Association of Plasma Cholesteryl Ester Transfer Protein Activity with Severity of Angiographically Confirmed Coronary Artery Disease

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ABSTRACT

Background: Cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein has a key role in high-density lipoprotein cholesterol (HDL-C) metabolism. It transfers cholesteryl esters from HDL to apolipoprotein-B containing particles in exchange for triglycerides, thereby reducing the concentration of HDL-C and increasing non-HDL-C, a lipoprotein distribution predisposing to atheroma formation. Plasma CETP activity has been associated with plasma HDL-C concentrations.

Objectives: To determine the association of plasma CETP activity and its associated plasma lipoproteins concentration with angiographically confirmed Coronary artery disease (CAD).

Materials and Methods: In this case-control study, we analysed the plasma CETP activity in 146 patients with angiographically proven Coronary atherosclerosis and 145 non-cardiac cases as control subjects. Plasma CETP activity was determined by fluorometry and serum lipoproteins were estimated by routine enzymatic end point methods using an autoanalyser.

Results: Patients with CAD had significantly high plasma CETP activity (90.72±15.83 pmol/μL/hr) than control subjects (65.23 ± 12.23 pmol/μL/hr, P=0.000). CAD patients with single, double or triple vessel disease had significantly high CETP activity when compared to control subjects. Significantly lower HDL-C (38.5+9.7 mg/dl versus 48.2+9.9 mg/dL, P=0.000) was observed in Coronary atherosclerosis patients than control subjects.

Conclusion: Our findings indicated that the plasma CETP activity was significantly associated with severity of Coronary artery disease and CETP activity may be an independent risk factor for Coronary atherosclerosis.

Keywords: Cholesteryl ester transfer protein, High density lipoprotein, Coronary artery disease

INTRODUCTION:

Coronary artery disease (CAD) has become a major public health problem in many developing countries.¹,² CAD is a multifactorial disease caused by genetic and environmental factors.³ Lipoproteins play a central role in the development of atherosclerotic cardiovascular disease in humans. The protective role of high density lipoprotein cholesterol (HDL-C) against atherosclerosis is well established.⁴,⁵ Numerous genetic, hormonal and environmental factors determine HDL-C levels within distinct populations. The cholesteryl ester transfer protein (CETP) plays a pivotal role in HDL metabolism.⁶,⁷ Cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein composed of 476 amino
Materials and Methods:

The study sample comprised 146 unrelated Coronary artery disease patients (131 male, 15 female) of Mean age 50.82 ± 9.3 years. Inclusion criteria were more than 50% stenosis of atleast one of the major coronary arteries. Patients with minimal obstruction were excluded. Patients with recent episode of Myocardial infarction (Less than 3 months) were also excluded. Controls were 145 non-cardiac cases and were recruited from outpatient department. Cases and controls were matched for Age, Sex and other confounding factors like diabetes, hypertension, smoking, alcoholism. For all diabetic controls, tread mill test was done. Only those with negative Tread Mill Test result were included in the study. This case-control study was done after obtaining the approval from Institutional Ethical Committee and informed consent was obtained from all the study participants.

Recumbent blood pressure and 12 lead ECG were recorded on each subject after a thirty minute rest on the couch. Height and weight were recorded and blood samples were collected by venipuncture after overnight fasting in two test tubes. One was collected into plain non-additive tube for lipid profile estimation and the other anticoagulated with EDTA for estimation of CETP activity.

Laboratory analysis - Lipid profile was measured by enzymatic methods with an auto analyser (XL 300) and manufacturers Agent kits. Serum total cholesterol was estimated by Esterase Oxidase method and triglycerides by colorimetric enzymatic method. High Density Lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C) were estimated by Immunoinhibition method which is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the

In view of this we have evaluated the association of Cholesteryl ester transfer protein activity and the associated serum lipid levels with coronary artery disease.
improvements in using optimized quantities of PVS/PEGME and selected detergents.

Plasma CETP activity was assayed by fluorometric method using BioVision Inc, Mountain View, CA assay kit. This kit includes donor and acceptor molecules. The fluorescent neutral lipid is present in a self-quenched state when contained within the core of the donor. The CETP-mediated transfer is determined by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the self-quenched donor to the acceptor. The reaction mixture is incubated for 1 hour at 37°C. The samples are diluted to 500 μL with 1X CETP assay buffer and read in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm. A standard curve was used, according to the manufacturer's guidelines, to derive the relation between fluorescence intensity and mass transfer.

**Statistical analysis:**

Age, BMI, serum lipid levels and plasma CETP activity were compared between control subjects and patients by Student's t test, p <0.05 was considered statistically significant. ANOVA was used to test for differences in mean levels of Total cholesterol, LDL cholesterol, HDL cholesterol, Triglyceride and plasma CETP activity between patients with single, double, triple vessel disease and controls. Logistic regression analysis was used to evaluate the interaction between the variables in relation to the prevalence of Coronary artery disease. Independent variables included in the analysis were Age (quantitative), Sex (male/female), Smoking (yes/no), Alcoholism (Yes/No), Hypertension (Yes/No), Diabetes (Yes/No), Serum Levels of Cholesterol, Triglycerides (Quantitative). The analysis was executed by SAS Statistical program Version 6.10 for Macintosh. Plasma CETP activity level and HDL-C levels were correlated by Pearson's Correlation analysis. Relationship between the number of Coronary arteries and the plasma CETP activity was assessed by Spearman's Rank Correlation analysis.

**RESULTS:**

Table-1 shows Age, Sex, BMI, Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein levels, CETP activity and conventional risk factor distribution among patients and control subjects. Since all the confounding factors were matched there were no significant differences between the two groups. There was a significant difference in the Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein levels and plasma CETP activity among patients and control subjects.

Table-2 shows Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein levels and plasma CETP activity among controls, single, double and triple vessel disease patients. There was a significant difference in the Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein levels and plasma CETP activity among groups.

Table-3 shows the correlation between High Density Lipoprotein levels and plasma CETP Activity. There was a strong negative correlation between High Density Lipoprotein levels and CETP activity among cases and controls.

Table-4 shows the correlation between plasma CETP activity and the number of vessels stenosed. Single vessel disease was ranked 1, double vessel disease 2, Triple vessel disease 3. There was a positive correlation between activity and the rank. i.e., as the activity increased number of vessels affected increased.

On multivariate analysis (Table 5), we obtained a
significant positive correlation coefficient for plasma CETP activity i.e., increase in the CETP activity would increase the possibility of atherosclerosis, whereas there was a significant negative correlation coefficient for HDL levels i.e., an increase in HDL levels decreased the possible occurrence of atherosclerosis.

**Table 1: Characteristics of patients with CAD and of controls**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case</th>
<th>Control</th>
<th>P Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.82 ± 9.3</td>
<td>50.81 ± 8.8</td>
<td>0.99</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>131 (89.7%)</td>
<td>128 (88.3%)</td>
<td>0.99</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>15 (10.3%)</td>
<td>17 (11.7%)</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>48 (31.1%)</td>
<td>46 (34.9%)</td>
<td>0.83</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>61 (48.8%)</td>
<td>64 (51.2%)</td>
<td>0.69</td>
<td>NS</td>
</tr>
<tr>
<td>DM-HT</td>
<td>23 (15.8%)</td>
<td>20 (13.8%)</td>
<td>0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>87 (54.5%)</td>
<td>74 (46.6%)</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>66 (32.8%)</td>
<td>59 (47.2%)</td>
<td>0.44</td>
<td>NS</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25.36 ± 2.25</td>
<td>24.99 ± 3.14</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>180.9 ± 76.7</td>
<td>159.9 ± 23.2</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>161.6 ± 42.8</td>
<td>128 ± 27.6</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>High Density lipoprotein (mg/dL)</td>
<td>38.5 ± 9.7</td>
<td>48.2 ± 9.9</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Low Density lipoprotein (mg/dL)</td>
<td>106 ± 25.3</td>
<td>82.1 ± 25.1</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Plasma CETP activity (pmol/μL/hr)</td>
<td>90.72 ± 15.83</td>
<td>65.23 ± 12.23</td>
<td>0.000</td>
<td>S</td>
</tr>
</tbody>
</table>

DM: Diabetes mellitus, HT: Hypertension, CETP: Cholesteryl ester transfer protein, S: Significant, NS: Not significant

**Table 2: Comparison of biochemical parameters in patients with Single, Double, Triple vessel disease and Controls**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control n=145</th>
<th>Single vessel disease n=55</th>
<th>Double vessel disease n=41</th>
<th>Triple vessel disease n=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>159.9 ± 23.23</td>
<td>177.6 ± 27.93</td>
<td>179.5 ± 28.9</td>
<td>185.5 ± 23.2</td>
<td>0.000 - S</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>128 ± 27.62</td>
<td>162.85 ± 39.41</td>
<td>165.01 ± 42.6</td>
<td>157.44 ± 47</td>
<td>0.000 - S</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>48.22 ± 9.92</td>
<td>39.64 ± 8.82</td>
<td>38.94 ± 9.7</td>
<td>37 ± 10.44</td>
<td>0.000 - S</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>82.07 ± 25.11</td>
<td>101.42 ± 25.53</td>
<td>103.62 ± 25.77</td>
<td>113.62 ± 23.4</td>
<td>0.000 - S</td>
</tr>
<tr>
<td>CETP Activity (pmol/μL/hr)</td>
<td>65.23 ± 12.23</td>
<td>83.7 ± 14.14</td>
<td>89.63 ± 14.8</td>
<td>99.82 ± 14.17</td>
<td>0.000 - S</td>
</tr>
</tbody>
</table>

HDL: High Density lipoprotein, LDL: Low Density lipoprotein, CETP: Cholesteryl ester transfer protein, S: Significant

**Table 3: Correlation between High Density Lipoprotein and CETP activity**

<table>
<thead>
<tr>
<th>Group</th>
<th>HDL Pearson correlation (mg/dL)</th>
<th>CETP activity (pmol/μL/hr)</th>
<th>N</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>- .716**</td>
<td>145</td>
<td>.000</td>
</tr>
<tr>
<td>CEP Activity Pearson correlation (pmol/μL/hr)</td>
<td>- .716**</td>
<td>1</td>
<td>145</td>
<td>.000</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).**

HDL: High Density lipoprotein, CETP: Cholesteryl ester transfer protein

**Table 4: Correlation between CETP activity and number of vessels stenosed**

<table>
<thead>
<tr>
<th>Kendall's tau b</th>
<th>CETP Activity (pmol/μL/hr)</th>
<th>Correlation Coefficient (Sig, 2-tailed)</th>
<th>N</th>
<th>Angiography Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>291</td>
<td>1.000</td>
</tr>
<tr>
<td>Angiography</td>
<td></td>
<td></td>
<td>146</td>
<td>1.000</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).**
controls (65.23±12.23 pmol/μL/hr). P value was 0.000. The HDL cholesterol levels are decreased in cases (38.5 +9.7 mg/dL) when compared to controls (48.2+9.9mg/dL). P value was 0.000, suggesting that the high CETP activity is associated with low HDL cholesterol concentration and this low HDL makes a person more susceptible to atherosclerosis. Hence high plasma CETP activity and the resultant low HDL cholesterol can be considered as independent risk factors for atherosclerosis. Several studies have reported an association between CETP activity and HDL cholesterol concentration and also with the results of the Framingham Study. 24

When the CETP activity was compared between single, double and triple vessel diseased patients there was a significantly high CETP activity among triple vessel disease when compared to double vessel (99.82+14.17 pmol/μL/hr Vs 89.03+14.8 pmol/μL/hr) and single vessel (83.7+14.14pmol/μL/hr) disease patients. P value was 0.000 showing that the CETP activity was associated with the severity of coronary artery disease.

The evidence available showed that there was a significantly low HDL cholesterol concentration among triple vessel disease patients (37+10.44 mg/dL) when compared to control (38.94+9.7mg/dL) and single vessel (39.64+8.85mg/dL) disease patients. P value was 0.000.

Strong epidemiological evidences show that the risk of cardiovascular disease is inversely related to the plasma HDL cholesterol concentration. HDL exerts its cardio protective function through a process called reverse cholesterol transport (RCT), in addition to anti-inflammatory and antioxidative
effects. RCT describes a metabolic pathway initiated by HDL-mediated efflux from peripheral tissues and subsequent delivery to the liver. The CETP mediates the exchange of cholesteryl ester from HDL-C to triglyceride-rich lipoproteins. Thus, the CETP decreases HDL cholesterol which increases the risk of CAD.

The cause for increased CETP activity observed in the CAD patients could be due to polymorphism in the CETP gene. Studies have shown that individuals with CETP TaqIB polymorphism and 5′ promoter region base changes like −629C>A were found to be associated with increased CETP activity.

The plasma CETP activity correlated negatively with high density lipoprotein levels among both cases and controls. Plasma CETP activity and the number of vessels blocked were found to be strongly positively correlated that is increase in the activity is associated with an increase in the number of vessels blocked. So it has got a poor prognostic value also.

On multivariate analysis, after adjusting other variables, the plasma CETP activity was significantly associated with CAD. The positive correlation coefficient for plasma CETP activity and LDL cholesterol indicates that the high plasma CETP activity and high LDL cholesterol will increase the susceptibility of atherosclerosis. The negative correlation coefficient for HDL cholesterol indicates that high HDL level is protective.

CONCLUSION:

In conclusion, we have examined the association of the plasma Cholesteryl Ester Transfer Protein activity with HDL levels and with CAD patients. We have found a significant association of high Cholesteryl Ester Transfer Protein activity with low HDL levels and with severity of CAD. The high plasma Cholesteryl Ester Transfer Protein activity may be an independent risk factor for Coronary Atherosclerosis.

LIMITATIONS OF THE STUDY:

Further studies are required in this regard with a larger sample, so that the significance of the study could be increased.

REFERENCES:


