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LEVELS OF MARKERS OF MYOCARDIAL INFARCTION AND ATHEROGENICITY IN INDIVIDUALS WITH SICKLE CELL TRAIT

Constance N. Nwadike[1] and Emmanuel Ifeanyi Obeagu[2]

1. **Department of Medical Laboratory Science, Imo State University Owerri, Nigeria.**
2. **Department of Medical Laboratory Science, Kampala International University, Uganda.**

ABSTRACT

Sickle cell anemia is associated with recurrent multi-organ ischemia and infarction. This study was aimed at determining the level of the markers of myocardial infarction and atherogenicity in subjects with sickle cell trait. A total of 30 subjects were recruited for the study of which 10 were subjects with HbAS, 10 were subjects with HbSS while 10 were healthy subjects. Five (5mls) of fresh venous blood was collected from all the participants by venepuncture using sterile needle and syringe. Lipid profile, aspartate amino transferase, lactate dehydrogenase and troponin were determined using enzymatic method while troponin was determined using ELISA method. All values were expressed as mean ± standard deviation. The statistical analysis was carried out using ANOVA. Values with level of significance ($p<0.05$) was considered to be statistically significant. There was a significant increase ($p=0.014$, $p=0.000$, $p=0.000$, $p=0.000$ respectively) in the mean values of triglyceride (127.5±31.21)mg/dl, lactate dehydrogenase (692±152.68)u/l, troponin (2.35±0.59)ng/ml and aspartate amino transferase (67.9±16.59)ug/ml in HbSS subjects compared to control subjects (95.92±22.79)mg/dl, (378.10±105.72)U/L, (0.11±0.06)ng/ml and (18.9±4.95) respectively. The mean value of T Chol, HDL and LDL were significantly reduced ($p=0.000$, $p=0.001$, $p=0.000$ respectively) in HbSS subjects (172.68±14.82)mg/dl, (60.75±7.12)mg/dl and (91.55±16.55)ug/ml when compared to control subjects total cholesterol (129.03±37.62)mg/dl, high density lipoprotein (41.10±10.85)mg/dl and low density lipoprotein (69.06±10.54)mg/dl. There was a significant increase ($p=0.002$, $p=0.000$, and $p=0.006$ respectively) in the mean value of lactate dehydrogenase (515.60±62.39)U/L, troponin (1.29±0.76)ng/ml and aspartate amino transferase (25.50±7.32)U/L in HbAS subjects compared to control subjects (378.10±105.72)U/L, (0.11±0.06)ng/ml and (18.9±4.95)U/L respectively. The mean value of total cholesterol and high density lipoprotein were significantly reduced ($p=0.001$ and $p=0.009$ respectively) in HbAS subjects (153.49±9.13)mg/dl and (52.45±5.99)mg/dl when compared to control subjects total cholesterol (172.68±14.82)mg/dl and high density lipoprotein (60.75±7.12)mg/dl. The mean value of triglyceride and low density lipoprotein showed no significant difference ($p=0.448$ and $p=0.192$) in HbAS subjects (94.27±21.47)mg/dl and (86.52±9.87)mg/dl when compared to control subjects (95.92±22.79)mg/dl and (91.55±16.55)mg/dl. There was a significant increase ($p=0.001$, $p=0.011$, $p=0.002$ respectively) in the mean value of total cholesterol (153.49±9.13)mg/dl, high density lipoprotein (52.45±5.99)mg/dl and low density lipoprotein (86.52±9.87)mg/dl in HbAS subjects when compared to HbSS subjects (129.03±37.62)mg/dl, (41.10±10.85)mg/dl and (69.06±10.54)mg/dl respectively. The mean value of Triglyceride, Lactate Dehydrogenase, Troponin and Aspartate Amino Transferase were significantly reduced ($p=0.002$, $p=0.005$, $p=0.002$ and $p=0.000$ respectively) in HbAS Patients (94.27±21.47)mg/dl, (515.60±62.39)u/l, (1.29±0.76)ng/ml and (25.50±7.32)u/L when compared to HbSS subjects (127.5±31.21)mg/dl, (692±152.68)u/l,

(2.35±0.59)ng/ml and (67.9±16.59)u/l. Though there were some differences in the individual results, sickle cell disease is associated with increased level of triglyceride, lactate dehydrogenase, troponin and aspartate amino transferase and a decreased level of T Chol, HDL and LDL. From these results, there is a clear indication of derangement of lipid metabolism and dyslipidaemia, which suggest association of sickle cell disease with cardiovascular diseases.
Keywords: myocardial infarction and atherogenicity in individuals with sickle cell trait

INTRODUCTION

Sickle cell disease is a blood disorder wherein there is a single amino acid substitution in the hemoglobin protein of the red blood cells, which causes these cells to assume a sickle shape, especially when under low oxygen tension. Sickling and sickle cell disease also confer some resistance to malaria parasitization of red blood cells, so that individuals with sickle-cell trait (heterozygotes) have a selective advantage in environments where malaria is present [1]. Sickle cell trait is a hemoglobin genotype AS and is generally regarded as a benign condition. The significance of the sickle-cell trait is that it does not show any symptoms, nor does it cause any major difference in blood cell count [2]. The trait confers about 30% protection against malaria and its occurrence appears to have risen tremendously in Africa, India and the Middle East [3].

Atherosclerotic lesion development takes place in the intimal layer of the arterial wall. Adult intima is a rather thick formation with complex architecture and heterogeneous cellular composition [4]. The intima is separated from the lumen of the vessel by a monolayer of endothelial cells. Endothelium plays a key role in the transport of cells and non-cellular components of blood from the arterial bed to the vascular wall [5]. The primary source of lipids that accumulate in foam cells is atherogenic modified low density lipoprotein (LDL). Particles of LDL circulate in the blood and undergo chemical modifications affecting the glycoconjugate, lipid, and protein moieties [6]. Cellular composition of the arterial wall undergoes profound changes in atherosclerotic lesion areas [7]. Lesion development is accompanied by a local increase of the number of cells (cellularity), especially the numbers of macrophages and hematogenous cells. Moreover, the cellular network of pericyte-like cells disintegrates, with loss of intercellular communication and changes of cellular phenotype.

Myocardial infarction (MI), commonly known as a heart attack occurs when blood flow decreases or stops to a part of the heart, causing damage to the heart muscle. The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck or jaw. Often it occurs in the center or left side of the chest and lasts for more than a few minutes. Many deaths that occur in sickle cell abnormality is closely linked to myocardial infarction of which lipid dyslipidaemia is implicated. Plasma non esterified fatty acids (NEFA) are also building blocks of erythrocyte membranes and its alteration could impact on the structure and function of red blood cells. Therefore, abnormal lipid homeostasis could alter red blood cell membrane fluidity and functions leading to a significant worsening in sickle cell anaemia. Abnormalities in total cholesterol either as an increased or decreased level is associated with increased mortality from all causes.

A study on lipid homeostasis and markers of myocardial infarction in SCA is thus necessary because myocardial infarction poses a serious threat to sickle cell patients and in addition, red blood cells membrane is made up approximately 50% of lipid and plasma phospholipids contributes significantly to the synthesis of erythrocytes membranes. The present study investigated the level of the markers of myocardial infarction and atherogenicity in subjects with sickle cell disease (SCD).

MATERIALS AND METHODS

Study Area

The study was carried out at the medical-out patients department (Antenatal clinic) of Federal Medical Center Owerri, Imo state. Owerri is the capital of Imo state and is located in the Eastern part of Nigeria between longitude 6°50 E and 7° 25' E and between latitude 4°45'N and 7°15' N of the equator with an area of about 5100sq km. It is bordered by Abia state to the North and River Niger and Delta on the West by Anambra state to the North and Rivers state to the south. It provides home for a population of 127,213 people of mainly Igbo ethnic group and a few other tribes. This population is made up of 62,990 males and 64,223 females [11].

Study Population

Population for the study were all sickle cell patients coming to the federal medical centre Owerri for their regular follow up and willing to participate in the study. A total of 30 subjects were recruited for the study of which 10 were subjects with sickle cell disease and another 10 were subjects with sickle cell trait while the remaining 10 were healthy subjects.

Study Design

This was a case controlled study. A total of 30 individuals with sickle cell trait; 10 HbSS, 10 HbAS and 10 HbA (Ccontrol) which were well sampled were recruited for the study. These subjects were recruited from haematology clinic of Federal Medical Center Owerri. Thin blood samples were collected after overnight fast and analyzed for

markers of myocardial infarction and artherogenicity. Markers of Myocardial Infarction (MI) include CK-MB, AST, Myoglobin, Troponin and Hb.

Specimen Collection

From an intravenous access, under aseptic conditions using a vacutainer needle, 5mls of blood was collected into a lithium heparin vacutainer bottle for lipid profile of all participants. Another 2mls of blood sample was collected into a vacutainer EDTA bottle from all participants and properly mixed with the anticoagulant for alkaline Hb electrophoretic analysis in all participants to ensure the participants are either sickle cell anaemia patients, SSC or HbA participants before the lipid profile assay. For myoglobin test, the specimens is blood then separated into serum in type, and the usual precautions in the collection of venipuncture samples should be observed.

Determination of Test Parameter

The sera of the volunteer subject were analyzed for lactate dehydrogenase, aspartate aminotransferase and lipid profile following the instruction as directed on their diagnostic kits.

Statistical Analysis

Data generated from the study was subjected to statistical analysis using the Statistical Package for Science (SPSS) software computer program version 25. All values were expressed as Mean +/- standard deviation (SD) following analysis using Student t test. Values with level of significance ($p<0.05$) were considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1 showed that there was a significant increase ($p=0.014$, $p=0.000$, $p=0.000$, $p=0.000$ respectively) in the mean values of triglyceride (127.5±31.21)mg/dl, lactate dehydrogenase (692±152.68)u/l, troponin (2.35±0.59)ng/ml and aspartate amino transferase (67.9±16.59)ug/ml in HbSS subjects compared to control subjects (95.92±22.79)mg/dl, (378.10±105.72)U/L, (0.11±0.06)ng/ml and (18.9±4.95) respectively. The mean value of T Chol, HDL and LDL were significantly reduced ($p=0.000$, $p=0.001$, $p=0.000$ respectively) in HbSS subjects (172.68±14.82)mg/dl, (60.75±7.12)mg/dl and (91.55±16.55)ug/ml when compared to control subjects total cholesterol (129.03±37.62)mg/dl, high density lipoprotein (41.10±10.85)mg/dl and low density lipoprotein (69.06±10.54)mg/dl.

Table 2 showed that there was a significant increase ($p=0.002$, $p=0.000$, and $p=0.006$ respectively) in the mean value of lactate dehydrogenase (515.60±62.39)U/L, troponin (1.29±0.76)ng/ml and aspartate amino transferase (25.50±7.32)U/L in HbAS subjects compared to control subjects (378.10±105.72)U/L, (0.11±0.06)ng/ml and (18.9±4.95)U/L respectively. The mean value of total cholesterol and high density lipoprotein were significantly reduced ($p=0.001$ and $p=0.009$ respectively) in HbAS subjects (153.49±9.13)mg/dl and (52.45±5.99)mg/dl when compared to control subjects total cholesterol (172.68±14.82)mg/dl and high density lipoprotein (60.75±7.12)mg/dl. The mean value of triglyceride and low density lipoprotein showed no significant difference ($p=0.448$ and $p=0.192$) in HbAS subjects (94.27±21.47)mg/dl and (86.52±9.87)mg/dl when compared to control subjects (95.92±22.79)mg/dl and (91.55±16.55)mg/dl.

Table 3 showed that there was a significant increase ($p=0.001$, $p=0.011$, $p=0.002$ respectively) in the mean value of total cholesterol (153.49±9.13)mg/dl, high density lipoprotein (52.45±5.99)mg/dl and low density lipoprotein (86.52±9.87)mg/dl in HbAS subjects when compared to HbSS subjects (129.03±37.62)mg/dl, (41.10±10.85)mg/dl and (69.06±10.54)mg/dl respectively. The mean value of Triglyceride, Lactate Dehydrogenase, Troponin and Aspartate Amino Transferase were significantly reduced ($p=0.002$, $p=0.005$, $p=0.002$ and $p=0.000$ respectively) in HbAS Patients (94.27±21.47)mg/dl, (515.60±62.39)u/l, (1.29±0.76)ng/ml and (25.50±7.32)u/L when compared to HbSS subjects (127.5±31.21)mg/dl, (692±152.68) u/l, (2.35±0.59)ng/ml and (67.9±16.59)u/l.

Table 4 showed increased artherogenicity index of Sickle Cell Disease subjects which exceeded the reference range. The artherogenicity indices of HbAS and HbA are within reference range. Myocardial infarction has been speculated to be a possible feature of sickle cell anemia in adults and children. Hypocholesterolemia and to a lesser extent, hypertriglyceridemia have been documented in SCD cohorts worldwide for over 40 years, yet the mechanistic basis and physiological ramifications of these altered lipid levels are yet to be fully elucidated.

Decreased TC and LDL-C in SCD has been documented in virtually every study that examined lipids in SCD adults with slightly more variable results in SCD children. Although it might be hypothesized that SCD hypocholesterolemia results from increased cholesterol utilization during the increased erythropoiesis of SCD, cholesterol is largely conserved through the enterohepatic circulation, at least in healthy individuals, and biogenesis of new RBC membranes would likely use recycled cholesterol from the hemolyzed RBCs. Westerman demonstrated that hypocholesterolemia was not due merely to increased RBC synthesis by showing that it is present in both hemolytic and non-hemolytic anemia [12]. The mean value of T Chol, HDL and LDL were significantly reduced ($p<0.005$) in HbSS subjects when compared to control subjects. Total cholesterol, in particular LDL-C, has a well-established role in atherosclerosis. The low levels of LDL-C in SCD are consistent with the low levels of total cholesterol and the virtual absence of atherosclerosis among SCD patients. Our results also clearly show a decrease in HDL-C in SCD vs. controls. Decreased HDL-C in SCD has been documented in some but not all previous studies.

As in lipid studies for other disorders in which HDL-C is variably low, potential reasons for inconsistencies between studies include differences in age, diet, weight, smoking, gender, small sample sizes, different ranges of disease severity, and other diseases and treatments. Decreased HDL-C and apoA-I is a known risk factor for endothelial dysfunction in the general population and in SCD, a potential contributor in SCD to PH, although the latter effect size might be small [13]. This study also convincingly show increased triglyceride levels in serum of SCD vs. control subjects. Triglycerides have not been as widely studied as cholesterol in SCD, and previous studies have given mixed results. Increased triglyceride have been reported in several previous studies of SCD adults. In addition, triglyceride levels were found to increase during crisis. In children, one study found increased triglyceride whereas another reported normal triglyceride in SCD. Potential reasons for inconsistencies between studies are similar to reasons for inconsistencies in HDL-C [13].

Studies in atherosclerosis have firmly established that lipolysis of oxidized LDL in particular results in vascular dysfunction. Lipolysis of triglycerides present in triglyceride-rich lipoproteins releases neutral and oxidized free fatty acids that induce endothelial cell inflammation. Many oxidized fatty acids are more damaging to the endothelium than their non-oxidized precursors; for example, 13-hydroxy octadecadienoic acid (13-HODE) is a more potent inducer of ROS activity in HAECs than linoleate, the nonoxidized precursor of 13-HODE. Lipolytic generation of arachadonic acid, eicosanoids, and inflammatory molecules leading to vascular dysfunction is a well-established phenomenon [14]. Although LDL-C levels are decreased in SCD patients, LDL from SCD patients is more susceptible to oxidation and cytotoxicity to endothelium and an unfavorable plasma fatty acid composition has been associated with clinical severity of SCD. Lipolysis of phospholipids in lipoproteins or cell membranes by secretory phospholipase A2 (sPLA2) family members releases similarly harmful fatty acids, particularly in an oxidative environment and in fact selective PLA2 inhibitors are currently under development as potential therapeutic agents for atherosclerotic cardiovascular disease.

Elevated triglycerides have been documented in autoimmune inflammatory diseases with increased risk of vascular dysfunction and pulmonary hypertension, including systemic lupus erythematosus, scleroderma, rheumatoid arthritis, and mixed connective tissue diseases. In fact, triglyceride concentration is a stronger predictor of stroke than LDL-C or TC. Even in healthy control subjects, a high-fat meal induces oxidative stress and inflammation, resulting in endothelial dysfunction and vasoconstriction. Perhaps having high levels of plasma triglycerides promotes vascular dysfunction, with the clinical outcome of vasculopathy mainly in the coronary and cerebral arteries in the general population, and with more targeting to the pulmonary vascular bed in SCD and autoimmune diseases. Using forearm blood flow physiological studies, we demonstrated a link between endothelial dysfunction and increased TG/HDL-C ratio, previously proposed as an index of vascular dysfunction [15]. The common factor in the above examples, regardless of mechanism, is an increase in plasma triglyceride levels. The combined data indicate that increased serum triglycerides and vascular dysfunction are linked not only to each other but to pulmonary hypertension as well.

The mechanisms leading to hypocholesterolemia and hypertriglyceridemia in plasma or serum of SCD patients are not completely understood. In normal individuals, triglyceride levels are determined to a significant degree by body weight, diet and physical exercise, as well as concurrent diabetes. While data on diet and physical exercise were not available for our study population, these factors very likely impact body weight and triglyceride levels in SCD patients. These findings indicate that standard risk factors for high triglycerides are also relevant to SCD patients. Mechanisms of SCD-specific risk factors for elevated plasma triglycerides are not as clear. RBCs do not have de novo lipid synthesis. In SCD the rate of triglyceride synthesis from glycerol is elevated up to 4-fold in sickled reticulocytes but SCD patients have defects in postabsorptive plasma homeostasis of fatty acids. Lipoproteins and albumin in plasma can contribute fatty acids to red blood cells for incorporation into membrane phospholipids, but RBC membranes are not triglyceride-rich and contributions of RBCs to plasma triglyceride levels have not been described, to our knowledge. Interestingly, chronic intermittent or stable hypoxia just by exposure to high altitudes, with no underlying disease, is sufficient to increase triglyceride levels in healthy subjects [16]. Thus, it is possible that hypoxia in SCD may contribute at least partially to the observed increase in serum triglyceride. Finally, there is a known link of low cholesterol and increased triglycerides that occurs in any primate acute phase response, such as infection and inflammation. Perhaps because of their chronic hemolysis, SCD patients have a low level of acute phase response, which is also consistent with the other inflammatory markers. Further studies are required to elucidate the mechanisms leading to hypocholesterolemia and hypertriglyceridemia in SCD.

The present study showed that there was a significant increase in the mean value of aspartate amino transferase HbAS subjects when compared to control subjects. The observed high activity of AST in patients with sickle cell anaemia could be as a result of the presence of numerous sickled red blood cells in the lobular parenchyma of the liver. The study showed also that there was a significant increase in the mean value of lactate dehydrogenase in HbSS and HbAS subjects when compared to control subjects. The present study showed that there was a significant increase in the value of mean artherogenicity index in HbSS subjects when compared to HbAS subjects and

HbAwhen compared to control subjects. This indicates increased risk of cardiovascular disease in sickle cell disease. Elevated lactate dehydrogenase (LDH) activity and artherogenicity index in SCD comes from various mechanisms, especially intravascular hemolysis, as well as ischemia-reperfusion damage and tissular necrosis. Intravascular hemolysis is associated with vasoconstriction. Platelet activation, endothelial damage, and vascular complications. LDH has used as a diagnostic and prognostic factor of acute and chronic complications [10].

Table 1: Lipids, Lactate Dehydrogenase, Troponin and Aspartate Amino Transferase of HbSS Subjects

Parameters	Control n=10	HbSS n=10	t-test	P-value
T Chol	172.68±14.82	129.03±37.62	5.907	0.000
T.G	95.92±22.79	127.5±31.21	2.613	0.014
HDL	60.75±7.12	41.10±10.85	4.129	0.001
LDL	91.55±16.55	69.06±10.54	4.913	0.000
LDH	378.10±105.72	692±152.68	5.971	0.000
TP	0.11±0.06	2.35±0.59	12.998	0.000
AST	18.9±4.95	67.9±16.59	6.835	0.000

Table 2: Lipids, Lactate Dehydrogenase, Troponin and Aspartate Amino Transferase of HbAS Subjects

Parameters	Control n=10	HbAS n=10	t-test	P-value
T Chol	172.68±14.82	153.49±9.13	4.751	0.001
T.G	95.92±22.79	94.27±21.47	0.134	0.448
HDL	60.75±7.12	52.45±5.99	2.913	0.009
LDL	91.55±16.55	86.52±9.87	0.915	0.192
LDH	378.10±105.72	515.60±62.39	3.888	0.002
TP	0.11±0.06	1.29±0.76	4.821	0.000
AST	18.9±4.95	25.50±7.32	3.177	0.006

Table 3: Lipids, Lactate Dehydrogenase, Troponin and Aspartate Amino Transferase in HbAS subjects and HbSS subjects.

Parameter	HbSS n=10	HbAS HbSS n=10	t-test	P-value
T Chol	129.03±37.62	153.49±9.13	4.148	0.001
T.G	127.5±31.21	94.27±21.47	3.888	0.002
HDL	41.10±10.85	52.45±5.99	2.751	0.011
LDL	69.06±10.54	86.52±9.87	3.839	0.002
LDH	692±152.68	515.60±62.39	3.196	0.005
TP	2.35±0.59	1.29±0.76	3.772	0.002
AST	67.9±16.59	25.50±7.32	5.739	0.000

Table 4: Artherogenicity Index of Control (HbA), HbAS and HbSS.

Parameter	**Artherogenicity Index**
Control	0.20
HbSS	0.49
HbAS	0.25

CONCLUSION

Sickle cell disease is associated with increased level of triglyceride,, lactate dehydrogenase, troponin and aspartate amino transferase and a decreased level of total cholesterol and high density lipoprotein.From these results, there is a clear indication of cardiac dysfunction and infarction associated with sickle cell disease.

CONSENT AND ETHICAL APPROVAL

Ethical approval of the study was obtained from Federal Medical Center Owerri, Nigeria. The consents of the volunteer subjects were duly sort. Each subject signed an informed consent form after which the procedure and implication were explained using a language the subjects would understand.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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