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Evaluation Of Lipid Profile In Sickle Cell Disease Subjects In Steady State Attending Nauth Nnewi Anambra State Nigeria

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Abstract:

Background: Lipid metabolism may be altered in red cell genetic disorders. The erythrocyte and serum lipids are defected which may increase the risk of cardiovascular disease. In the present study, we hypothesized a possible association between severity of anemia and altered lipid profile in SCD.

Aim: The major aim of the present study was to assess serum lipid profiles in SCA (SS) adult subjects at steady state, and to compare them to those of sickle cell trait HbAS and healthy controls HbAA.

Methods: This is a cross- sectional study. A total of one hundred and fifty aged matched participants were recruited for this study and grouped as follows: 50 SCD subjects with sickle cell anaemia (SS), 50 subjects with sickle cell trait (AS), and 50 Subjects with normal haemoglobin (AA). The control group consisted of 50 healthy individuals. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were enzymatically measured and LDL-C was calculated using friedewalds standard formulae.

Results: The result of analysis of variance (ANOVA) showed that the mean TC, TG, HDL-C and LDL-C levels were significantly different amongst the groups ($F=33.645$, 16.558, 11.277 and 18.958; $p<0.05$), respectively. Total cholesterol, Triglyceride, HDL-C and LDL-C were significantly lower ($p< 0.05$) in SS and AS subjects compared to AA controls. However, the mean serum TC, TG, HDL-C and LDL-C levels did not differ significantly in SS individuals than in the AS group ($p>0.05$) respectively.

Conclusions: This study revealed significant alterations in lipid profile levels among the SCD participants studied. Thus, further prospective studies should examine the contribution of an altered lipid profile to the severity and clinical complications in SCD patients even when in their steady state.

Keywords: Lipid profile, Sickle cell disease, Steady state.

I. INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder, which is caused by mutation of the hemoglobin subunit beta gene (*HBB*), most commonly seen in African descendants [1]. SCD is associated with significant tissue/organ damages and reduced life expectancy [2-4]. Sickle cell disease (SCD) is a monogenetic disorder resulting from a point mutation in the β-

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globin gene leading to the synthesis of abnormal hemoglobin S (HbS), [5]. Sickle cell anemia (SCA), that is, the homozygous state of the β^S allele, is the most frequently encountered genotype worldwide, far beyond sickle cell SC disease, that is, the heterozygous composite state of the β^S and β^C alleles (SCC). The sickling of red blood cells (RBCs), due to polymerization of HbS when deoxygenated, is the main pathophysiological mechanism at the origin of several vaso-occlusive-like events resulting from the entrapment of poorly deformable and fragile sickle red blood cells in small vessels [6]. SCD is characterized by chronic hemolysis, inflammation, exacerbated oxidative stress, frequent vaso-occlusive complications, multiple organ damage and reduced patient survival [6]. There are large variations in the nature and incidence of complications affecting SCA and SCC patients, and the clinical severity of SCC is often considered to be milder than that of SCA [7]. By contrast, it has been recently shown that SCC patients may also frequently experience similar vaso-occlusive-like events than SCA patients, that is, vaso-occlusive crisis (VOC), acute chest syndrome (ACS) and osteonecrosis (OTN), and may also develop more frequently specific complications such as retinopathy and otologic disorders [8]. The exact pathophysiological mechanisms, which lie at the origin of the heterogeneous clinical severity in SCA subjects, have yet to be fully elucidated.

Previous study has shown that dyslipidemia, abnormally elevated cholesterol or lipids in the blood including hypocholesterolemia and hypertriglyceridemia, is a common comorbid feature of SCD, and is significantly associated with serious SCD complications, for example, haemolytic severity, vascular dysfunction and pulmonary hypertension [8, 9, 10]. Hypocholesterolemia in SCD includes low levels of total plasma cholesterol and low-density lipoprotein (LDL) cholesterol [1, 8, 11]. However, to date, detailed profiling information as well as the mechanistic basis of dyslipidemia in SCD is lacking. From a clinical viewpoint, SCD manifestations can be roughly attributed to two phenomena: hemolysis and Vaso occlusion, disturbing microcirculation resulting in oxidative and inflammatory stress [12]. The vasculopathy seen in SCD is similar to that of atherosclerosis and coronary heart disease [9, 13] where lipid monitoring is an important guide. It is reported that in SCD besides elevated markers of endothelial dysfunction, such as vascular cell adhesion molecule-I, interleukin-6, C-reactive protein, selectins and vascular endothelial growth factor, decreased apolipoprotein A-1 (apoA-1) levels, reduced nitric oxide (NO) bioavailability, and decreased HDL-C are similar in both diseases. The vasculopathy observed in SCD is not attributable precisely to total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, since both have been shown to be decreased in SCD patients compared to healthy individuals. Even though LDL-C levels are diminished in SCD, the proportion of small dense LDL is an important factor to consider in SCD vasculopathy, since it can easily leak into the subendothelial space, be held by proteoglycans, and easily be oxidized [11]. There is epidemiologic evidence that hypocholesterolemia

The pathophysiology of sickle cell disease (SCD) and the variability of its clinical expression remain not fully understood, whether within or between different SCD genotypes [3,13,16].

Lipids have been very recently hypothesized to play a role in the pathophysiological mechanisms of SCA. SCA patients have unique plasma lipid profile characterized both in adults and children, by decreased levels of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), apolipoprotein A (apoA), and apolipoprotein B (apoB), compared to controls or to the general population [9, 12-14]. Contrasting results have been reported regarding the level of triglycerides (TG) in SCA compared to controls [9, 11, 15]. This dyslipidemia has been associated with the severity of hemolysis and would be involved in vascular dysfunction [9, 3, 16]. In addition, it has been also shown that patients with the highest TG levels would be prone to develop complications like pulmonary hypertension [9, 3, 16] and acute chest syndrome [11]. Furthermore, two recent studies showed evidence of an enhanced production of deleterious pro-inflammatory HDL-C that has been associated with endothelial cell injury in SCA subjects [17]. Altogether, these data support a deleterious impact of dyslipidemia on endothelial cells function in SCA subjects and suggest that alterations in lipids profile could be modulate or reflect the disease severity.

II. METHODOLOGY

STUDY DESIGN

This study adopted a cross-sectional study approach. One hundred and fifty (150) subjects grouped and age matched (SS = 50, AS =50 and AA = 50) attending NAUTH for routine clinic in the Hematology unit were recruited for the study. The AA group was used as control. The SS subjects were in their steady state. The steady-state condition was defined as follows: no blood transfusion in the previous three months, and absence of acute episodes (infection, VOC, ACS, stroke, priapism) at least two months before inclusion into the study. Healthy subjects were excluded if they have chronic disease, acute infectious, dyslipidemia, and obesity.

Ethical Clearance

Ethical approval for the study was obtained from the Nnamdi Azikiwe University Teaching Hospital ethics committee, and all participants had been informed about the purpose and procedures of this study, for which they had given a written consent in accordance with the guidelines.

SAMPLE COLLECTION

3ml of fasting blood samples were obtained from all participants for determination of biochemical parameters. Venous blood was collected in plain bottles, allowed to clot, centrifuge at 3000 rpm for 10 minutes, aliquots was drawn and used for determination of serum lipid parameters.

METHODS

The sickle cell phenotype in sickle cell anaemia (SS) patients was assessed by Hb electrophoresis and confirmed by

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Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Concerning the control subjects, the absence of a sickle cell trait was confirmed by the presence of a normal Hb electrophoresis.

Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined enzymatically using ChemWell chemistry analyzer, Low-density lipoprotein cholesterol (LDL-C) levels were estimated from the Friedewald formula (FF): LDL-C = TC – ½HDL-C + TG/5.

III. STATISTICAL ANALYSIS

Descriptive statistics including mean and standard deviation (SD) were calculated for all variables using SPSS software (version23, IBM SPSS Statistics, Chicago, IL). Data obtained was statistically tested using one-way ANOVA and posthoc test. The significance level was defined as $p < 0.05$.

IV. RESULTS

The result of analysis of variance (ANOVA) showed that the mean TC, TG, HDL-C and LDL-C levels were significantly different amongst the group (F=33.645, 16.558, 11.277 and 18.958; p<0.05), respectively.

The mean serum TC, TG, HDL-C and LDL-C levels did not differ significantly in SS individuals than in the AS group (p>0.05) respectively.

There were statistically significantly lower (p-value= 0.000; 0.003; 0.000 and 0.000) mean serum TC, TG, HDL-C and LDL-C levels in the SS individuals compared to the AA participants respectively.

Additionally, there were statistically significantly lower (p-value= 0.000; 0.000; 0.029 and 0.000) mean serum TC, TG, HDL-C and LDL-C levels in the AS individuals compared to the AA participants respectively.

GROUP	TC	TG	HDL-C	LDL-C
SS	3.60±0.71	1.02±0.22	0.95±0.15	2.23±057
AS	3.57±0.50	0.95±0.11	1.00±0.14	2.38±0.59
AA	4.41±0.55	1.13±9.11	1.08±0.11	2.86±0.43
f-value	33.645	16.558	11.277	18.958
p-value	0.000	0.000	0.000	0.000
SS vs AS	1.000	0.058	0.109	0.496
SS vs AA	0.000	0.003	0.000	0.000
AS vs AA	0.000	0.000	0.029	0.000

**Statistically significant at p<0.05; KEY: HDL-C- high density lipoprotein-cholesterol, TC- total cholesterol, TG-triglyceride, LDL-C -low density lipoprotein-cholesterol.*

Table 1: Levels of TC, TG, HDL-C and LDL-C in SS, AS and AA groups (MEAN ±SD)

V. DISCUSSION

Sickle cell disease is a hereditary and life-threatening condition that causes ongoing vascular damage and repeated injury to the blood vessels and organs including the heart and lungs.

In this study, participants from the SS and AS groups had lower total cholesterol (TC), HDL-C, TG, and LDL-C values than those from the AA group. Nearly every study that looked at lipids in SCD adults found that they had lower TC and LDL-C levels. Uche *et al*. in their study on lipid profile and disease severity in sickle cell disease patients in Lagos State Nigeria documented low TC, LDL-C and HDL-C levels in SCD patients compared with local laboratory reference values [18] which agrees with the present results. Samarah *et al*. also observed that total cholesterol and LDL-C were significantly lower in SS and sickle *β*-thalassemia patients compared to AS individuals and AA controls in keeping with the present findings whereas they noted that the HDL-C was significantly higher in AS individuals compared to AA controls which is in contrast with the current reports [19]. Decreased TC and LDL-C in SCD have been reported in many previous studies that examined lipids in SCD patients [20, 21] while some other similar studies showed lower HDL-C and TC levels among the SCD patients than in controls [22]. Lipid metabolism disorders, especially hypocholesterolemia and hypertriglyceridemia, are linked to clinical events observed in SCA, suggesting they play a relevant role in the multifactorial pathogenesis of this disease [23]. However, it might be hypothesized that SCD hypocholesterolemia results from cholesterol utilization during increased erythropoiesis of SCD, Cholesterol is largely conserved through enterohepatic circulation at least in healthy individuals. We found significantly lower levels of cholesterol, LDL-C, and HDL-C in SCD cases, which are consistent with earlier research by other investigators [24, 22]. (Previous study has shown that cholesterol is closely related to haematocrit values, and hypocholesterolemia is also commonly seen in different types of anaemia (16). The observed hypocholesterolemia may be due to decreased reservoir storage of cholesterol related to the decreased total red cell mass in SCD anaemia [1,23]. Hypocholesterolemia have been documented in SCD worldwide for over forty years yet the mechanistic basis and physiological aspects of these altered lipid levels have yet to be fully elucidated [25]. TC in particular and LDL-C has a well-established role in atherosclerosis. The low levels of LDL-C in SCD are consistent with low levels of TC and the absence of atherosclerosis among SCD individuals [21].

The present study result also clearly shows a decrease in HDL-C in SCD (SS and AS) vs Controls (AA). Lower HDL-C in SCD has been documented in some but not all previous studies [16, 19]. In studies on Lipid in which HDL-C is low this may be suggestive for inconsistencies between studies includes differences in age, diet, bodyweight, smoking, gender, diff ranges of disease and severity of other diseases and other diseases and treatments [16]. Decreased HDL-C is a known risk factor endothelial dysfunction in the general population and in SCD [24].

Furthermore, the current study showed that TG level was significantly lower in SS and AS compared to AA. In normal individuals, TG levels are determined to a significant degree by bodyweight, physical exercise diet. Mechanisms for SCD specific risk factors for delayed TG clearance are not clear. In SCD the rate of TG synthesis from glycerol is elevated up to four-fold in sickled reticulocytes [26] but SCD patients have defects in postabsorptive homeostasis of fatty acids [12].

Some Previous studies have given mixed results regarding TG in SCD. Increased TG levels have been reported in several studies of SCD adults [27, 28,29]. However, two studies did not find increase TG levels in SCD adults [14, 29] which are in conformity with our findings. Lipolysis of TG present in TG-rich lipoproteins releases neutral and oxidized free fatty acids that induce endothelial cell inflammation [2,28,29].

VI. CONCLUSION

Since dyslipidemia increases the incidence of atherosclerosis, coronary heart disease, cardiovascular disease, cholestasis and xanthomatosis, the individuals with sickle cell anaemia may be at an advantage in having protection against these disorders. Further prospective studies should examine the contribution of an altered lipid profile to the severity and clinical complications in sickle cell anaemia individuals even when in their steady state.

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