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Oxidative Modification of Lipids and Lipoproteins in Steady State Sickle Cell Anemic Patients from South-Eastern Nigeria

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Abstract: Sickle cell anemia is a genetic disease associated with constant medical crises. These crises are due to many biochemical and hematological changes arising from multi-organ damages. This study was aimed to assess changes in the plasma levels of lipids and specific lipoproteins in sickle cell anemia patients who were in their steady states. The study is a cross-sectional one involving 75 patients with sickle cell anemia, who were in their stable states while attending their routine check-up at the sickle cell clinic of University of Nigeria Teaching Hospital, Enugu, Nigeria and 72 age and sex-matched apparently healthy school children with normal blood genotypes. The patients were made up of 40 males and 35 females while the controls were 36 males and 36 females. The results of the study showed that patients have the following; total cholesterol 135 ± 38.6mg/dL, HDL-cholesterol 22.7 ± 8.1mg/dL, LDL-cholesterol 91.4 ± 41.2mg/dL, VLDL-cholesterol 20.5 ± 5.0mg/dL and triglycerides 102.7 ± 25.2mg/dL, while the results from the controls were; total cholesterol 162.2 ± 27.4mg/dL, HDL-cholesterol 36.6 ± 7.9mg/dL, LDL-cholesterol 100.2 ± 23.9mg/dL, VLDL-cholesterol 25.4 ± 3.7mg/dL and triglycerides 127.0 ± 18.4mg/dL. These results showed that there were significant decreases ($p<0.001$ in all) in these parameters in sickle cell anemia except LDL-cholesterol ($p=0.28$), indicating oxidative modifications of the lipids and the lipoproteins. However, there were no significant differences ($p>0.05$) between the parameters obtained in males and females in both patients and controls. Lipids and lipoproteins were significantly reduced in steady state sickle cell anemia, causing the patients to depend mainly on limited carbohydrates and proteins for the provision of energy. This may account for constant lack of energy as well as the frail and fragile appearances of these patients.

Keywords: Oxidative Modification, Lipids and Lipoproteins, Sickle Cell Anemia

1. Introduction

Sickle cell disease is a genetically inherited disease usually associated with multi-organ damage, with increased risk of early mortality [1, 2]. The most common and deadly type is sickle-cell anemia (SCA), which causes abnormality in the oxygen-carrying capacity of the red blood cells resulting in many organ and system failures with attendant consequences. The multi-organ damages are as a result of complications arising from numerous derangements associated with this disease. These derangements are products of many biochemical and hematological changes associated with the disease with attendant complications. Some of these complications include vaso-oclusive episodes (precipitated by stressed reticulocytes) and sickled erythrocytes, with participation of other blood cells like leukocytes and platelets, as well as endothelium activation [2, 3-6]. Other complications include priapism, leg ulceration, pulmonary hypertension and death. All these are associated with nitric oxide resistance and vascular hemolysis [7, 8]. Nitric oxide, which is a mediator, usually interacts with oxidative products - reactive species like reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chloride species (RCS) to produce some of the crippling conditions seen in sickle cell anemia [9].

Lipids and lipoproteins are affected physiologically by many factors including age, sex, physical activity, weight, eating habit/diet and heredity. While cholesterol generally rises slightly with increasing age, physical activity helps to lower LDL-cholesterol [10, 11]. Abnormalities in metabolism of lipids and lipoproteins are associated with many disease conditions including hypertension, diabetes, liver disease, atherosclerosis etc. These disease conditions are associated with increase in total cholesterol and, particularly, low density lipoprotein. On the other hand, some disease conditions are known to lower the levels of these lipoproteins through oxidative modification. For instance, it has been documented that during malaria infection in children, lipoproteins are oxidatively modified, causing reduced lipoproteins in parasitized children [9, 12]. This modification seems to be as a result of acute phase response, and the degree of the modification is thought to be related to the severity of the malaria infection Another research [13] has revealed a higher occurrence of pneumonia and cardiac abnormalities among those with lower HDL-cholesterol levels. This was attributed to the production of auto-antibodies specific to oxidized phospholipids. These auto-antibodies inhibit the uptake of oxidized LDL-cholesterol by macrophages, which would have in turn provided protection against virulent pneumococcal infection. Low levels of HDL-cholesterol are an important cardiovascular risk factor. This is related to the reduction of oxidized lipids and the enhancement of reverse cholesterol transport A study [15] has demonstrated the important role of the apo-lipoprotein pathway and its association with endothelial dysfunction in sickle cell disease patients with pulmonary hypertension. Furthermore, in malaria parasitemia, increased oxidative stress which accounts for the degradation of these lipoproteins is thought to originate from intracellular of parasitized erythrocytes, extracellular of hemolysed erythrocytes or host immune responses Therefore, in sickle cell disease where these conditions are equally obtainable when the patients are in crisis, it is expected that oxidative degradation of lipoproteins is a possibility or even a certainty. Hence, understanding the basal levels of these lipoproteins at steady state of the sickle cell patients will afford the clinicians the basic strategy for protecting the sufferers from going into dyslipidemia-precipitated crisis. Furthermore, the levels of these lipoproteins during routine hospital visitation may serve as a biomarker for a looming crisis. Moreover, apart from sickle cell disease, it has earlier been suggested that obese children are in need of cholesterol screening irrespective of family history or other risk factors This is because of their high risk of developing coronary vascular disease later in life. Therefore, it is expedient that sufferers of all conditions with potential risk of dyslipidemia later in life or during crisis, should be monitored early and routinely to prevent the possible derangement and subsequent complications. We report the result of a study on the levels of lipids and specific lipoproteins in sickle cell patients at steady state.

2. Materials and Methods

2.1. Ethical Clearance

The ethical clearance for this study was obtained from the Ethical Committee of University of Nigeria Teaching Hospital, Enugu, Nigeria, while additional consent was sought and obtained from the patients' mothers.

2.2. Sample Population/Subjects

Our patients included seventy-five (75) sickle cell anemia patients who were not having crisis at the time of the study but were attending the sickle cell clinic of University of Nigeria Teaching Hospital, Enugu, Nigeria on routine medical visitation. They were children and young adolescents aged between 8 and 15 years. Another group of apparently healthy non-sicklers – seventy-two (72) in number, were used as controls. These controls were sex and age matched with the patients.

2.3. Exclusion Criteria

All sickle cell anemia patients, whether in crisis or on steady state, attending sickle cell clinic of University of Nigeria Teaching Hospital, Enugu, usual undergo screening for malaria parasitemia and also have their packed cell volume measured. These are in addition to other laboratory tests the pediatrician may deem necessary. Those who were in crisis were not considered for the study, and those on steady state but were found to have malaria infections were excluded from the study since malaria parasitemia is known to increase lipid oxidation [9, 12].

2.4. Sample Collection

After cancelling and obtaining oral consent of the patients and their mothers, 3.0ml of blood was collected from each subject and dispensed into sequestered container and mixed by invasion for five times. From this, the packed cell volume was estimated for the patient's routine check while the remaining was then centrifuged at 3000 rpm for five minutes and the plasma separated immediately. The plasma was stored frozen till needed for analysis.

2.5. Laboratory Analysis

The plasma concentrations of the lipids and lipoproteins were determined. Total Cholesterol (Total-Chol), High Density Lipoprotein Cholesterol (HDL-Chol) and Triglycerides were determined by spectrophotometric methods using reagent kits (Cromatest®) prepared by Linear Chemicals SL (Spain) and sourced from local science equipment company – Ceejay Global Resources Limited. Laboratory analyses were done in batches of 15 samples each. Duplicate analysis was done for each sample, to ensure proficiency and reproducibility, and the average value taken while product instruction manuals were strictly followed to ensure reliability of the results. The Low Density Lipoprotein Cholesterol (LDL-Chol) values were calculated using Friedewald formula [17]while Very Low Density Lipoprotein Cholesterol (VLDL-Chol) were taken as one fifth of the triglycerides in milligram per deciliter.

2.6. Data Analysis

Generated data were analyzed using the statistical package for social sciences (SPSS) software version 21.0. Descriptive values were analyzed and differences between means were calculated. The level of statistical significance was set at $p < 0.05$.

3. Results

Table 1 is the demographic distribution of both patients and controls while table 2 shows the results of plasma lipids and lipoproteins in both patients and controls. These results indicated significant decrease of all the parameters in sickle cell anaemia when compared with those of the controls except LDL-cholesterol. Tables 3 and 4 are the results obtained from male and female patients and controls respectively, indicating non-significant sex-dependent changes in the plasma concentrations of these parameters.

Table 1. *Socio-demographic distribution of both patients and controls.*

Age	Patients (n=75)	Controls (n=72)
7 – 9	23(30.7)	21(29.2)
10 – 12	25(33.3)	25(34.7)
13 – 15	27(36.0)	26(36.1)
Sex		
Males	40 (53.3)	36 (50.0)
Females	35 (46.7)	36 (50.0)
Education		
Primary	38 (50.7)	39 (54.2)
Secondary	37 (49.3)	33 (45.8)

Table 2. *Mean (±SD) of the lipids and lipoproteins in patients and controls.*

Parameter(mg/dL)	Controls(n=72)	Patients(n=75)	R^2	P-value
T-Chol	162.2(27.4)	135.0(38.6)	0.142	<0.001
HDL-Chol	36.7(7.9)	22.7(8.0)	0.437	<0.001
LDL-Chol	100.2(23.9)	94.4(37.9)	0.008	0.270
VLDL-Chol	25.4(3.7)	20.5(5.0)	0.237	<0.001
Triglycerides	127.0(18.4)	102.7(24.9)2	0.237	<0.001

Table 3. *Means (±SD) of lipids and lipoproteins in male and female patients.*

Parameter(mg/dL)	Males(n=35)	Females(n=40)	R^2	P-value
T-Chol	129.1(6.3)	140.2(6.2)	0.021	0.213
HDL-Chol	23.4(1.4)	22.2(1.3)	0.005	0.534
LDL-Chol	88.6(6.0)	99.5(6.2)	0.021	0.213
VLDL-Chol	21.3(0.8)	19.9(0.8)	0.018	0.247
Triglycerides	106.3(3.9)	99.6(4.1)	0.018	0.247

Table 4. *Means(±SD) of lipids and lipoproteins in male and female controls.*

Parameter(mg/dL)	Males(36)	Females(36)	R^2	P-value
T-Chol	160.0(5.1)	162.3(4.2)	2.513	0.967
HDL-Chol	38.0(1.4)	35.7(1.3)	0.021	0.221
LDL-Chol	98.5(4.3)	101.5(3.7)	0.004	0.606
VLDL-Chol	25.6(0.7)	25.2(0.6)	0.003	0.645
Triglycerides	128.2(3.6)	126.1(2.8)	0.003	0.644

4. Discussion

From the above results, all the lipids and lipoproteins studied decreased significantly in sickle cell anemia patients when compared with apparently normal subjects (controls). Exception is low density lipoproteins which though decreased in sickle cell patients but the decrease was not statistically significant. The results from this study are in agreement with an earlier study [19] on the same class of

patients. They are also in agreement with our earlier study of these parameters in malaria patients [9] where we were of the opinion that the significant decreases may be as a result of oxidative modification of the lipids. Since sickle cell anemia patients are always under oxidative stress, the same modification could have accounted for these significant lower values. These results however are not in total agreement with some previous studies on the same subjects [20-22]. These previous studies had reported either significant or non-significant increase in the levels of triglycerides in sickle cell anemia patients as against non-significant decrease in low density lipoproteins obtained from the present study. The difference between these studies may be as a result of environmental, socio-economic, nutritional and medical differences between the areas of study as well as the subjects, particularly the level of stability of the patients. However, despite the significant decrease in the levels of these parameters in the present study, none of the results obtained from the patients was lower than its established reference value. This seems to imply that these patients at steady state are neither prone to the consequences of low lipids and lipoproteins nor at high risk of consequences of high values. Though such lower levels have been reported in sickle cell anemia patients with vaso-occlusive crisis [23], it was not clear whether the lower level was the cause or effect of vaso-occlusion. On the other hand, it is known that oxidative metabolism of lipids yields more than twice the energy of an equal weight of carbohydrate or protein. Likewise, hydrolysis of these fats in adipose tissue, accomplished by hormone-sensitive triacylglycerol lipase, yields free fatty acids that are released into the blood stream where they bind to proteins (produced from the liver and mucosa cells), forming lipoproteins. Therefore, reduced lipids will subsequently/automatically lead to reduced lipoproteins, while adequate intake of fats spares carbohydrates and proteins by providing bulk of the energy need of the body. In addition to oxidative modification, many factors that include nutritional, environmental, socio-economic and cultural, might have contributed to the significant decrease in the levels of these parameters in sickle cell patients. For instance, though globalization, resurgence of middle class, urbanization and high propensity to consume cheap, high energy dense foods seem to be exposing young Nigerians to obesity, physical, psychological, medical and socio-economic consequences of sickle cell anemia is so daunting that sufferers hardly meet up with "living" nutritional requirements. Moreover, most of these patients are seen within low economic class, hence many of them depend largely on carbohydrate-rich foods as their only source of energy. Unfortunately, because of the reduced man/hour occasioned by physical condition of the patients with its attendant low socio-economic situation, neither the cheap high energy dense foods nor the carbohydrate-rich foods are easily and sufficiently accessed by these patients. To worsen the matter, because of the economic conditions, accessing protein-rich foods by these patients is even more remote than accessing the other two. Thus, while the medical condition reduces food intake by these patients - anorexia, the little that are consumed are almost completely oxidized for provision of energy, causing energy-food crisis in the patients. No wonder most of them always look worn-out, frail, fragile and devastated, falling into crisis more often than expected, making them and their families visit hospital more frequently than any known inherited disease. Furthermore, that LDL-cholesterol showed no significant decrease in this study may be related to the reduced physical activity of the patients. One of the basic precautions observed by sickle cell patients and their families is avoidance of strenuous physical activity, but it is known that good physical activity is a major factor that helps to lower LDL-cholesterol [10, 11]. Hence, no significant change is expected as a result of the disease condition.

5. Conclusion

The results of this study showed that there is constant oxidative modification of lipids and lipoproteins in sickle cell anemia, hence plasma lipids and lipoproteins were significantly reduced in these patients even at steady state. This condition probably makes it mandatory that the patients depend largely on carbohydrates and proteins for the provision of energy. And because of poor supply of these food materials, they get exhausted easily leading to emaciation, frail and weak appearances of the patients. We advocate that intake of cheap high energy dense foods (derogatorily referred to as "junk foods") should always form substantial part of nutritional requirements for sickle cell patients. Most importantly, governments should legislate against marriage between two people with sickle cell trait, so that by gradual long term elimination, this excruciating condition can be eliminated from the face of the earth.

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