Alteration of Lecithin: cholesterol acyl-transferase activity correlates with estimated glomerular filtration rate in adult Sickle cell disease patients

Abstract

Background: Glomerular filtration rate is an important clinical indicator of kidney function. Sickle cell disease (SCD) is regarded as a chronic vasculopathy marked by endothelial injury and lipoprotein metabolism may be affected. 

Objective: To correlate the activities of lecithin:cholesterol acyl-transferase (LCAT) and lipoprotein lipase (LPL) in adult SCD patients with estimated glomerular filtration rate (eGFR).

Methods: One hundred and eighty confirmed SCD patients were consecutively recruited for the study. They consisted of 120 males aged: 26.4 ± 2.4 years and 60 females aged 25.4 ± 1.6 years. They were grouped into 3 categories based on their eGFR viz: eGFR >90mL/min/1.73m2; eGFR 60 to 89mL/min/1.73m2 and eGFR <60mL/min/1.73m2. Serum triglyceride and total cholesterol were determined using reagent kits by Randox laboratories, UK, while HDL- cholesterol was determined in the supernatant after precipitation with magnesium chloride/phosphotungstic acid solution. LCAT was measured using the Anasolv LCAT assay kit supplied by DAICCHI chemicals, Japan and LPL was assayed by colorimetric method as previously described.

Results: Serum LPL and LCAT were significantly lower (p<0.001) with decreasing eGFR. The eGFR correlated negatively with urea (r=−0.174;p<0.02), creatinine (r=−0.195;p<0.01) and triglyceride (r=−0.145;p<0.05) while HDL-c (r=0.149;p<0.05), LPL (r=0.148;p<0.05) and LCAT (r=0.209;p<0.005) correlated positively with eGFR.

Conclusions: The activities of LCAT and LPL were lower with decreasing levels of glomerular filtration rate. The importance of adequate lipid and lipoproteins metabolism cannot be overemphasized in the management of SCD patients.

Keywords: Lecithin: cholesterol acyl-transferase, lipoprotein lipase, lipoproteins, sickle cell disease

INTRODUCTION

Glomerular filtration rate is an important clinical indicator of kidney function and may be used as an independent predictor of long-term survival and clinical outcome of several diseases (Jin, R. et al., 2008). It measures the volume of water filtered out of the plasma through the glomerular capillary walls per unit time by the functional units of the kidney. The measurement of endogenous creatinine clearance involves the assay of creatinine levels in serum and urine in addition to 24hours urine volume (Chan, A. K. et al., 2003; & Cockcroft, D.W., & Gault, M.H. 1976). This takes time and can be influenced by muscle mass. To eliminate the associated clinical difficulties estimated glomerular filtration rate (eGFR) was introduced. The eGFR is calculated using several verified equations taking other definable factors (serum creatinine, age, gender, race, serum and/or urine urea and albumin concentrations) in to consideration (Cockcroft, D.W., & Gault, M.H. 1976; Levey, A. S. et al., 1999; & Levey, A.S. et al., 2000). In this study, we have used modified Cockcroft-Gault formula (Cockcroft, D.W., & Gault, M.H. 1976) to calculate eGFR. The eGFR measures the excretery function of the kidneys and may be regarded as the gold standard used to assess renal function. According to the National Kidney Foundation’s guidelines for chronic kidney disease, a GFR >90mL/min/1.73m2 is considered normal; GFR of 60-89 mL/min/1.73m2 is mildly decreased; GFR 30-59 mL/min/1.73m2 is moderately decreased and may indicate renal insufficiency while a GFR 29 mL/min/1.73m2 is considered severe.
regarded as severely decreased and a GFR <15 mL/min/1.73m² is considered kidney failure (National Kidney Foundation. 2002).

Sickle cell disease (SCD) is regarded as a chronic vasculopathy characterized by endothelial injury and the oxidative environment of sickle plasma may be a major contributor to high density lipoprotein-cholesterol dysfunction (Soupene, E. et al., 2016). Abnormalities in lipoprotein metabolizing enzymes in SCD could exacerbate the development of vascular endothelial dysfunction which may be influenced by decreasing renal function in SCD.

Lecithin: cholesterol acyl-transferase (LCAT) and Lipoprotein lipase (LPL) are important enzymes involved in lipid metabolism. Abnormal lipid metabolism is often reported in renal impaired individuals but may not necessarily lead to hyperlipidaemia (Attman, P. O. et al., 1993). The abnormal lipoprotein levels in renal impaired individuals are thought to develop over time during the asymptomatic to advanced stages of the disease. Reduced activity level of LCAT can exacerbate the development and progression of renal disease since the enzyme play vital role in plasma lipoprotein metabolism. Low activity of LCAT in renal impairment can also lead to remarkable alterations in a wide range of lipids and lipoprotein levels (Gillett, M. P. et al., 2001). LCAT binds to HDL to catalyze the conversion of unesterified cholesterol and phosphotidylcholine (PC) to esterified cholesterol and lysophosphatidylcholine (LPC). Sickle cell disease patients are susceptible to the development of glomerulopathy and progressive decrease of glomerular filtration rate leading to severe end-stage-disease (Emokpae, M. A. et al., 2011). We previously reported lower LCAT and LPL activities in SCD patients than subjects with normal haemoglobin (Emokpae, M.A. et al., 2010), sex differences in their activities (Attman, P. O. et al., 1993) and their association with c-reactive proteins in SCD patients (Emokpae, M.A. et al., 2013). Lipoprotein lipase (LPL) is involved in the modulation of fatty acid influx, which is responsible for the breakdown of triglyceride-rich chylomicrons, very low density lipoproteins (VLDL) and intermediate density lipoproteins to liberate free fatty acid and glycerol for uptake in the target tissue. The LPL is functional at the vascular surface of the capillary endothelial anchored by proteoglycan chains of heparin sulphate (Vaziri, N.D., & Parks, J.S. 2001). The present study correlates the activities of LCAT and LPL in adult SCD patients with different eGFR.

**MATERIALS AND SUBJECTS**

**Study Population**

This cross sectional study was conducted at Aminu Kano Teaching Hospital, Kano. One hundred and eighty confirmed sickle cell anaemia (SCA) patients were consecutively recruited from the Sickle cell disease clinic of the hospital. They consisted of 120 males aged: 26.4 ± 2.4 years and 60 females, aged 25.4 ± 1.6 years. They were grouped into 3 categories based on their eGFR viz: eGFR >90mL/min/1.73m² (group A); eGFR 60 to 89mL/min/1.73m² (group B) and eGFR <60mL/min/1.73m² (group C).

**Ethical Consideration:**

The protocol used was approved by the ethics committee of the hospital (code no AKTH/EC/2007/017) and the patients gave informed consent.

**BLOOD COLLECTION AND PREPARATION**

Fasting blood specimens were obtained from the patients and were allowed to clot at 4°C for 30 min. The specimens were centrifuged at 3000 rpm for 10 min to obtain sera. The sera were stored at -20°C until analyses were performed.

**Specimen Analyses:**

Serum triglyceride and total cholesterol were determined using enzymes catalyzed colorimetric method by Randox laboratories, UK, while Friedewald (Friedewald, W. T. 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 (Goldberg, I.J. 1996), 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. The LPL was determined by incubating the serum in glyceryl trioleate substrate, the glycerol liberated was measured in an aliquot of the incubating mixture (Eisenberg, S. 1997; & Anurag, P., & Anuradha, C. V. 2002). The substrate was incubated with 0.2 ml of serum at 37°C for 60 min and 0.1 ml 0.1 NH₄SO₄ 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.

**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 icebath for 8 min with alternating 60s sonication with a 60s 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**

Statistical analysis was performed using GraphPad Prism statistical analysis software (version 6, GraphPad, San Diego CA, USA). Measured parameters were expressed as mean± standard error of mean (SEM). Differences between group mean values were assessed for statistical significance by one-way ANOVA and Pearson correlation coefficient was used to assess the statistical significance of association between eGFR and other measured variables. A p-value was considered statistically significant at P<0.05.

**RESULTS**

Table 1 shows the mean values of measured parameters in SCD patients based on eGFR. The mean age of the subjects increased with decreasing eGFR. The mean age of those with eGFR 60-89mL/min/1.73m² and <60mL/min/1.73m² were significantly higher (p<0.005) than those with eGFR >90mL/min/1.73m². The means eGFR were significantly lower in group B(p<0.01) and group C(p<0.001) compared to group A. Serum urea was significantly higher in group B and group C than group A while creatinine was significantly higher in group C compared to group A and group B. Serum LPL and LCAT were significantly lower (p<0.001) with decreasing eGFR in group B and group C than group A.

Table 2 shows the correlation of eGFR with measured parameters. The eGFR correlated negatively with urea (r=-0.174; p<0.02), creatinine (r=-0.195; p<0.01) and triglyceride (r=-0.145; p<0.05). On the other hand, HDL-c (r=0.149; p<0.05), LPL (r=0.148; p<0.05) and LCAT (r=0.209; p<0.005) correlated positively with eGFR.

**Table 1:** Comparison of measured variables between sickle cell disease patients based on calculated estimated glomerular filtration rate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(Group A) eGFR &gt;90mL/min/1.73m²</th>
<th>(Group B) eGFR 60-89mL/min/1.73m²</th>
<th>(Group C) eGFR &lt;60mL/min/1.73m²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects</td>
<td>144</td>
<td>20</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.3±1.8</td>
<td>27.6±1.6a</td>
<td>28.6±1.7a</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Urea(mmol/L)</td>
<td>2.63±0.9</td>
<td>3.5±0.2b</td>
<td>14.5±0.8b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine(µmol/L)</td>
<td>60.2±2.8</td>
<td>64.0±2.7</td>
<td>492±7.8b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR(mL/min/1.72m²)</td>
<td>101±5.6</td>
<td>76.2±7.2c</td>
<td>14.8±2.0b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.12±0.2</td>
<td>1.10±0.2</td>
<td>1.72±0.04b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T.Chol(mmol/L)</td>
<td>3.25±0.04</td>
<td>3.21±0.20</td>
<td>3.94±0.03b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-c(mmol/L)</td>
<td>0.75±0.02</td>
<td>0.66±0.08</td>
<td>0.60±0.02c</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL-c(mmol/L)</td>
<td>1.92±0.02</td>
<td>1.80±0.04a</td>
<td>1.82±0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LPL(µmol glycerol lib/L)</td>
<td>3.76±0.02</td>
<td>3.54±0.05d</td>
<td>2.98±0.04b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LCAT(µmol cholesterol lib/L)</td>
<td>62.9±1.3</td>
<td>60.1±0.5b</td>
<td>55.0±0.6b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

eGFR=estimated glomerular filtration rate; TG=triglyceride; T.Chol= total cholesterol; HDL-c=high density lipoprotein cholesterol; LDL-c=low density lipoprotein cholesterol; LPL=lipoprotein lipase; LCAT= lecithin:cholesterol acyltransferase; Level of significance (a=0.005; b=0.001; c=0.01; d=0.05).
Table 2: Association of measured variables with estimated glomerular filtration rate in Sickle cell disease patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR/urea</td>
<td>-0.174</td>
<td>0.02</td>
</tr>
<tr>
<td>eGFR/creatinine</td>
<td>-0.195</td>
<td>0.01</td>
</tr>
<tr>
<td>eGFR/triglyceride</td>
<td>-0.145</td>
<td>0.05</td>
</tr>
<tr>
<td>eGFR/total cholesterol</td>
<td>0.121</td>
<td>0.10</td>
</tr>
<tr>
<td>eGFR/HDL-c</td>
<td>0.149</td>
<td>0.05</td>
</tr>
<tr>
<td>eGFR/LDL-c</td>
<td>0.126</td>
<td>0.10</td>
</tr>
<tr>
<td>eGFR/lipoprotein lipase</td>
<td>0.148</td>
<td>0.05</td>
</tr>
<tr>
<td>eGFR/lecithin:cholesterol acyl-transferase</td>
<td>0.209</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table: Association of measured variables with estimated glomerular filtration rate in Sickle cell disease patients

Dysfunctional cholesterol metabolism have been reported in SCD patients (Emokpae, M. A. et al., 2011; Emokpae, M.A. et al., 2010; Emokpae, M.A. et al., 2013; Emokpae, M. A. et al., 2010; Emokpae, A. M., & Kuliya-Gwarzo, A. 2014; Mathias Emokpae, A. et al., 2010; Monnet, D. et al., 1996; Shores, J. et al., 2003; & Zorca, S. et al., 2010). But known has associated the activities of LCAT and LPL with changes in eGFR. The alterations in the levels of lipoproteins in SCD patients are reflections of the low activities of HDL-bound proteins such as LCAT and LPL which is functional at the vascular surface of capillary endothelial anchored by proteoglycan chains of heparin sulphate (Soupene, E. et al., 2016; & Homan, R. et al., 2013). The lower LCAT and LPL activities with decreasing eGFR may be attributed to disturbances such as high levels of inflammatory mediators (Subbaiah, P. V. et al., 1985; Subbaiah, P. V. et al., 1982; Subbaiah, P. V. et al., 1980; & Albers, J. J. et al., 1981) that affect their synthesis in the liver, increased excretion and structural defects (Soupene, E. et al., 2016) in the enzymes. The down-regulation of their synthesis is due to inflammatory events (Lopez-Ruiz, A. et al., 2008) and the impact of progressive renal injury which could exacerbate atherosclerosis (Emokpae, M. A. et al., 2011). It was reported that LCAT plays important role in oxidized PC catabolism, transforming various oxidized products of PC and preventing oxidation of LDL-c. But in LCAT deficiency, oxidatively modified LDL is formed and oxPC accumulate in the glomeruli by unknown mechanisms (Vaziri, N. D. 2008). These modified products cannot be removed by LCAT-deficient plasma thereby leading to progressive renal dysfunction (Vaziri, N. D. 2008).

The LCAT activity depends on the presence of apo A-1 (cofactor) that facilitates free cholesterol uptake by HDL particles from the peripheral tissues and maturation of lipoproteins poor HDL₃ to cholesterol ester rich HDL₂ particles (Glomset, J. A. et al., 1980). The acyl-CoA independent acyl-transferase LCAT can produce different acylated products depending on the presence or absence of Apo A-I or LDL-c (Subbaiah, P. V. et al., 1985; Subbaiah, P. V. et al., 1982; & Subbaiah, P. V. et al., 1980). If Apo-A-I is present for example, LCAT esterifies cholesterol by transferring the Sn-acyl group to PC to generate cholesterol ester but if a lipophilic acyl acceptor is absent, it transfers the acyl group to water and acts like a PC hydrolyzing phospholipase A₂. On the other hand, the presence of LDL, the enzyme can transfer an acyl group from PC to LPC. However, LCAT is mainly bound to HDL particles in the plasma and LCAT activity has been reported to positively correlates with total cholesterol in individuals with normal haemoglobin (Albers, J. J. et al., 1981). Some authors observed a positive association between plasma LCAT activity and plasma total cholesterol in SCD but no significant association was reported between LCAT activity and total cholesterol in normal haemoglobin (Monnet, D. et al., 1996; Shores, J. et al., 2003; & Zorca, S. et al., 2010) Soupene et al., (2016) suggested that the lower LCAT activity observed in SCD may be as result of combination of lower proteins concentrations and structural damage that occurred in SCD.

Low LPL activity may alter free fatty acid distribution and regulates the component and concentration of plasma lipids. Low LPL activity observed with decreasing eGFR is consistent with previous studies (Lechleitner, M. 2000; Kwan, B. C. et al., 2007; & Cavigiolio, G. et al., 2010). This alteration may be due to depletion of enzyme pool, down regulation of biosynthesis in the liver. Others have attributed the low levels to increased plasma apoC-III/apoC-II ratio and the presence of some lipase inhibitors which accumulate with decreasing GFR.
ApoC-II is an activator of LPL while apoC_III is an inhibitor of the enzyme (Emokpae, M.A. et al., 2013). Recent studies have observed accelerated atherosclerosis in renal impairment and failure of Statins in advanced stages of renal disease are not due to the traditional cardiovascular risk factors but HDL dysfunction which play prominent role in the set-up of cardiovascular disease in renal disease. In conclusion, LCAT and LPL activities were lower with decreasing levels of glomerular filtration rate. The importance of adequate lipid and lipoproteins metabolism cannot be overemphasized in the management of SCD patients.

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Conflicts of interest: None declared.

REFERENCES


