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Full Length Research Paper

Lecithin: Cholesterol acyltransferase, lipoprotein lipase and lipoproteins in adult Nigerians with sickle cell disease

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Lecithin: cholesterol acyltransferase (LCAT) is a glycoprotein enzyme involves in the esterification of plasma cholesterol to form cholesteryl ester and is important in the maturation of high density lipoprotein. Lipid metabolism in sickle cell anaemia patients may be affected. The objective of this study was to determine the activities of serum lecithin: cholesterol acyltransferase, lipoprotein lipase, lipids and lipoprotein levels in sickle cell anaemia patients in a steady state. The study population consisted of 144 confirmed sickle cell disease patients; 68 males aged 22.2 ± 3.8 years and 76 females aged 21.0 ± 3.0 years. Fifty age matched males; 25 sickle cell trait (HbAS), 25 normal haemoglobin (HbAA) and fifty females; 25 HbAS and 25 HbAA were used as controls. Statistically significant decrease in LCAT and LPL activities ($p < 0.001$) were observed in sickle cell anaemia patients compared with HbAS and HbAA controls in both sexes. Decrease levels of cholesterol ($p < 0.001$) and HDL cholesterol ($p < 0.001$) were also observed in sickle cell anaemia patients. The activities of lecithin: cholesterol acyltransferase and lipoprotein lipase were lower in subjects with sickle cell anaemia than sickle cell trait and normal haemoglobin. This may contribute to the changes observed in lipid metabolism in sickle cell anaemia. The exact cause is not known but appears to be multifactorial.

Key words: Sickle cell disease, Lecithin: cholesterol acyltransferase, lipoprotein lipase, Lipoproteins.

INTRODUCTION

Lecithin: cholesterol acyltransferase (LCAT) is a 63KDa glycoprotein enzyme which is synthesized in the liver and secreted in the plasma (Francone and Fielding, 1991; Wang et al., 1997). LCAT plays an important role in reverse cholesterol transport. It binds to high density lipoprotein (HDL-cholesterol) to catalyze the transfer of a fatty acid residue from the sn-2 position of lecithin to cholesterol to form cholesterol ester and lysolecithin (Saku et al., 1999).The enzymatic activity of LCAT depends on the presence of apolipoprotein A-1 which serves as cofactor (Vaziri and Parks, 2001). It thus faciletates free cholesterol uptake by HDL particles from the peripheral tissues and maturation of lipoprotein poor HDL_3 to cholesterol ester rich HDL_2 particles (Glomset, 1968). A lack of LCAT activity leads to increase in free cholesterol and phospholipids and decrease in esterified cholesterol. This phenomenon results in abnormalities of all kinds of lipoprotein particles (Nichols et al., 1975; King et al., 1980) which are observed in some organs such as kidney, cornea and erythrocytes in patients with LCAT deficiency (Gjone, 1988; Godin et al., 1978; Hosoda et al., 1984). These changes clinically correspond to renal insufficiency, cornea opacity and haemolytic anaemia respectively (Saku et al., 1999). Lipoprotein lipase (LPL) is an enzyme involved in the regulation of fatty acid influx, responsible for the hydrolysis of triglyceride rich chylomicrons, very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL), liberating free fatty acid and glycerol for uptake in the in the target tissue. LPL is functional at the vascular surface of the capillary endothelium anchored by proteoglycan chains of heparin sulphate (Vaziri and Parks, 2001). Sickle cell anaemia

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Table 1. Lipid, lipoproteins, LCAT and LPL in male sickle cell disease subject s compared with HbAS and HbAA controls.

	HbSS males	HbAS Males	P-value	HbAA males	P-value
No of subjects	68	25		25	
Age of years	22.2 ± 3.8	28.7 ± 7	-	28.8 ± 7	-
Triglyceride (mmol/L)	1.10 ± 0.4	1.19 ± 0.18	N/S	1.4 ± 0.12	P < 0.001
Total cholesterol (mmol/L)	3.06 ± 0.5	4.05 ± 0.06	P < 0.001	4.3 ± 0.12	P < 0.001
HDL cholesterol (mmol/L)	0.72 ± 0.17	1.18 ± 0.03	P < 0.001	1.2 ± 0.06	P < 0.001
LDL cholesterol (mmol/L)	1.92 ± 0.54	2.15 ± 0.14	NS	2.52 ± 0.16	P < 0.001
VLDL cholesterol (mmol/L)	0.48 ± 0.06	0.42 ± 0.07	N/S	0.41 ± 0.08	NS
LPL (µmol/glycerol liberated/hr/L)	4.12 ± 1.2	5.12 ± 0.4	P < 0.001	5.56 ± 0.23	P < 0.001
LCAT(µmol/cholesterol liberated/hr/L)	66.8 ± 2.8	69.2 ± 3.0	P < 0.001	70.2 ± 2.96	P < 0.001

(SCA) is a chronic haemolytic disorder and manifests by a wide variety of clinical, biochemical and haemotological features. The chronic haemolytic anaemia often leads to limited oxygen availability to peripheral tissues. This lack of oxygen has the potential to modify events in the intracellular metabolism (Buchwoski et al., 2007) and metabolism of lipids may be altered in individuals with SCA (Rahimi et al., 2006).

Sickle cell disease (SCD) is the most common and most important haemoglobin variant accounting for over 60% of the world's major haemoglobinopathies. It is estimated that 2 - 3 millions Nigerians are affected (Olatunji, 2002). Plasma lipids have been studied in Nigerians (Oforofuo and Adedeji, 1994; Akinyaju and Akinyaju, 1976) and adult African Americans (Shores et al., 2003) with SCD and none has studied the activities of lecithin: cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL) in conjunction with lipoproteins in this group of individuals. The objective of this study is to determine the concentration of plasma lipids and lipoproteins, the activities of LCAT and LPL in adult sickle cell disease patients in Kano, northern Nigeria.

MATERIALS AND METHODS

The study was conducted in Aminu Kano teaching hospital, Kano from January 2006 to December, 2008.

The study was approved by the ethical committee of the hospital and consent was also obtained from the patients. The study population was made up of 144 confirmed sickle cell disease subjects. They consisted of 68 males aged 22.2 ± 3.8 years and 76 females aged 21.0 ± 3.0 years. They were SCD patients on steady state attending sickle cell disease clinics of the hospital. The controls subjects consisted of 25 HbAS males aged 28.7 ± 7.0, 25 HbAA males aged 28.8 ± 7 years, 25 HbAS females aged 29.0 ± 7 years and 25 HbAA females aged 26.8 ± 8 years.

Fasting blood specimens were obtained from the patients and were allowed to clot at room temperature for 30 min. The specimens were centrifuged at 3000 rpm for 10 min to obtain sera. Serum triglyceride and total cholesterol were determined using enzymes catalyzed colorimetric methods by Randox laboratories, UK. While friedwald (Friedwald et al., 1972) formula was used to calculate LDL cholesterol levels. HDL- cholesterol was determined in the supernatant after precipitation with magnesium chloride-phosphotungstic acid solution. LCAT was measured using the Anasolv LCAT assay obtained from DAICCHI chemicals, japan (Saku et al., 1999; Korn, 1955), in which proteoliposome was used as substrate. The substrate (0.2 ml) was incubated with 0.02 ml plasma at 37°C for 60 min. Aliquot was used at 30 min interval to measure free cholesterol. Isopropanol was added as an arresting agent. Then 0.01 ml of the incubating mixture was added to 1.0 ml cholesterol oxidase reagent and was incubated for 10minutes at 37°C. The absorbance was read at 540 nm against reagent blank. Recrystallized cholesterol solution (500 µmol/L) in isopropanol was used as the calibrator. The enzyme activity was expressed as the function of free cholesterol liberated during the incubation period. LPL was determined by incubating the serum in glyceryl trioleate substrate, the glycerol liberated was measured in an aliquot of the incubating mixture (Korn, 1955; Anurag and Anuradha, 2002). The substrate was incubated with 0.2 ml of serum at 37°C for 60 min and 0.1 ml 0.1 NH2SO4 was added directly in a centrifuge tube. The content of the tube was allowed to equilibrate at room temperature and 0.1 ml of 0.5 M sodium periodate was added. After 5 min, 0.1 ml of 0.5 M sodium arsenite was added to reduce the excess periodate. After additional 10 min, 9 ml chromotrophic acid reagent was added and the reaction tube place in covered boiling water for 30 min. The cool tube was adjusted to a volume of 10 ml with distilled water and the absorbance read at 570 nm. Glycerol standards were used as calibrators. The results were expressed as micromole glycerol liberated per hour per litre.

Student's t-test for unpaired means was used for statistical comparison of the means. Results were expressed as mean ± SD and value was considered significantly different at $p < 0.05$.

RESULTS

Results are as indicated in Tables 1 and 2. In the male SCD patients, there was a decrease in the mean triglyceride level compared with male HbAS (NS) and male HbAA ($p < 0.001$). Statistically significant decrease in mean cholesterol level was observed in male SCD patients compared with male HbAS ($p < 0.001$), and HbAA ($p < 0.001$). The mean HDL-cholesterol level was also significantly decreased in male SCD patients compared with HbAS ($p < 0.001$) and HbAA ($p < 0.001$). The mean LDL cholesterol level in male SCD patients was also decrease compared with male HbAS (NS) and HbAA ($p < 0.001$). The mean LPL activities in male SCD patients were significant lower than those of the male HbAS ($p < 0.001$) and HbAA ($p < 0.001$). In the same vein, the mean activity of LCAT in SCD patients was

Table 2. Lipid, lipoproteins LCAT and LPL in female SCD patients compared with HbAS and HbAA controls.

	HbSS females	HbAS females	P-value	HbAA females	P-value
No of subjects	76	25		25	
Age (years)	21 ± 3.0	29.0 ± 7	-	26.8 ± 8	-
Triglycerides (mmol/L)	1.05 ± 0.56	1.3 ± 0.18	P < 0.001	1.42 ± 0.12	P < 0.001
Total cholesterol (mmol/L)	3.43 ± 0.4	4.1 ± 0.2	P < 0.001	4.4 ± 0.14	P < 0.001
HDL cholesterol (mmol/L)	0.78 ± 0.15	1.26 ± 0.05	P < 0.001	1.31 ± 0.03	P < 0.001
LDL cholesterol (mmol/L)	2.0 ± 0.39	2.62 ± 0.04	P < 0.001	2.68 ± 0.04	P < 0.001
VLDL cholesterol (mmol/L)	0.41 ± 0.08	0.62 ± 0.08	NS	0.42 ± 0.07	NS
LPL (µmol/glycerol liberated/hr/L)	3.39 ± 0.21	5.22 ± 0.5	P < 0.001	5.36 ± 0.3	P < 0.001
LCAT (µmol/cholesterol liberated/hr/L)	59 ± 1.3	69 ± 3.2	P < 0.001	71.2 ± 2.2	P < 0.001

significantly lower than those the male HbAS ($p < 0.001$) and HbAA ($p < 0.001$) controls (Table 1).

In the female SCD patients, there was statistical significant decrease in the mean triglyceride compared with the female HbAS ($P < 0.001$) and HbAA ($P < 0.001$). The mean total cholesterol level was significantly lower than the female HbAS ($P < 0.001$) and HbAA ($P < 0.001$). The mean LDL cholesterol was lower than the female HbAS ($P < 0.001$) and HbAA ($P < 0.001$).

Similarly, the mean LPL activity in the female SCD patients was significantly lower than HbAS ($P < 0.001$) and HbAA ($P < 0.001$).

The LCAT activity in SCD patient was also significantly lower than HbAS ($P < 0.001$) and HbAA ($P < 0.001$) controls (Table 2). Comparing Tables 1 and 2, it was observed that mean triglyceride level was significantly lower in female SCA than Hb AS and HbAA whereas in the male subjects, the concentration was only significantly lower in HbAA and not in HbAS.

DISCUSSION

The finding of low serum cholesterol and other lipoprotein levels in SCD patients is consistent with other reports both in Nigeria (Oforofuo and Adedeji, 1994; Akinyaju and Akinyaju, 1976) and elsewhere (Rahimi et al., 2006; Zailare et al., 2003; Elmehdawi, 2008). It is important to mention that the levels in Nigerian adult sickle cell disease patients is much more lower compared with the lipid levels reported in both African American16 and Saudi Arabian20 patients with SCD. There is an indication of progressive decrease in plasma cholesterol level wherever HbS gene is inherited as sickle cell trait (HbAS) or as SCD (HbSS) irrespective of sex. This is an indication that the low cholesterol and other lipoproteins levels are intrinsic to the disease. The exact etiology of hypocholesterolaemia in SCA is not known but several studies postulated different mechanisms (Oforofuo and Adedeji, 1994; Shalev et al., 2007; Lakhani et al., 2001).

Hypocholesterolaemia tends to occur in patients with chronic anaemia, increased erythropoietic activity and it has been suggested that this may be due to increase cholesterol requirements by the proliferative erythroid cells (Elmehdawi, 2008).

The chronic anaemia may leads to an increased plasma volume and having “dilution effect” on plasma consistuents 21. There may be down regulation in the pathway of cholesterol biosynthesis in the hepatic cells of SCD subjects (Shalev et al., 2007).

A Low LCAT and LPL activity was observed in SCD subjects compared with HbAS and HbAA subjects in this study. Little is known about LCAT metabolism and regulation in health and disease (Lakhani et al., 2001). LCAT appears to be exclusively secreted by the liver and few perturbations such as inflammatory mediators affect its biosynthesis (Saku et al., 1999). It was observed that SCA patients in steady state may undergo constant inflammatory process which may occur as a result of subclinical vaso-occlusion which may in turn leads to inflammatory response (Bourantas et al., 1998). The biosynthesis of this enzyme may be down regulated in sickle cell disease due to subclinical inflammatory episodes. Because LCAT plays an important and central role in plasma lipoprotein metabolism, the effects of a significant decrease in LCAT activity in SCD patients as observed in this study may be seen in a wide range of lipid and lipoprotein changes.

These altered lipolytic enzymes and lipoproteins reflect changes in composition and concentration of different lipoproteins and can also lead to alterations in lipid composition of membrane such as in red blood cells. The low LCAT activity in SCA patients may affect reverse cholesterol transport, HDL maturation and oxidation of atherogenic lipids in SCA patients.

Conclusion

Lipid metabolism in SCD appears to be different from that in sickle cell trait and normal haemoglobin in adult Nigerian SCD patients. The exact cause is not known but

appears to be multifactorial.

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