Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.2, No.7, 2012

LIVER ENZYMES IN NORMAL AND SICKLE CELL SUBJECTS

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ABSTRACT

The activity of the liver enzymes, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were compared in serum samples obtained from normal (HbAA), heterozygous (HbAS), and homozygous (HbSS) blood. The enzyme activities (measured in international units, IU per litre) for normal subjects were 8.0 ± 4.0, 11.0 ± 5.0 and 56.0 ± 43.0 respectively for ALT, AST and ALP. While those for sickle cell subjects were 18.0 ± 9.0, 24.0 ± 12.0 and 151.0 ± 21.0 respectively for ALT, AST and ALP. The activities of these were compared during sickle cell crisis and in steady state. Results show that enzyme activities increased during painful crisis. The difference in activities of the enzyme in HbAA and HbAS blood was not statistically significant ($p \geq 0.05$). ALP showed the highest activity in both normal and sickle cell conditions. In normal and sickle blood, the order of activity of the enzymes was ALP>AST>ALT. ALT, AST and ALP activities of sickle blood ranged from 125 to 135% of the normal.

Keywords: alanine transaminase, aspartate transaminase, alkaline phospatase, genotype, heterozygous, homozygous, sickle cell, anaemia.

1. Introduction

Sickle cell disease is a severe disease of the blood. It is a hereditary disorder that affects several million people in tropical Africa, the Middle East and United States of America (U.S.A.) (Dean and Schechter, 1978). It involves the possession of two abnormal allelemorphic genes related to haemoglobin (Hb) formation, at least one of which is the sickle cell gene (Konotey-Ahulu, 1974). Examples include SS, SC, SE, SF and SD. Both genes must be normal and so the genotype comparising one normal (A) and the abnormal gene (S) is not included in the definition of sickle cell disease, since the criterion of two abnormal gene is not met.

Although the genetic nature of sickle cell disease (SCD) has long been identified, its pathophysiology has remained diffuse and complex. Clinical and pathologic data indicates the intravascular sickling does occur in several vital organs such as the liver, kidney, spleen and so on.

One of the most convenient methods for assessing hepatic function is the laboratory measurement of some blood stream enzymes. The enzymes when assayed in parallel with some non-enzyme compounds are particularly useful in the differential diagnosis of a hepatic ailment.

Sickle cell disease has long been associated with liver damage. In view of the problem pose by sickle cell disease, the liver enzymes, alanine transaminase, aspartate transaminase and alkaline phosphatase levels in plasma or serum can give some insight into the state of the liver in either “steady state” or “crisis” situations.

2. Materials and Methods

2.1 Materials: Materials, test kits and equipments used were of laboratory standards.

10ml of blood samples from each pediatric and adult HbSS subjects were collected by venipuncture into heparinized tubes during routine clinical visits at the Braitwaite Memorial Hospital, Port Harcourt and University of Port Harcourt Teaching Hospital Sickle Cell Clinic.

2.2 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.

3. Assay of Enzyme Activity

3.1 Alanine transaminase (ALT) activity:

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 546nm. The optical density (O.D) of the reaction mixture was taken colorimetrically at 546nm as a measure of the enzyme activity.

3.2 Aspartate transaminase (AST) activity:

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 546nm. This measures the activity of aspartate transaminase.
The enzyme activity was measured by a procedure described in the RANDOX GOT kit.

3.3 Alkaline phosphatase (ALP) activity:
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 405nm. The intensity of the colour due to p-nitrophenol during a fixed time is measured at 405nm 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 420nm.

4. Result

4.1 Electrophoresis
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.

5. Discussion
The liver is a very important organ in the body. Metabolic disturbances in hepatic disease are therefore quite characteristic and may be useful in diagnosis. Sickle cell disease is usually associated with liver damage.

5.1 Alanine transaminase (ALT) activity:
Alanine transaminase (ALT) is widely distributed in various tissues of the human body but it is mainly found in the liver. It has been observed in this study that there is variation in alanine transaminase activity for normal and sickle cells. As shown in table 3.1 above, normal subjects exhibited a lower activity of 8.0 ± 1.2µL. Sickle cell subjects had a higher activity than normals (18.0 ± 1.4). The ALT activity of heterozygous subjects is not statistically different ($p \geq 0.05$) from that of normal (HbAA) subjects.
Sickle cell subjects in steady state showed an activity of 18.0 ± 0.9µL, while those in crisis had alanine transaminase activity of 33.7 ± 1.0. This showed a 94.4% increase.
Alanine transaminase constitutes one of the most commonly determined enzymes due to higher concentrations. ALT is however reliable, specific and sensitive for the diagnosis of sickle cell disease and is very much indicative of liver disease.

5.2 Aspartate transaminase (AST) activity:
The level of aspartate transaminase in serum is used as an index in the diagnosis of various disease conditions of the cells of the liver, heart, kidney, skeletal muscles, etc.
Lower aspartate transaminase activity was observed in HbAA subjects. The order of activity of AST was SS>AS>AA. Statistical activity however showed no significant difference ($p \geq 0.05$) in AST activity between HbAA and HbAS subjects. AST activity has been reported in many tissues (Nygren, 1967). Although the enzyme is presumably present in these tissues, it has been extensively studied and used as an index in liver disease. There are several reports showing that AST activity of sickle cell subjects is higher than that of normal individuals (Ekeke and Ibeh, 1990). The result reported here may not be specific to sickle cell disease. Elevated levels of AST have been observed in sickle cell crisis, malaria tumors, kwashiorkor and general state of malnutrition.

5.3 Alkaline phosphatase (ALP) activity:
Sickle cell (SS) subjects exhibited alkaline phosphatase activity of 124.0 ± 6.2µL as compared to normal (AA) subjects with 56.0 ± 4.2µL. ALT activity of heterozygous (AS) is not significantly different from that of normal (AA) subjects. HbSS subjects who were in steady state exhibited enzyme activity of 108.2 ± 4.3µL compared 142.2 ± 4.8 ALP activity shown by subjects in crisis (see table 3.2).
Since ALP is a rather non-specific phosphohydrolase enzyme, we postulate after confirming hyperphosphataemia in sickle cell disease that there could well be consequent hyperphosphataemia. Work is currently in progress to determine if the observed hyperphosphatasaemia could result to hyperphosphataemia.
The findings of this study have conclusively demonstrated that there is a considerable variation in the enzyme activities reported for normal and sickle cell subjects.

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4.2 Enzyme Activity

The ALT, AST and ALP activities of sickle cell and normal subjects is shown in table 3.1 as well as their steady and crisis state as shown in table 3.2 below.

Table 3.1: ALT, AST and ALP activities in normal (AA), heterozygous (AS) and sickle cell (SS) subjects.

Genotype	ALT activity (µL)	AST activity (µL)	ALP activity (µL)
AA n = 24	8.0 ± 1.2	11.0 ± 0.5	56.0 ± 4.2
AS n = 16	8.4 ± 1.2	12.8 ± 0.4	63.0 ± 5.8
SS n = 24	18.0 ± 1.4	24.0 ± 1.2	124.0 ± 6.2

n = number of samples analysed.
All experiments were done in duplicate and results are mean ± SD.

Table 3.2: ALT, AST and ALP activities in HbSS subjects in crisis and steady state

HbSS	ALT activity (µL)	AST activity (µL)	ALP activity (µL)
Steady state n = 12	18.0 ± 0.9	24.0 ± 1.2	108.2 ± 4.3
Crisis n = 12	33.7 ± 1.0	40.0 ± 1.0	142.2 ± 4.8

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