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Assessment of major liver enzymes activities among sickle cell subjects in Enugu South East of Nigeria

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Abstract

Objective: Sickle cell anemia is a condition resulting from inheritance of two abnormal allele of genes controlling β-globin formation. This haemoglobinopathy affects multiple organ system in the body. The hepatobiliary system is most commonly affected in sickle cell disease. This study assessed liver enzymes activities in sickle cell subjects in Enugu, Nigeria.

Methods: We recruited about hundred subjects age (20-40years) for this study. Fifty sickle cell subjects (Male =22, Female=28) as test subjects and fifty non-sickle cell subjects (Male=24, Female=26) as control. Anthropometric indices were measured and five milliliters of blood sample collected from each participant for analysis of Alanine transaminase (ALT), alkaline phosphatase (ALP), and Aspartate transaminase (AST) using autoanalyser. Data were analysed using statistical package for the social sciences (SPSS) version 25.

Results: The sickle cell subjects showed a significant decrease (P<0.05) in mean ± SD of systolic BP, diastolic BP and body mass index compared to control subjects. The liver enzymes analysis showed a statistical increase (P<0.05) in ALP and AST while a non-significant increase (P>0.05) exist in ALT of sickle cell subjects compared to non- sickle cell subjects. There is a positive correlation (P<0.05) in SBP vs. DBP, ALT vs. AST, ALT vs. BMI among the test subjects.

Conclusion: This study suggests that there is increase in liver enzymes activities in sickle cell subjects compared to non- sickle cell subjects, which could lead to liver complications and hence, need for regular assessment.

Keywords: Liver-enzymes; Haemoglobin SS; Sickle cell; Haemoglobinopathy; Enugu

1. Introduction

Sickle cell disease (SCD), is a genetic disease associated with the human blood and it is one haemoglobinopathy that is most common among the other haemoglobinopathies, and hence, a major public health problem with over 200,000 babies born per year with SCD in Africa [1,2,3].

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Sickle cell anemia is a hereditary blood disease caused by defective gene in position six of β- globin chain in which valine replaces glutamate producing abnormal haemoglobin [4].

Human blood is made up of haemoglobin which is responsible for the shape of red blood cells. It usually appears like doughnuts but with a thin center rather than a hole [5]. Haemoglobin is a tetramer composed of two α globin and two beta globin chains in normal adults [6]. These chains work in conjunction with heme which reversibly binds with oxygen and transports it throughout the body.

In sickle cell disease, haemoglobin as a component of red blood cell responsible for transporting oxygen from the lungs to tissues becomes fragile, distorted, and deformed when the oxygen pressure decreases in the tissue .The distorted cells are called the sickle cells and appears sickle or crescent shape in well stained blood smear when viewed microscopically [4]. Millions of people in the whole world lives with Sickle cell disease and its endemic in sub-Saharan Africa, South America, Caribbean, Central America, Saudi Arabia, and Mediterranean countries such as Turkey, Grease, Italy [4].

Nigeria is the most sickle cell endemic country in Africa with 2-3% of the total population affected [7], with an estimated 24% prevalence of sickle cell trait, 100,000 annual SCD births, and 100,000 annual SCD infant deaths [8]. Sickle cell anemia is probably the most common hereditary blood disorder in Nigeria [9].

The most common clinical phenotype is the homozygous form (HbSS or sickle cell anemia) [10]. Compound heterozygous SCD includes HbSC, HbSD, HbSO-Arab, and HbS/beta-thalassemia [11]. Heterozygotes are generally less symptomatic than homozygous [10]. This homozygous form results from the substitution of the amino acid, valine (GTG), for glutamic acid (GAG). Glutamic acid is an amino acid in the sixth position in the globin chain of the adult haemoglobin (HbA) [12].

Sickle cell anemia is the most common form of sickle cell disease [13]. It is characterized by chronic haemolysis and painful vaso-occlusion, which often results in organ dysfunction. Sickling, which is associated with haemoglobin S (HbS) alongside vascular occlusion and erythrocyte haemolysis, affects the overall biochemical balance in sickle cell patients [14] and will lead to various biochemical abnormalities affecting hepatobiliary organs in the body such as liver and others.

Liver enzymes are produced by the liver. They play vital role in catalyzing and enhancing biological processes in the body. Some of these enzymes are produce only in the liver where as some are produce in other organs in the body. The increase in any of these enzymes should be carefully examined and not misinterpreted as many other organs could produce similar enzymes [15]. The liver enzymes includes; Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Gamma glutamyl transferase (GGT). These enzymes are also referred to as enzymes found within the liver and biliary tract. The level of these enzymes is measured as part of liver function test and elevation of any of these enzymes above normal range indicates different pathologies including liver disease [15].

Significant raised form of any of this enzyme is of clinical important in detecting physiological and pathological condition of various organs where they are located [16]. Many clinical enzymes in the blood have continued to serve as diagnostic biomarkers for assessing necrosis of the liver cells, Many of these enzymes, such as Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) are implicated [17,15].

Serum ALT and AST are 1-3 times above normal in acute sickle cell hepatic crisis, sickle cell lead to hepatomegaly and elevated liver enzymes levels [18]. Mortality from liver related complications in sickle cell disease is about 7% of all sickle cell disease death [18].

Sickle cell hepatopathy (SCH) describes any hepatobiliary dysfunction in sickle cell disease has an estimated incidence of 10-40% in patients with sickle cell disease [19, 20].

Sickle cell disease been a genetically acquired disease has long been of medical concern since Sickle cell anemia is probably the most common hereditary blood disorder in Nigeria [9]. Therefore the outcome of this research work will help physicians/clinicians to manage liver related complications in sickle cell subjects and reduce mortality rate from hepatopathy caused by sickle cell diseases.

2. Materials and methods

2.1. Study Design/Selection

This was a cross sectional study involving a total number of one hundred (100) adult human volunteers aged (20-40 years) from Enugu metropolis. Fifty (22 males, 28 females) sickle cell subjects attending clinic at University of Nigeria Teaching Hospital Ituku-Ozalla Enugu were recruited as Test group, while fifty (24 males, 26 females) as control group. Informed consent was obtained from each participant. Questionnaires were distributed and duly filled by the participants. Inclusion criteria for the test group were sickle cell anemic patients in stable state between ages 20-40 years while control groups were apparently healthy non-sickle cell subjects between ages 20-40 years. We excluded subjects on haematinics or other special therapy, hypertensive, alcoholics and those suffering from other comorbidity.

2.2. Ethical Considerations and Informed Consents

Ethical approval was duly obtained from the ethics committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu with Ref no: UNTH/HREC/2022/02/328. Written consent of willingness to participate in the study as subject was obtained from all the participants.

2.3. Sampling Techniques

Venous blood was collected into appropriately labelled five millilitre (5ml) plain tube. Sample was allowed to clot and retract, centrifuged at 5,000 rpm and the supernatant (serum) was separated into another labelled vial and stored at -20^0C until analysed, and the analyses were carried out within 48 hours of collection. All analysis of the samples was done by the researchers at the laboratory of University of Nigeria Teaching Hospital Ituku-Ozalla Enugu.

2.4. Anthropometric Measurements

2.4.1. Measurements of Body Mass Index (BMI)

The weight, height and BMI of the respondents were recorded. A digital weighing scale (Camry BR 9011) was used to measure the body weight (kg). A stadiometer was used to measure height (m); and the BMI was calculated by dividing the weight (kg) by the square of height (m^2).

2.4.2. Measurements of Blood Pressure (BP)

A standardized automatic BP monitor (Reli-On HEM 8724) was used to take the blood pressure measurements in two readings and the average record was used.

2.5. Biochemical Analysis

All analysis was done using reagent kits manufactured by Randox Laboratories Ltd, Antrim, United Kingdom. Aspartate and Alanine transaminase were estimated using Reitman-Frankel Colorimetric method [21]. Alkaline Phosphatase was assayed using Phenolphthalein monophosphate Substrate method [22].

2.6. Statistical Analysis

Data obtained from this study was analysed using SPSS version 25. Data were presented as mean and standard deviation. Student's t-test was used to calculate differences between the means. All hypotheses tests were performed using two-tailed test and p-value <0.05 considered statistically significant.

3. Results

Table 1 Anthropometric parameters of sickle cell subjects and Non- sickle cell subjects

Groups	SystolicBlood pressure(mmHg)	DiastolicBlood pressure(mmHg)	BMI(kg/m^2)
Sickle cell subjects N = 50	109.50 ± 9.13	67.27 ± 8.05	21.93 ± 1.86
Non-Sickle cell subjects. N = 50	113.83 ± 7.89	71.97 ± 6.38	23.07 ± 2.29
T – statistic	-2.170	-2.503	-2.403
P-values	0.038*	0.018*	0.023*

Values are given as Mean ± SD   * = Significant values (P < 0.05)

Table 2 The liver enzymes activities in sickle cell and non- sickle cell subjects

Groups	Alkaline phosphatase (ALP) (U/L)	Alanine transaminase (ALT)(U/L)	Aspartate transaminase (AST)(U/L)
Sickle cell subjects. N=50	84.96 ± 31.97	28.87 ± 13.02	47.18 ± 30.38
Non-sickle cell subjects N =50	64.10 ± 20.52	24.20 ± 8.98	27.47 ±4.60
t- statistics	2.679	1.487	3.599
p- value	0.012*	0.148	0.001*

Values are given as Mean ± SD   * = Significant values (P < 0.05)

Table 3 The Correlations between parameters in sickle cell subjects

Parameters N=50	r (Pearson)	P-Value
SBP vs. DBP	0.562	0.001**
SBP vs. ALP	-0.241	0.199
SBP vs. ALT	0.298	0.109
SBP vs. AST	0.140	0.462
SBP vs. BMI	0.161	0.396
DBP vs. ALP	-0.075	0.693
DBP vs. ALT	0.136	0.473
DBP vs. AST	0.037	0.844
DBP vs. BMI	0.0570	0.766
ALT vs. ALP	-0.076	0.689
ALP vs. AST	-0.003	0.988
ALP vs. BMI	0.325	0.080
ALT vs. AST	0.857	0.000**
ALT vs. BMI	0.392	0.032*
AST vs. BMI	0..331	0.074

*= Weak correlation (P<0.05)   ** =Strong correlation (P<0.01)

4. Discussion

Sickle cell anemia is probably the most common hereditary blood disorder in Nigeria [9]. Mortality from liver related complications in sickle cell disease is about 7% of all sickle cell disease death [18]. Serum ALT and AST are 1-3 times above normal in acute sickle cell hepatic crisis, sickle cell lead to hepatomegaly and elevated liver enzymes levels [18].

The result presented in table 1 showed a significant decrease (P < 0.05) in the mean ± standard deviation of systolic blood pressure (109.50 ± 9.13, 113.83 ± 7.98), Diastolic blood pressure (67.27 ± 8.05, 71.97 ± 6.38) and BMI (21.93 ± 1.86, 23.07 ± 2.29) in the test subjects compared to the control subjects respectively. This decreased blood pressure seen in the sickle cell subjects could be as a result of haemolytic anemia, dehydration, haemodilution and reduced angiotensinogen. When there is haemolysis, most of red cell components are lost and further sequestered by the spleen. Cellular components present in blood stream like angiotensinogen are affected. The release of enzyme renin from the kidney will not have much effect to act on limited angiotensinogen to increase the low blood pressure in a sickle cell subject. This finding is in line with previous studies by [1; 23-26].

The decreased BMI could be attributed to micro-nutrient deficiencies, loss of appetite, and increase demand from high metabolic rates due to increased red blood cell turnover due to hyper haemolysis, reduced absorption, and increased degradation of nutrients that occurs in sickle cell subjects. This is in line with studies by [1; 27- 29] but in contrast with the study by Hall *et al.* [30], who recorded a high body mass index in high-income countries.

In table 2, the liver enzyme analysis showed a statistical increase (P<0.05) in mean ± SD of Alkaline phosphatase (84.96 ± 31.97, 64.10 ± 20.52) and Aspartate transaminase (47.18 ± 30.38, 27.47 ± 4.60) while a non-significant increase (P>0.05) exist in ALT (28.87 ± 13.03, 24.20 ± 8.98) of sickle cell subjects compared to non- sickle cell subjects respectively. The Increase level in alkaline phosphatase could be due to cholestasis or vaso occlusive crises involving bones and biliary system in sickle cell subjects. This observation is consistent with the study by Johnson *et al* [16] and Kotila *et al* [15]. Again the increases in AST and ALT among the sickle cell subjects compared to the non-sickle cell subjects could be attributed to the effects of sickling of erythrocytes within the vasculature of the liver, with consequent hypoxic liver injury particularly affecting the centrilobular region and biliary system. Also complications related to the multiple blood transfusions including viral hepatitis and iron overload in sickle cell subjects could affect the enzymes. This is in agreement with many previous studies by [15; 16; 31; 32] who reported increases in liver enzymes among sickle cell subjects.

This study also observed the relationship between the different parameters in sickle cell subjects (in table 3). There exists a positive correlation between SBP vs DBP, ALT vs. AST, ALT vs. BMI. This shows that systolic BP is increasing as Diastolic Bp increases; also ALT is increasing as AST increases too. This finding is in agreement with the study by [18; 27]

5. Conclusion

This study has shown that there is increase in serum liver enzymes (ALP, AST, and ALT) levels among sickle cell subjects compared to non-sickle cell subjects. These variations in liver enzymes levels between the two groups which were attributed to the effects of sickling of erythrocytes within the vasculature of the liver, with consequent hypoxic liver injury, as well as complications related to multiple blood transfusions including viral hepatitis and associated vasoocclusive crises seen in sickle cell subjects. Therefore there is need for regular assessment of liver enzymes in sickle cell subjects while they are managed. This will reduce or avert liver dysfunction and other hepatic complications associated with sickle cell subjects.

This study recommend further research on other liver function parameters in sickle cell subjects relating to sex and age, this will provide a better understanding and management of sickle cell patients in relation to associated complications involving the liver.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Statement of ethical approval

Ethical approval was duly obtained from the ethics committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu with Ref no: UNTH/HREC/2022/02/328.

Statement of informed consent

Informed consent was obtained from all participants in this study before commencement.

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