

Mots-clés: crise de drépanocytose, de façon irréversible les cellules falciformes, les enzymes hépatiques, des plaquettes, des oligo-éléments.

Departments of *Medicine, †Physiology, College of Medicine, University of Lagos, Lagos. Nigeria.

Correspondence: **Prof. S. I. Jaja,** Department of Physiology, College of Medicine, University of Lagos, PMB 12003, Lagos. Nigeria.
E mail: sjaja4@yahoo.com

Abbreviations: ALP, Alkaline Phosphatase; **ALT,** Alkaline Transaminase; **AST,** Aspartate Aminotransaminase; **NSCA,** Non Sickle Cell Anaemia; **SCA,** Sickle Cell Anaemia; **SCAC,** Sickle Cell Anaemia Patients in Crisis; **SCD,** Sickle Cell Disease; **SCASS,** Sickle Cell Anaemia Subjects in the Steady State.

INTRODUCTION

In sickle cell disease (SCD) progressive injury to the liver with decreased liver function by adulthood has been reported.[1] The pathophysiology of liver disease in this condition is complex because of the interrelated multifactorial causes. Hepatic lesions that are mainly vascular have been suggested as being responsible for the resultant acute and/or chronic ischaemia in the liver[2] which may then trigger liver dysfunction. Other factors that may compound the pathophysiology of the liver in SCD are iron overload, viral damage and cholelithiasis.[3,4]

The persistence of hepatomegaly , a frequent finding in sickle cell anaemia, is associated with increased disease severity. Gurkan *et al*[4] demonstrated intrasinusoidal sickling and Kupffer cell hyperplasia in all biopsy specimens studied by them. In addition they showed that all their patients had hepatomegaly while 27% of the patients showed liver function test abnormalities. Oparinde *et al*[5] also showed that increase in liver enzymes was associated with hepatomegaly. They demonstrated a significant increase in serum alkaline transaminase, alanine phosphatase, and gamma-glutamyl transferase levels in sickle cell anaemia with persistent hepatomegaly over those without hepatomegaly.

In the acute state, hepatic functional derangements in children have been associated with increases in alanine aminotransferase, alkaline phosphatase and bilirubin.[6] Kotila *et al* [7] have however attributed the higher levels of alkaline phosphatase to the associated vaso-occlussive crises involving the bones rather than a pathology of the liver. In addition, acute vaso-occlusive involvement of the liver with hepato-cellular – cholestatic or purely cholestatic injury which are benign in nature has been reported.[8]

Trace elements are required for the differentiation, activation and performance of numerous functions of the immune cells. Trace elements may therefore be important in the pathophysiology of sickle cell disease. In comparison to non sickle cell anaemia subjects, plasma zinc concentration has been shown to be lower in sickle cell anaemia patients in the steady state.[9–13] However, serum copper and magnesium levels were higher in sickle cell anaemia patients than in their non sickle cell anaemia counterparts.[9,10] It is not clear whether zinc deficiency is related to a deficient stature in sickle cell anaemia patients or not.[9,11] Also, reduced plasma zinc is related to increased vascular cell adhesion molecule-1, (VCAM-1), a protein that is involved in vaso-occlusion.[13]

Chronic supplementation with zinc has been shown to reduce incidence of infections and vaso-occlussive pain crisis, generation of tumor necrosis factor-alpha and also decreased levels of oxidative stress markers.[14,15] It is not clear from the literature what the relationship of liver enzymes to plasma trace elements in the acute state of sickle cell anaemia is.

This study therefore determined the plasma levels of some hepatic enzymes and certain trace elements (Zn^{++}, Cu^{++}, Mn^{++} and Fe^{++}) during pain crisis in sickle cell anaemia patients. Comparisons between plasma levels of the measured hepatic enzymes, and trace elements in non sickle cell disease, sickle cell disease in the steady state and during pain crisis have also been made. The study, in addition, set out to investigate the relationship between the measured hepatic enzymes and trace elements during painful crisis in sickle cell anaemia.

SUBJECTS, MATERIALS, AND METHODS

Paticipants

Fifty-eight subjects participated in the study. Twenty subjects were non-sickle cell anaemia (NSCA) subjects. They were students of the College of Medicine of the University of Lagos, Lagos, Nigeria. The subjects were of genotype HbAA, selected on the basis of their medical records on admission into the University. The NSCA subjects were non-smokers, non-alcoholics and were not on any medication at least six months to the time of the study. All physical and cardio-vascular measurements and the collection of blood were performed in the Research Laboratory of the Department of Physiology of the College of Medicine of University of Lagos, Lagos, Nigeria.

Twenty other subjects were sickle cell anaemia subjects in the steady state (SCASS) who attended the Out-Patient Sickle Cell Clinic of the Lagos University Teaching Hospital, (LUTH), Lagos, Nigeria. Anthropometric and blood pressure measurements and the collection of blood from this group were made at the clinic. None of the subjects with SCA had been admitted to the ward for pain crisis in the preceding six months. There was also no past history of blood transfusion in the last twelve months.

The last group of 18 subjects with sickle cell anaemia. Patients admitted to the ward with pain crisis (SCAC). All measurements and collection of blood were made on arrival at the Emergency Room and before admission to the ward.

The principles outlined in the Helsinki declaration were followed, including informed consent from each subject.

Measurement of Anthropometry and Blood Pressure

The following anthropometric measurements were made in each subject and recorded: height (in centimeters and without shoes) with a meter rule and weight (kilograms and in light clothing) with a bathroom weighing scale.

Arterial blood pressure was measured using the brachial artery of each subject using the sphygmomanometric – auscultatory method. Diastolic blood pressure was taken as the fifth phase (disappearance of the Korotkoff's sounds).

Estimation of Haematological Parameters and Irreversibly Sickled Cells

Haemoglobin concentration [Hb, g/dl], haematocrit (Hct, %), red blood cell count (RBC count, mm^3) and platelet count (mm^3) were estimated using an automated counter (Swelab AC 910EO+, Boule Medical AB, Stockholm, Sweden).

A modification of the method of Jensen *et al* [16] was used in the identification and determination of the percentage of irreversibly sickled cells.[17] Briefly, after equilibration with air, 0.01 ml of the blood sample was put in a test tube containing 5 ml of normal saline and thoroughly mixed by gentle inversion. With a capillary tube, a drop of the mixture

was placed on a slide and covered with a cover slip and then observed under a light microscope using the X40 objective. The percentage of irreversibly sickled cells was determined by counting the number of cells that remained sickled and dividing by the total number of cells counted. Four hundred cells were counted per slide.

Determination of Plasma Levels of some Hepatic Enzymes and Trace Elements

From each subject, 10 ml of blood was withdrawn from an ante-cubital vein and stored in heparinized EDTA (ethylene diamine tri-acetic acid) bottles until assayed. Colorimetric method was used in the determination of hepatic enzymes (aspartate aminotransferase, μ/L, alanine aminotransferase, μ/L and alkaline phosphatase, μ/L). Atomic absorption spectrometry (AAS) method (Perking Emer Analyst, 2000) was used for the determination of plasma levels of copper (Cu^{++}, mg/l), iron (Fe^{++}, mg/l), zinc (Zn^{++}, mg/l) and manganese (Mn^{++}, mg/l).

Data Analyses: Statistical analyses were performed with the software package Origin MicroCal. (Version 8.0). Results are expressed as mean and standard error of mean (Mean ± SEM). Comparison of means was by ANOVA. Correlation coefficient (r) was calculated between measured hepatic enzymes and trace elements in SCAC patients. Statistical significance was set at $p < 0.05$.

RESULTS

A summary of anthropometric and blood pressure values is shown in Table 1. There were no significant differences in the measured variables between non-sickle cell anaemia (NSCA) subjects, the sickle cell anaemia subjects in the steady state (SCASS) and in crisis (SCAC).

Table 2 shows a comparison of some blood parameters in the three groups of subjects with NSCA subjects serving as controls. Red blood cell count (RBC) was significantly higher in NSCA subjects than in SCASS ($p<0.05$) and SCAC subjects ($p< 0.001$). Haemoglobin concentration [Hb] and Hct values were significantly higher in NSCA subjects than in SCASS and SCAC ($p<0.001$ in each case). Platelets were significantly higher in SCAC than in SCASS ($p<0.01$) and NSCA subjects ($p<0.001$).

Table 1: Anthropometric and Blood Pressure Measurements in Participants

Parameter	NSCD (a)	SCASS (b)	SCAC(c)	(a) Vs(b)*	(a) Vs(c)*	(b) Vs(c)*
Age (yr)	24.0±0.8	26.1±1.4	24.9±1.4	NS	NS	NS
Weight (kg)	62.0±4.8	63.3±5.6	61.1±2.8	NS	NS	NS
Height (m)	1.7 ± 0.1	1.7±0.04	1.7±0.01	NS	NS	NS
BMI	22.3±2.7	22.0±1.7	21.6±1.1	NS	NS	NS
SBP	115.0±2.0	120.0±1.8	127.0±2.6	NS	NS	NS
DBP	74.0±2.0	74.0±2.0	73.0±3.1	NS	NS	NS
MAP	88.0±1.7	89.0±1.6	91.0±2.3	NS	NS	NS

**ANOVA, Values are mean ± SD. BMI, Body Mass Index in Kg^{-2}; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MAP, Mean Arterial Blood Pressure in mmHg.*

Table 2: Summary of Haematological Parameters

Parameter	NSCD (a)	SCASS (b)	SCAC (c)	(a) Vs(b)*	(a) Vs(c)*	(b) Vs(c)*
RBC (x $10^6/mm^3$)	5.9±0.1	4.8±0.2	3.8 ± 0.1	<0.01	<0.001	<0.01
Hb (g/dl)	13.2±0.6	8.1±0.2	7.8 ± 0.07	<0.001	< 0.001	NS
Hct (%)	39.0±1.5	22.0±0.6	24.0 ± 0.2	< 0.001	< 0.001	NS
Platelets (x $10^3/mm^3$)	148.0±15.0	280.0±29.0	359.0±34.0	< 0.001	< 0.001	< 0.05
Irreversibly sickle cell count (%)	0	7.0 ± 0.6	30.0 ± 0.6	< 0.001	< 0.001	< 0.001

**ANOVA*

Table 3: Summary of Levels of Enzyme Markers and Trace Elements

Variable	NSCA (a)	SCASS (b)	SCAC (c)	(a) Vs(b)*	(a) Vs(c)*	(b) Vs(c)*
AST (μ/L)	36.4 ± 4.1	31.8 ± 5.2	60.0 ± 8.1	NS	< 0.01	< 0.01
ALT (μ/L)	14.2 ± 0.8	13.3 ± 1.3	22.1 ± 3.7	NS	< 0.05	< 0.05
ALP (μ/L)	19.3 ± 2.1	31.8 ± 3.0	32.3 ± 2.5	< 0.01	< 0.01	NS
Cu^{++} (mg/l)	0.07 ± 0.03	0.06 ± 0.02	0.17 ± 0.03	NS	< 0.01	< 0.01
Fe^{++} (mg/l)	0.70 ± 0.04	0.80 ± 0.10	1.0 ± 0.20	NS	NS	NS
Zn^{++} (mg/l)	0.70 ± 0.07	0.70 ± 0.06	3.4 ± 0.70	NS	< 0.001	< 0.001
Mn^{++} (mg/l)	0.10 ± 0.04	0.07 ± 0.005	0.20 ± 0.07	NS	< 0.05	< 0.01

**ANOVA, Values are mean* ± SD

Table 3 shows that plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher in SCAC subjects than in NSCA and SCASS subjects ($p<0.001$ in each case). Also, plasma levels of alkaline phosphatase (ALP) were significantly higher in SCAC and SCASS than in NSCA subjects ($p < 0.01$ in each case). Plasma ALP levels were similar in SCAC and SCASS subjects. Table 3 also shows that plasma levels of Cu^{++} and Zn^{++} were significantly higher in SCAC subjects than in NSCA and SCASS subjects ($p<0.001$ in each case). Plasma level of Mn^{++} was also significantly higher in SCAC subjects than in NSCA ($p<0.05$) and SCASS ($p<0.001$) subjects. Plasma levels of Fe^{++} were however similar in the three groups of subjects.

Correlation coefficients (r) between measured enzymes and plasma trace elements during SCAC are presented in Table 4. Significant and positive correlations were seen between AST and ALT ($r = 0.7$; $p = 0.03$), AST and ALP ($r = 0.9$; $p = 0.001$) and Zn^{++} and Fe^{++} ($r = 0.9$; $p = 0.001$).

Table 4: Correlation Coefficients between Measured Hepatic Enzymes And Trace Elements During SCA-Cr

	AST	ALT	ALP	Cu^{++}	Fe^{++}	Zn^{++}	Mn^{++}
AST r	1.0	0.7	0.9	0.2	0.03	–0.01	–0.3
p	–	0.03	0.001	0.6	0.9	0.9	0.6
ALT r	1.0	0.5	0.2	– 0.1	–0.1	–0.5	
p	–	0.1	0.6	0.8	0.8	0.2	
ALP r		1.0	0.1	–0.01	–0.09	–0.03	
p	–	0.8	0.9	0.8	0.5		
Cu^{++} r		1.0	0.4	0.5	0.3		
p		–	0.3	0.2	0.5		
Fe_{++} r			1.0	0.9	0.6		
p		–	0.001	0.1			
Zn^{++} r			1.0	0.5			
p		–	0.2				
Mn^{++} r						1.0	
p					–		

r, Correlation coefficient; p, Level of significant.

DISCUSSION

Our results show an elevated platelet count in the SCAC subjects. Platelet count in NSCA and SCASS subjects were however similar. Platelets and leucocytes have been implicated in acute sickle cell pain crisis. Platelet activation with ADP release is known to occur during acute sickle cell pain crisis.[18] Activated platelets secrete ADP which cause adherence to sickle erythrocytes contributing to platelet plug and formation of microvascular obstruction.[18–20]

Leucocytes also contribute to the acute phase of SCA by adhesion to blood vessel walls and obstructing the lumen. They aggregate with other blood cells and stimulate the vascular endothelium to increase its expression of ligands for adhesion molecules on blood cells thus causing more effective blockage of the lumen. In this way, they cause tissue damage and inflammatory reaction which predispose to vaso-occlusion.[21]

The lower RBC, [Hb] and Hct values seen in the SCAC subjects are as a direct consequence of the destruction of RBCs. During vaso-occlusive crisis, red blood cells are destroyed leading to low RBC count or anaemia, low haemoglobin concentration and reduced haematocrit. Proportions of irreversibly sickle cell count in SCASS and SCAC seen in this study are similar to what have been reported earlier in these patients.[22–24] Irreversibly sickled cells are known to play an initiating role in the pathogenesis of sickle cell crisis.[22,25]

The SCAC patients were examined in the Emergency Room of the Out Patients Department of LUTH. The elevated levels of AST, ALT and ALP seen in SCAC patients agree with an earlier study.[6] Due to illness the patients had abstained from food even before hospitalization. Other workers have shown in man[26] and in rats[27] that prolonged starvation increases the activities of ALT and AST. During fasting, transaminases favoring gluconeogenesis are induced making the liver much more effective in synthesizing glucose.[28,29] It is therefore likely that the elevated transaminases may be due to reduced intake of food. It has also been suggested that the source of the elevated ALP in sickle cell anaemia sufferers may be the bone rather than liver.[7] The present results however show that the plasma level of AST was positively and significantly related to those of ALT and ALP during SCAC. There was no significant relationship between any of the measured liver enzymes and the trace elements.

Our results show that plasma concentrations of the measured trace elements were similar in NSCA and SCASS subjects. This is similar to the earlier result of Alayash.[30] However other authors have shown that while plasma Zn^{++} was lower,[9–11] plasma Cu^{++} was higher[9] in SCASS subjects than in NSCA subjects. Further, our results show that apart from plasma Fe^{++} that was similar in all the groups, plasma concentrations of all the other trace elements measured were higher in the SCAC group than in SCASS or NSCA group. Although comparative studies were not found in the literature, Elia *et al* [31] have shown that after a short-term fast by healthy non-obese young adults, plasma Zn^{++} increased markedly and returned to normal after refeeding. Also, after a long fast (44 days) by non-obese men, it has been shown that plasma Zn^{++} increased while plasma Cu^{++} was normal.[32] The elevated plasma levels of these trace elements during fasting have been attributed to breakdown of lean tissues and also from bone.[31] It is therefore likely that the elevated levels of trace elements in the SCAC group were caused by abstinence from food brought about by ill health.

As earlier pointed out, there was no significant relationship between the plasma levels of any of the measured liver enzymes and the trace elements. Apart from Zn^{++} that positively and significantly related to Fe^{++}, there was no significant relationship between the plasma levels of the other measured trace elements.

In conclusion, the results of this study show that during SCAC the expression of alanine aminotransaminase, aspartate amino-tranaminase and alkaline phosphatase from the liver were elevated. There is a positive and significant relationship between the plasma level of AST and ALT and also between AST and ALP. No liver enzyme was significantly related to any trace element. Plasma levels of Cu^{++}, Zn^{++} and Mn^{++} were also elevated. Plasma levels of Zn^{++} and Fe^{++} were positively and significantly related.

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