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Research article

LIVER ENZYMES AND ITS ASSOCIATION WITH AGE AND SEX IN SICKLE CELL ANAEMIA PATIENTS AND HAEMOGLOBIN S TRAIT CARRIERS.

Chuku L C[1], Chinaka N C[2]

[1, 2] *Department of Biochemistry, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria*

**Corresponding Author: cn_chinaka@yahoo.com*

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ABSTRACT

The activities of the liver enzymes, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase were compared between males and females of various age groups ($0–25^{+}$) during sickle cell crisis and in steady state. Results show that enzyme activities increased during painful crisis. The difference in activities of the enzymes in normal homozygous HbAA and heterozygous (HbAS) blood was not statistically significant ($p \geq 0.05$). The activities of the enzyme increased with age in all the genotypes studied. There was no significant difference ($p \geq 0.05$) in the activities of ALT, AST and ALP between males and females for all the age groups studied.

KEY WORDS: Alanine transaminase, alkaline phospatase, anaemia, aspartate transaminase, genotype, heterozygous, homozygous, sickle cell.

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INTRODUCTION

Sickle cell anaemia is a form of sickle cell disease in which both abnormal genes are for the formation of HbS (Konotey-Ahulu, 1974). Sickle cell haemoglobin (HbS) is the major abnormal haemoglobin. Its solubility is sufficiently altered to produce a serious disease when present in the homozygotes (White, *et al*., 1978). Sickle cell anaemia (SCA) has been a great clinical problem in the history of West Africans since the 7th century. The disease was first described by a Chicago physician, J.B. Herrick (Herrick, 1910). Herrick observed peculiar elongated and sickled-shaped red cells in a case of severe anaemia of an ailing West Indian student.

The term "sickle cell trait" is used to describe a person who has inherited one normal haemoglobin gene (A) from one parent and one abnormal gene (S) from the other parent. Sickle cell disease arose as a result of mutation which caused a single amino acid substitution (valine for glutamic acid) at the sixth position of the β-globin chain of the haemoglobin molecule (Acquaye, *et al*., 1981). This change has an adverse effect on the haemoglobin molecule. Hence, the research was undertaken to study any variation that might occur in the enzymes in normal and sickle cell subjects over a wide age range and sexes (male and female).

MATERIALS AND METHODS

Materials, test kits and equipments used were of laboratory standards. Blood from normal and heterozygous individuals was obtained at the University of Port Harcourt Teaching Hospital and LABMEDICA laboratory, Port Harcourt. Samples were then grouped according to their ages and sex.

The normal subjects (HbAA and HbAS) were aged between 0–70 years and of both sexes. It was not possible to obtain HbSS blood from patients beyond 20 years of age. Blood was also collected from HbSS patients who were in haemolytic crisis.

Electrophoresis

In all cases, blood samples from different individuals were genotyped. Different molecular species of Hb were separated from each other by electrophoresis at pH 8.4 on cellulose acetate. The separated haemoglobin bands were then stained by Ponceau S dye and identified by comparison with known haemoglobin standards separated stained in the same manner.

Different types of Hb were separated from one another by electrophoresis on cellulose acetate paper. Separation was clear at the buffer pH of 8.4. Sixty four (64) blood samples were separated into 24 AA, 16 AS and 24 SS.

Assay of enzyme activity

Alanine transaminase (ALT) activity: By Ratman and Frankel, (1957).

Alanine transaminase activity was estimated by the procedure described in the RANDOX kit for the determination of alanine transaminase activity in serum or plasma at 546 nm. The optical density (O.D) of the reaction mixture was taken colorimetrically at 546 nm as a measure of the enzyme activity.

Aspartate transaminase (AST) activity: By Ratman and Frankel, (1957).

Aspartate transaminase catalyses the reaction (transamination) between α-ketoglutarate and L-aspartate. The oxaloacetate formed in the reaction reacts with 2, 4-dinitrophenylhydrazine (DNPH), which in alkaline medium gives a red-brown colour. This is measured in a colorimeter at 546 nm. This measures the activity of aspartate transaminase. The enzyme activity was measured by a procedure described in the RANDOX GOT kit.

Alkaline phosphatase (ALP) activity: By Ratman and Frankel, (1957).

Alkaline phosphatase (ALP) hydrolyses p-nitrophenyl phosphate in alkaline conditions to

yield phosphate and p-nitrophenol. The nitrophenol is yellow in colour and absorbs maximally at 405 nm. The intensity of the colour due to p-nitrophenol during a fixed time is measured at 405 nm colometrically and is proportional to the ALP activity in the sample. This was estimated by the procedure described in the "BESSEY LOWRY" colour method kit for the determination of ALP activity in plasma or serum at 420 nm.

RESULTS

Enzyme Activity

The effect of age on ALT, AST and ALP activities of HbAA, HbAS and HbSS subjects are shown in tables 3.1 as well as their steady and crisis state as shown in table 3.2.1, 3.2.2 and 3.2.3 respectively.

The effect of sex on ALT, AST and ALP activities in HbAA and HbAS subjects are is illustrated in table 3.3.1 respectively. The effect of sex on AST and ALP activities of HbSS and its crisis state is shown on table 3.3.2 and 3.3.3 respectively.

DISCUSSION

Alanine transaminase (ALT):

The activity of ALT of sickle cell subjects was found to be higher than normal. There was however no significant difference ($p \geq 0.05$) in the specific activity of ALT for normal (AA and AS) subjects. It was also observed that the activity of ALT did not increase steadily with age and did not also depend on sex. Serum ALT is highly variable in sickle cell disease. Values obtained for subjects between 11–25 years were far lower than those obtained for subjects between 6–10 years although higher than the values obtained for subjects between 0–5 years. This was observed in all the genotypes.

In crisis and steady state, ALT activity in HbSS increased with age (11–20 years) in steady state.

However, with the age range studied there was no significant difference ($p \geq 0.05$) in mean enzyme activity values when compared with normals. The pattern could change in older subjects, but for age 0–25 years at least hepatic dysfunction based on this enzyme alone could not be definitely deduced (see tables 3.1, 3.2.1).

Table 3.1: Effects of age on ALT, AST, and ALP activities in HbAA, HbAS and HbSS subjects.

		ALT Activty (µL)			AST Activty (µL)			ALP Activty (µL)	
Age (yrs)	AA	AS	SS	AA	AS	SS	AA	AS	SS
0-5 n = 5	5.3 ± 1.0	5.5 ± 1.2	30.1 ± 2.0	7.5 ± 0.2	15.75 ± 0.4	19.75 ± 0.6	137.66 ± 23.1	102.8 ± 18.2	148.75 ± 14.9
6-10 n = 5	11.7 ± 0.8	13.2 ± 0.9	36.9 ±1.8	13.33 ± 0.4	14.70 ± 0.3	10.5 ± 0.5	100.33 ± 15.2	102.8 ± 12.6	126.0 ± 8.49
11-15 n = 5	7.8 v 1.2	7.5 ± 1.0	26.8 ± 1.4	5.5 ± 0.2	11.0 ± 0.1	31.0 ± 1.9	66.75 ± 6.7	70.0 ± 5.6	88.7 ± 26.6
16-20 n = 5	7.2 ± 0.6	10.5 ± 0.8	43.5 ± 1.2	9.67 ± 0.7	14.5 ± 0.2	39.1 ± 2.0	40.8 ± 13.3	49.5 ± 0.71	72.4 ± 13.6
21-25 n = 5	8.0 ± 1.0	7.5 ± 0.9		12.0 ± 0.2	13.5 ± 0.4		31.5 ± 0.7	28.0 ± 2.8	
>25 n = 5	8.0 ± 1.0	8.8 ± 1.0		10.25 ± 0.7	10.67 ± 0.5		29.8 ± 2.4	31.0 ± 16.2	

n = number of samples analysed.
Values are Mean ± SD.

Table 3.2.1: Effect of age on ALT activity in HbSS subjects (Crisis and steady state).

	ALT Activity (µL)		
Age (yrs)	Steady state	Age (yrs)	Crisis
0–5 n = 5	15.3 ± 0.5	0–5 n = 3	30.0 ± 0.7
6–10 n = 4	15.3 ± 0.6	6–10 n = 3	36.8 ± 1.4
11–15 n = 4	23.7 ± 0.4	11–15 n = 3	26.8 ± 0.4
16–20 n = 5	28.4 ± 0.2	16–20 n = 3	43.5 ± 0.8

n = number of samples analysed.
Values are Mean ± SD.

Table 3.2.2: Effect of age on AST activity in HbSS subjects (Crisis and steady state).

AST Activity (µL)		
Age (yrs)	Steady state	Crisis
0–5 n = 5	19.75 ± 0.6	30.0 ± 0.7
6–10 n = 5	10.5 ± 0.5	36.8 ± 1.4
11–15 n = 5	31.0 ± 0.4	26.8 ± 0.4
16–20 n = 5	33.2 ± 0.6	43.5 ± 0.8

n = number of samples analysed.
Values are Mean ± SD.

Table 3.2.3: Effect of age on ALP activity in HbSS subjects (Crisis and steady state).

ALP Activity (µL)		
Age (yrs)	Steady state	Crisis
0–5 n = 5	148.75 ± 14.9	164.4 ± 4.3
6–10 n = 5	126.0 ± 8.49	155.6 ± 7.6
11–15 n = 5	88.7 ± 26.6	150.0 ± 9.9
16–20 n = 5	72.4 ± 13.6	119.0 ± 4.4

n = number of samples analysed.
Values are Mean ± SD.

Table 3.3.1: Effect of sex on ALT, AST and ALP activities in HbAA and HbAS subjects

ALT Activity			AST Activity			ALP Activity			
Sex	AA	AS	Sex	AA	AS	Sex	AA	AS	SS
Males n = 15	6.8 ± 0.4	7.6 ± 0.4	Males n = 5	9.0 ± 4.7	13.0 ± 4.0	Males n = 5	57.5 ± 3.9	68.0 ± 5.9	125.6 ± 3.8
Females n = 9	9.0 ± 0.52	8.0 ± 0.4	Females n = 9	10.0 ± 6.85	12.0 ± 5.6	Females n = 9	56.8 ± 2.9	62.0 ± 4.6	121.3 ± 2.9

n = number of samples analysed.
Values are Mean ± SD.

Table 3.3.2: Effect of sex on AST and ALP activity in HbSS subjects (Crisis and steady state).

AST Activity				ALP Activity		
Sex	Steady State	Sex	Crisis	Sex	Steady state	Crisis
Males n = 5	23.8 ± 13.54	Males n = 8	43.88 ± 11.56	Males n = 5	125.6 ± 3.8	144.6 ± 2.7
Females n = 5	23.5 ± 12.18	Females n = 7	35.14 ± 6.36	Females n = 5	10.0 ± 6.85	157.3 ± 7.8

n = number of samples analysed.
Values are Mean ± SD.

Aspartate tarnsaminase (AST):

The level of AST activity in sickle cell anaemia (HbSS) subjects was higher than normal. In HbAS there was a slight decrease in activity as age increased. For HbSS subjects, AST activity was high at age 15. Subjects in the age bracket 6–10 years exhibited a decrease in AST activity when compared to those between 0–5 years.

In steady and crisis situation, AST activity was highest in HbSS subjects when compared to HbAA and HbAS subjects. However, during crisis a higher activity was observed for all age groups studied which appears to be no age dependent trend in AST activity for HbSS subjects under crisis situation with mean values for each age group increase progressively (see table 3.2.2). AST activity in steady state increased with age up to age 20. Subjects in steady state between 6–10 years exhibited a low AST activity when compared to subjects between 0–5 years. However, there was no significant difference ($p \geq 0.05$) in AST activity in HbSS subjects between 11–15 years and 16–20 years (see table 3.1).

On the effect of sex, statistical analysis showed no significant difference ($p \geq 0.05$) between both sexes in AST activity for HbAA, HbAS and HbSS (crisis and steady state) subjects.

Alkaline phosphatase (ALP):

From table 3.1 above, ALP activity of heterozygous (AS) is not significantly different ($p \geq 0.05$) from that of homozygous (AA) subjects. As shown in table 3.2.3, ALP activity increased during crisis compared to steady state condition.

The effect of ALP activity in normal (AA) and heterozygous (AS) subjects decreased with age significantly ($p \leq 0.05$), while a steady decrease in ALP activity was observed with increase in age for HbSS subjects. However, the difference in activity between the various age groups is statistically significant ($p \leq 0.05$).

Table 3.3.1 shows the effect of sex on ALP activity of normal and sickle cell subjects. The mean value for each group did not depend on sex. There was no significant difference ($p \geq 0.05$) in ALP activity between both sexes.

Table 3.3.2 shows the effect of sex on ALP activity in HbSS subjects in steady state and painful crisis. Sex did not seem to affect the enzyme activity whether in steady state or crisis.

In this work, ALP had the highest levels of activity, especially in growing children – in both normal and sickle cell subjects. This may be as a result of its involvement in the calcification of bone and teeth. Also, raised serum alkaline phosphatase accompanies rickets of various etiologies.

CONCLUSION

In conclusion, the ALT and AST activities in sickle cell disease as regards age are raised from childhood to adolescence and that in the steady state of sickle cell, there were elevations which were further increased during painful crisis. The sexes may not lead to any major hepatic dysfunction, but should be considered as a diagnostic parameter in sickle cell management.

REFERENCES

Acquaye, C., Wilchek, M. and Gorecki, M. (1981). Strategies for tackling sickle cell disease. Trends in Biochemical Sciences, 146–148.

Attah, E.B. (1975). Pathology of sickle cell anaemia. Dokita. 7:19–21.

Dacie, J.V. (1960). The haemolytic anaemia, congenital and acquired anaemia. (2nd ed.). J.A. Churchill Ltd. Part 1. London: 200–230.

Dean, J. and Schechter, A.N. (1978). Sickle cell anaemia. Molecular and cellular basis of therapeutic approaches. The New England Journal of Medicine. 229:753–755.

Ekeke, G.I. and Ibeh, G.O. (1990). Liver function enzymes and serum inorganic phosphate levels in sickle cell disease. 2: 6–9.

Emmel, V.E. (1917). A study of the erythrocyte in the case of severe anaemia with elongated and sickle shape red blood corpuscles. Arch. Intern. Med. 20: 586.

Hahn, E.V. and Gillepspie, G.E.G. (1927). Sickle cell anaemia. Arch. Intern. Med. 39: 233–236.

Herrick, J.B. (1910). Peculiar elongated and sickle shaped red blood corpuscle in a state of severe anaemia. Arch. Intern. Med. 6: 517–521.

Konotey-Ahulu, F.I.D. (1974). The sickle cell diseases, clinical manifestations including the "sickle crisis". Arch. Intern. Med. 133: 611–619.

Lehmann, H. and Raper, A.B. (1949). Distribution of sickle cell trait in Uganda, and its ethnological significance. Nature, 164: 494–495.

Nygren, A. (1967). SGOT in chronic alcoholism. Act. Med. Scand. 182–383.

Smith, C.H. (1972). Blood disease of infancy and childhood (3rd ed.). C.V. mosby and Co., St. Louis, 353–449.

Sofowora, A.O. (1993). Medicinal plants and traditional medicine in Africa. 2nd ed. Ibadan: Spectrum books limited; pp 150.

White, A., Handler, P., Smith, E.L., Hill, R.L. and Lehmann, I.R. (1978). Principles of Biochem., 6th ed. 947–1003.

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