Sex Dimorphism in Serum Lecithin: Cholesterol Acyltransferase and Lipoprotein Lipase Activities in Adult Sickle Cell Anaemia Patients with Proteinuria

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Abstract Proteinuria in subjects with sickle cell anaemia (SCA) is an indication of an ongoing renal insufficiency and its prevalence varies between sexes. We evaluated sex differences in the activities of Lecithin: cholesterol acyltransferase (LCAT), Lipoprotein lipase (LPL) and the levels of lipoproteins in SCA patients with proteinuria. Fifty SCA patients (30 males aged: 26.4 ± 7.3 years and 20 females, aged 25.4 ± 2.6 years) and 50 age and sex matched control SCA patients were recruited for the study. Random urine specimens were collected and tested for the presence of albumin by urine dipstick technique. A 24 h urinary protein was quantitated using sulphosalicylic acid technique. Fasting serum total cholesterol, triglyceride, urea and creatinine were determined using enzymes catalyzed colorimetric methods. HDL cholesterol was determined in the supernatant after precipitation with manganese chloride–phosphotungstic acid solution. LCAT was measured using the Anasolv LCAT assay with proteoliposome as substrate. LPL was determined by incubating the serum in glyceryl trioleate substrate, the glycerol liberated was measured in an aliquot of the incubating mixture. In male SCA controls there was 18.2 and 6.9% increase in the activities of LPL and LCAT respectively

when compared with females but in SCA patients with proteinuria there was 8.4 and 5.2% decreases in the male SCA patients compared with females. The concentration of 24 h urine protein in the SCA male subjects with proteinuria was significantly higher (0.25 g/day; $P < 0.001$) compared with the SCA female patients with proteinuria (0.09 g/day). There are sex differences in the activities of LCAT and LPL in SCA patients with proteinuria. Metabolism of these lipolytic enzymes may be modulated differently in SCA patients with proteinuria.

Keywords Sick cell Anaemia · Proteinuria · Lecithin: cholesterol acyltransferase · Lipoprotein lipase

Introduction

The activities of Lecithin: cholesterol acyltransferase (LCAT) and Lipoprotein lipase (LPL) have earlier been reported to be different in SCA patients compared with sickle cell trait and normal haemoglobin [1]. Sex differences in the activities of these enzymes in SCA patients have not been previously reported. Proteinuria is not only an early marker of renal injury but also predicts disease progression. Several types of renal disease progress at a faster rate in men compared with women [2]. Biochemical parameters such as antioxidant enzymes, oxidative stress indices and immune functions have been reported to show sex dimorphism [3–7]. These sex dimorphisms have been reported to be regulated mainly by sex hormones. In this study, we evaluated sex differences in the activities of Lecithin: cholesterol acyltransferase (LCAT), Lipoprotein lipase (LPL) and the levels of lipoproteins in sickle cell anaemia (SCA) patients with proteinuria. Lecithin: cholesterol acyltransferase and LPL are very important

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enzymes involved in lipid metabolism. Lipid metabolism is altered in a majority of patients with renal insufficiency and renal failure, but may not necessarily lead to hyperlipidaemia [8]. This dyslipoproteinaemia of renal disease has characteristic abnormalities of the apolipoprotein profile and lipoprotein composition. It is believed to develop during the asymptomatic stages of renal insufficiency and becomes more pronounced as renal failure advances [8]. Decreased in LCAT activity is most important in the development and progression of kidney disease. Because LCAT plays an important and central role in plasma lipoprotein metabolism, the effect of a significant decrease may be seen as a wide range of lipid and lipoprotein changes [9]. LCAT binds to HDL to catalyze the conversion of unesterified cholesterol and phosphatidylcholine (PC) to esterified cholesterol and lysophosphatidylcholine. In other words, a low LCAT activity causes an increase in unesterified cholesterol which results in abnormalities of all kinds of lipoprotein particles and structures [10–18]. Individuals with SCA may develop glomerulopathy with proteinuria and progressive renal insufficiency leading to end stage renal disease [19]. Although there is no gender predilection for renal failure, we observed male predominance of sickle cell nephropathy in SCA patients in Nigeria (in press).

Materials and Methods

Study Population

This cross sectional study was conducted at Aminu Kano Teaching Hospital, Kano. The protocol used was approved by the committee on Ethics of the hospital and all the patients and controls gave informed consent. Fifty sickle cell anaemia (SCA) patients with proteinuria attending the Sickle cell disease clinic of the hospital were consecutively recruited for the study. They consisted of 30 males aged: 26.4 ± 7.3 years and 20 females, aged 25.4 ± 2.6 years. Random urine specimens were collected and tested for the presence of albumin by urine dipstick technique. Significant proteinuria was defined as $\geq 1+$ proteinuria in urine on dipstick examination. A 24 h urinary protein was determined using 3% sulphosalicylic acid. Fifty SCA patients on steady state (25 males; aged 24.2 ± 2 years, 25 females aged 23.6 ± 2 years) who tested negative for urinary albumin were selected and used as controls. All subjects were evaluated in steady state with no acute illness during assessment.

Blood Collection and Processing of Serum

Fasting blood specimen was collected from both study group and controls which were allowed to clot at 4°C for

30 min. The specimen was centrifuged at 3,000 rpm for 10 min to obtain serum. The sera were stored at -20°C until analyses were performed. Serum total cholesterol, triglyceride, urea and creatinine were determined using enzymes catalyzed colorimetric methods by Randox laboratories, UK. Friedwald [20] formula was used to calculate LDL cholesterol levels. HDL cholesterol was determined in the supernatant after precipitation with manganese chloride–phosphotungstic acid solution. LCAT was measured using the Anasolv LCAT assay obtained from DAICCHI chemicals, Japan [21] in which proteoliposome was used as substrate. LPL was determined by incubating the serum in glyceryl trioleate substrate, the glycerol liberated was measured in an aliquot of the incubating mixture [22].

Preparation of LPL Substrate

The substrate contains the following components: 0.3 ml ammonium chloride, ammonia buffer (0.25 M, pH 8.5), 0.1 ml calcium chloride (1.0 M), 0.2 ml of substrate (13.2 mg of glyceryl trioleate and 180 mg serum bovine albumin emulsion in 2 ml), 1.5 ml of 0.2 M tris HCl buffer; pH 8.6 were made up to 6 ml with water in a beaker. The microtip of a Branson (MSE London) sonicator was placed approximately 5 mm below the surface of the solution and the mixture was sonicated in an ice-bath for 8 min with alternating 60 s sonication with a 60 s pause. The substrate was activated by incubating with 12 ml heated fasting human serum for 30 min at 37°C in a water bath with a shaker (The fasting human serum was first heated for 10 min at 62°C in a water bath to inactivate any endogenous LPL that might have been present in the body).

Statistical Analysis

Students' *t* test was used for comparison of the means. Results were expressed as mean $\pm$ SD and value was considered statistically significant at $P < 0.05$.

Results

Table 1 shows studied parameters in SCA patients with proteinuria compared with control subjects. In the control SCA patients, the age and BMI of the patients were not significantly different in male compared with the female. The LPL and LCAT activities were significantly higher ($P < 0.001$) in the male compared with female while triglyceride ($P < 0.001$), total cholesterol ($P < 0.001$), LDL cholesterol ($P < 0.005$) and VLDL cholesterol ($P < 0.001$)

Table 1 Sex differences in studied parameters in SCA patients with proteinuria and controls

	Control males	Control females	<i>P</i> value	Males with proteinuria	Females with proteinuria	<i>P</i> value
Number of subjects	25	25		30	20	
Age (years)	24.2 ± 2.0	23.6 ± 2.0	NS	26.4 ± 7.3	25.4 ± 2.6	NS
BMI (kg/m ²)	21.5 ± 4.0	21.0 ± 3.2	NS	22.1 ± 3.0	23.2 ± 2.0	NS
Urea (mmol/l)	2.6 ± 0.6	2.84 ± 0.9	NS	8.07 ± 2.2	3.01 ± 0.2	<i>P</i> < 0.001
Creatinine (μmol/l)	62.1 ± 9.8	61.8 ± 11.4	NS	269 ± 19.0	71 ± 14.2	<i>P</i> < 0.001
eGFR (min/ml)	106 ± 18	98 ± 40	NS	71 ± 6.8	100 ± 2.6	<i>P</i> < 0.001
Triglyceride (mmol/l)	1.19 ± 0.3	1.40 ± 0.5	NS	1.23 ± 0.2	1.52 ± 0.3	<i>P</i> < 0.001
T. cholesterol (mmol/l)	3.15 ± 0.4	3.57 ± 0.4	<i>P</i> < 0.001	3.47 ± 0.8	3.56 ± 0.3	NS
HDL-cholesterol (mmol/l)	0.75 ± 0.2	0.85 ± 0.2	NS	0.64 ± 0.2	0.78 ± 0.1	<i>P</i> < 0.001
LDL-cholesterol (mmol/l)	1.84 ± 0.4	2.1 ± 0.4	<i>P</i> < 0.005	1.82 ± 0.3	2.30 ± 0.7	<i>P</i> < 0.005
VLDL-cholesterol (mmol/l)	0.47 ± 0.01	0.60 ± 0.07	<i>P</i> < 0.001	0.52 ± 0.05	0.68 ± 0.02	<i>P</i> < 0.001
LPL (μmol/glycerol lib/h/l)	4.12 ± 1.0	3.51 ± 0.2	<i>P</i> < 0.001	3.7 ± 0.4	4.0 ± 0.06	<i>P</i> < 0.001
LCAT (μmol cholesterol lib/h/l)	67.0 ± 2.6	63 ± 0.8	<i>P</i> < 0.001	62 ± 3.0	65 ± 1.4	<i>P</i> < 0.001

LPL lipoprotein lipase, *LCAT* lecithin: cholesterol acyltransferase, *eGFR* estimated glomerular filtration rate, *BMI* basal metabolic index

were significantly lower in male compared with female controls. There were no significant differences in the levels of urea, creatinine, eGFR and HDL cholesterol in the male compared with female controls.

In SCA male patients with proteinuria there were no statistically significant differences observed for age and BMI, but significant increases were observed for urea (*P* < 0.001), creatinine (*P* < 0.001) and triglyceride (*P* < 0.001) compared with the female while significant decreases were observed for eGFR (*P* < 0.001), HDL cholesterol (*P* < 0.001), VLDL cholesterol (*P* < 0.001), LPL and LCAT (*P* < 0.001) compared with the female. The mean activity levels of LPL and LCAT were higher in control males compared with female, but in patients with proteinuria, enzymes activities were significantly lowered in the male compared with female.

The concentration of 24 h urine protein in the SCA male subjects with proteinuria was significantly higher (0.25 g/day; *P* < 0.001) compared with the SCA female patients with proteinuria (0.09 g/day).

Discussion

This study shows sex differences in LCAT and LPL activities in SCA patients both in steady state and proteinuria. The salient observation is that in male SCA controls there was 18.2 and 6.9% increases in the activities of LPL and LCAT respectively when compared with females but in SCA patients with proteinuria there was 8.4 and 5.2% decreases in the male SCA patients compared with females. In other words, the activities of these enzymes were decreased in male subjects with proteinuria; the activities in the female SCA subjects were increased

compared with controls. The metabolism of these enzymes appears to be differently modulated in both male and female with macroalbuminuria. This observed sex differences (women > men with proteinuria) in LCAT and LPL is in agreement with other investigators [23, 24] although their study population were not SCA patients with proteinuria. We earlier reported low LCAT and LPL activities in SCA subjects compared with sickle cell trait (HbAS) and normal haemoglobin (Hb AA) individuals [1]. The reason for this decreased activity was adduced probably to perturbations such as inflammatory mediators that affect their biosynthesis in the liver. Their biosynthesis may be down regulated in SCA patients because of subclinical inflammatory episodes [25] and it may have implications for progression of renal lesion and accelerated atherosclerosis. In macroalbuminuria, the observed sex differences in the activities of these enzymes suggest that the metabolisms of these enzymes are affected differently. Our findings is partially different from that of Magkos et al. [26] in some aspects. They reported that plasma LCAT concentration was 5% lower and LPL 35% higher in women than men in obese individuals, but in lean subjects there was no different in LPL concentration between men and women who were matched on percent body fat. They however recorded 20% lower plasma LCAT in women than men of similar percent body fat. The sex difference in plasma LPL (women greater than men) was adduced to secondary male/female phenotype (greater body fat in women than men). Their observation was based on an earlier report by Jiang et al. [27] that LPL mRNA is highly expressed in mammalian adipose tissue and the positive association between percent body fat and LPL concentration in plasma. In our study there was no significant different in the BMI of the SCA patients.

It was reported that males with non diabetic renal disease exhibit a faster rate of decline in renal function compared with women [1–6]. These sex differences were also observed in the mechanisms underlying renal injury which was linked to sex hormones, suggesting that androgens may permit or accelerate renal damage while estrogen may provide renoprotection [1–6]. This observation was supported by the fact that the incidence and rate of progression of non diabetic renal disease appears to be higher in postmenopausal women compared with age-matched men [28]. Other mechanisms by which women with SCA seems to be protected from developing renal disease compared with men may be an augmented renal haemodynamic response to chronic anaemia which is similar to renoprotection in female type 1 diabetes mellitus. Studies have showed that in humans with type 1 diabetes, examination of the renal haemodynamic responses in early stages of diabetic nephropathy showed that men do not exhibit changes in renal haemodynamics during hyperglycaemia, whereas women exhibit a reduction in effective renal plasma flow and renal flow and decrease renal vascular resistance [28]. This may connote a reduced glomerular capillary pressure in female kidneys compared with male kidneys. The female sex hormone (estradiol) is thought to have antioxidant properties. It was reported that estradiol increases superoxide dismutase and glutathione peroxidase expression and decreases NADPH oxidase enzyme activity and superoxide production [29]. Renal Nitric oxide synthesis (NOS) was reported to be affected in the male whereas no effect on NOS was observed in the female kidney [1]. Stress situations are associated with increased nitric oxide (NO) and reactive oxygen species secretions. Because superoxide reacts with NO in rapid manner, it plays important role in modulating NO levels.

The male SCA patient was a risk factor for the development of macroalbuminuria compared with women. The 24 h urinary protein estimation was greater in the male SCA patients compared with females. The analysis of significance of abnormal albuminuria in SCA, several authors demonstrated by physiologic and pathologic studies that macroalbuminuria may accelerate kidney disease progression and hence a clinical manifestation of an underlying glomerulopathy [18, 30].

But it was postulated that LCAT may play a role in oxPC catabolism [16]. It is capable of metabolizing a variety of oxidized products of PC and also prevent the modification of LDL. In LCAT deficiency, oxidatively modified LDL is readily formed and oxPC preferentially accumulates in glomeruli by unknown mechanisms [16]. Such oxPC cannot be removed by LCAT-deficient plasma, which may ultimately cause renal dysfunction [16]. Although oxPC was not examined in this study, it is suggested that LCAT activity which was increased in the female SCA patients with macroalbuminuria may have

provided some protective effects against the progression of renal disease. The study population is small because this is an extension of the previous study [1], further study in this area is therefore suggested.

This study shows sex differences in lipolytic enzymes activities which are independent of differences in total body fat accumulation between male and female. These altered enzymes may reflect changes in composition and concentration of difference lipoproteins and can also lead to alteration in lipid composition of membrane and may also accelerate renal disease progression in male SCA patients.

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