Role of Lecithin Cholesterol Acyl Transferase Activity and Apolipoprotein A-1 Level in Type 2 Diabetes

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ABSTRACT

Aims: Role of Lecithin Cholesterol Acyl Transferase Activity and Apolipoprotein A-I Level In Type 2 Diabetes

Background: Diabetes mellitus is a group of metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes continues to be one of the most common and important public health crises worldwide. LCAT secreted by the liver and enzyme producing most plasma cholesteryl esters and a key participant in the process of reverse cholesterol transport. The protein associated with lipoproteins, called Apolipoproteins. Apolipoproteins activates enzymes important in lipoprotein metabolism and act as legends for cell surface receptors. This study therefore aims to determine the activity of LCAT & APO A-I among diabetes mellitus patients.

Methods: forty patients (Age range 25-70 years) with type 2 diabetes and forty patients (Age range 25-70 years) age-matched controls were studied. LCAT activity was assessed by measuring the difference between esterified and free cholesterol. Determination of free and esterified cholesterol was done by using digitonin precipitation method. Apolipoprotein A-I was measured by immune turbidemetric method using semi auto analyzer. HDL cholesterol level was measured by CHOD-POD method.

Results: In this study, Total cholesterol, LDL cholesterol, triglyceride and small, dense lipoprotein cholesterol were all significantly higher in diabetes patients than controls except HDL compared to control subjects. It also shows that there were significantly decreased LCAT activity & Apolipoprotein A-I level in type 2 diabetes subjects as compared to control subjects.

Conclusion: In the Present study, we found strong correlation of LCAT activity and Apolipoprotein A-I with type 2 diabetes mellitus. Thus, decreased concentration of LCAT activity may be due to glycation of LCAT, due to this, undesirable structural changes occurs in HDL, that reduced the functional capacity of HDL in type 2 diabetes mellitus patients and it also contributes in the development of atherosclerosis in detected type 2 Diabetes Mellitus.

Keywords: Diabetes Mellitus, LCAT, Apolipoprotein A-I, Reverse Cholesterol Transport (RCT).

1. INTRODUCTION

Diabetes Mellitus (DM) had long been recognized as rapidly emerging global health problem that threatens to reach pandemic levels by 2030, the number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030. [¹]

Diabetes mellitus is a group of metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is
associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Type 2 diabetes (T2DM) accounts for >90% of cases and is characterized by insulin resistance, often accompanied by relative insulin deficiency.

Type 2 diabetes continues to be one of the most common and important public health crises worldwide. It has been estimated that the global health expenditure on diabetes is at least $376 billion in 2010 and will be $490 billion in 2030. Type 2 diabetes is a major risk factor for cardiovascular disease (CVD). Patients with type 2 diabetes have a 2–4 times higher risk of CVD mortality than those without diabetes. CVD accounts for approximately 70% of death among patients with type 2 diabetes. A report published by the World Health Organization (WHO) stated that the three countries with the most T2DM patients are India (With 31.7 million in 2000 and 79.4 million in 2030), China (20.8 million in 2000 and 42.3 million in 2030) and the USA (17.7 million in 2000 and 30.3 million in 2030).

Lecithin cholesterol acyl transferase (LCAT) is mainly, secreted by the liver. It is the enzyme producing most plasma cholesteryl esters and a key participant in the process of reverse cholesterol transport. Lecithin cholesterol acyl transferase (LCAT) is a key enzyme for the production of cholesteryl esters in plasma and promotes the formation of high density lipoprotein (HDL). LCAT was proposed to promote the Reverse Cholesterol Transport (RCT), the anti atherogenic mechanism by which excess cholesterol is removed from cells by HDL and delivered to the liver for excretion.

LCAT, a lipoprotein-associated enzyme, is a key player in the RCT pathway, which promotes the transfer of excess cellular cholesterol from peripheral tissues to the liver for excretion. It is generally accepted that LCAT activity is necessary for the formation of mature high density lipoprotein (HDL) and for remodeling of HDL lipoprotein particles. LCAT deficiency represents another rare, recessive genetic disorder that underlies HDL deficiency. LCAT is a plasma enzyme that esterifies free cholesterol, primarily at the surface of the HDL particle, after which the cholesteryl ester molecules migrate to the inner core of this lipoprotein. Through this action, LCAT plays a key role in the maturation of HDL particles.

Atherosclerosis is an important complication of diabetes mellitus. This is a multi-step process and includes the uptake of free cholesterol (FC) by HDL from cell membranes, the esterification of FC at the HDL surface by LCAT, which is mainly activated by apolipoprotein (apo) A-I. HDL plays a central role in reverse cholesterol transport because it not only promotes the efflux of cholesterol from peripheral tissues but is also the major site for the esterification of cholesterol by LCAT. The activity modulates cholesterol transfer from lipoproteins and cell membranes to HDL. Therefore, decreased activity of LCAT promotes the accumulation of free cholesterol at cell membranes, and of remnant lipoprotein in plasma, both factors being strongly related to atherosclerosis.

Apo (a) is polymorphism glycoproteins that contain repeating domains of varying length that are homologous to kringle IV of plasminogen. The protein associated with lipoproteins, called Apolipoproteins, is required for the assembly, structure and function of lipoproteins. Apolipoproteins activates enzymes important in lipoprotein metabolism and act as legends for cell surface receptors. ApoA-I, which is synthesized in the liver and intestine, then secreted into the plasma and lymph, is found on virtually all HDL particles. Apo A-I is a single polypeptide containing 243 amino acids, derived from a precursor, pre-a. Cardiovascular disease (CVD) continues to be the leading cause of
morbidity and mortality in subjects with diabetes. The major lipoprotein constituents of HDL are apolipoprotein (apo) A-I and A-II. As apo A-I has cardioprotective properties.\textsuperscript{[17]}

2. MATERIALS AND METHODS

2.1 Sources of the data

The study was carried out in age group of 25-70 years (both male and female diabetic) visiting medicine out Patient department of MGM Hospital, Navi mumbai. The present study is the Cross - Sectional study, carried out in the department of Biochemistry and department of Medicine, MGM Medical College, Navi Mumbai study duration between period of February 2015 to February 2016.

2.2 Sample Collection

After 12 hours overnight fast, 6.0 ml of blood was collected from each subject by venipuncture with standard blood collection technique in a plane vial for serum separation, sodium fluoride vial for plasma and EDTA vial for HbA1c estimation. Plasma was collected again after two hours of post meal for the postprandial glucose estimation.

2.3 Inclusion and Exclusion criteria

Inclusion criteria:
- Age: 25-70 years (both male and female diabetic).
- Patient diagnosed with Diabetes Mellitus as per WHO criteria.

Exclusion criteria:
- Patients with hypolipidemia were excluded.
- Patients with known case of hypothyroidism, Cushing’s syndrome.
- Patients with kidney disease, hepatic diseases.
- Patients with type 1 Diabetes Mellitus were also excluded.
- Patients < 25 years age or with known / suspected pregnancy.
- Diabetic patients with complications other than obesity will be excluded.

Ethical clearance was obtained from Institutional Ethics Review Committee (IERC). All healthy volunteers were enrolled and a written informed consent was taken. The proforma included, name, age, sex, dietary habit, personal history of disease (if any), smoking habit, drinking habit, socio-economic status and occupation.

2.4 Methods

Plasma LCAT activity was estimated initially by following the removal of one substrate, the reduction of free cholesterol, after incubation of the plasma at 37 °C. Free cholesterol was estimated after Digitonin-precipitation by the colorimetric method.(1960).\textsuperscript{[18]}

Quantitative determination of APO-A1 was done by an Immunoturbidimetric method, by semi- automatic analyzers. This method was based on the reaction of a sample containing human Apo AI and specific antiserum to form an insoluble complex which can be measured turbidimetrically at 340nm. By constructing a standard curve from the absorbance of standards, the concentration of Apo AI can be determined.\textsuperscript{[19]}

Reagents: The above APO A1 reagents were available as kits Ben - Biochemical Enterprise S.r.l. - via Toselli, Milano, Italy. Estimation of serum Cholesterol, serum Triglyceride & serum HDL was done by CHOD – POD method, GPO-POD Method & CHO-POD Method respectively. VLDL and LDL were calculated by Friedewald’s Formula.

The assays were performed according to the manufacturers’ instructions.

2.5 Statistical Analysis

Results were statistically analyzed by ‘GraphPad QuickCals t-test calculator’. The results were further subjected to students ‘t’ test for Comparisons between the groups and further expressed as mean ± SD. A ‘p’ value of less than 0.001 was considered significant.
3. RESULTS

Table 1: Comparison of LCAT and Apolipoprotein A-I in Control and Diabetes Group

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONTROL MEAN ±SD</th>
<th>DIABETES MEAN ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCAT (mg/dl)</td>
<td>93.15±5.13</td>
<td>57.34 ± 10.37</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dl)</td>
<td>124.23±17.31</td>
<td>38.31±13.05</td>
</tr>
</tbody>
</table>

*p≤0.05 Significant, **p≤0.001 Highly Significant, # p > 0.05

The result indicates that there was a highly significant decrease of LCAT activity and Apolipoprotein A-I level in diabetes group as compared to Control group.

Table 2: Comparison of diabetic parameters level in Control and Diabetes Group

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONTROL MEAN ±SD</th>
<th>DIABETES MEAN ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. Cholesterol (mg/dl)</td>
<td>178.6±30.60</td>
<td>204.77 ±51.55*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>131.05±27.87</td>
<td>162.13±73.33</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41.91±6.71</td>
<td>34.77±10.18</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>110.69±29.20</td>
<td>131.98±47.10*</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>91.30 ±9.17</td>
<td>161.52 ±51.52*</td>
</tr>
<tr>
<td>Post prandial glucose (mg/dl)</td>
<td>127.8±10.91</td>
<td>258.51 ±75.68*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.1525±0.21</td>
<td>9.30±1.58</td>
</tr>
</tbody>
</table>

*p≤0.05 Significant, **p≤0.001 Highly Significant, # p > 0.05

The result indicates that there was a highly significant increase of T cholesterol, TG, LDL, Fasting glucose, Post Prandial Glucose and HbA1c level in diabetes group as compared to Control group.

Table 3: Correlation of LCAT with various parameters

<table>
<thead>
<tr>
<th>S.N</th>
<th>PARAMETERS</th>
<th>CONTROL (r value)</th>
<th>PATIENTS (r value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>HbA1c</td>
<td>-0.142</td>
<td>-0.202</td>
</tr>
<tr>
<td>2.</td>
<td>T Cholesterol</td>
<td>-0.134</td>
<td>-0.110</td>
</tr>
<tr>
<td>3.</td>
<td>TG Glycerides</td>
<td>-0.126</td>
<td>-0.269</td>
</tr>
<tr>
<td>4.</td>
<td>HDL</td>
<td>0.162</td>
<td>0.307</td>
</tr>
<tr>
<td>5.</td>
<td>LDL</td>
<td>-0.094</td>
<td>-0.110</td>
</tr>
<tr>
<td>6.</td>
<td>Apolipoprotein A-I</td>
<td>0.161</td>
<td>0.102</td>
</tr>
</tbody>
</table>

*p≤0.05 Significant, **p≤ 0.001 Highly Significant, #p > Not Significant

Graph 1: Mean level of LCAT levels in control and Diabetes group

Graph 2: Mean level of Apo AI in control and Diabetes group

Graph 3: correlation values between LCAT & Apo A-I in control group

Graph 4: correlation values between LCAT & Apo A-I in diabetic group

4. DISCUSSION

Diabetes is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from insufficient action of insulins. [20] Diabetes
mellitus is the most common cause of macrovascular and microvascular complications, posing a huge international health burden. [21]

The aim of our study was to find an association between LCAT, Apolipoprotein A-I and lipid profile, BMI, and HbA1c in healthy control and type 2 diabetic subjects. Ethical clearance was granted by the scientific and ethical committee of the institution. The study was conducted in MGM Medical College; patients were selected from medicine OPD. Consent was taken before sample collection from the patients. The present study included total 80 subjects, a control group of 40 subjects and study group of 40 subjects diagnosed with Type 2 Diabetes mellitus.

In our study out of 40 patients in each group, there were 17 females and 23 males in healthy group and 15 female with 25 male in diabetic group. In present study, we estimated BMI of both control and type 2 diabetes mellitus patients. We found significantly increased BMI level in diabetes group as compare to control healthy group. (p<0.001)

In Present study, we estimated Fasting & Post Prandial. We found FBS and PPBS level were significantly increased in study groups as compared to control group. (p<0.001) (Table 2)

In Present study, Mean values of HbA1c were 5.15 % and 9.30 % in healthy individual and type-2 diabetic respectively. They were found to be significantly increased in study group as compared to control subjects. (Table 2) (p<0.001)

Our findings are concurrent with Zhang Y et al & Haddadinezhad S et al. Zhang Y et al 2012 found that every 1% increase in GHb was associated with a 15% increase in hazard of all-cause mortality, 25% in CVD mortality, 17% in CVD, 15% in CHD, 17% in fatal CHD, 11% in heart failure, 11% in stroke, and 29% in PVD event their result also suggest that chronic hyperglycemia is associated with increased risks for cardiovascular outcomes and all-cause mortality among patients with type 2 diabetes and independent from other conventional risk factors. [4] Haddadinezhad S et al (2010) showed that increasing of HbA1c has shown more dependency with postprandial plasma glucose as compared to with fasting plasma glucose. [22]

Lipid abnormalities are common in diabetics and frequently seen in type-2 diabetics. Dyslipidaemia make diabetics prone to develop CHD and other complications of atherosclerosis.

In present study, we found highly significant increase in Total cholesterol, Triglyceride level and LDL level in type 2 diabetic group as compare to healthy control. There is significant decrease level of HDL in type 2 diabetic group as compare to healthy group. (Table 2) (p<0.001)

Our study is concurrent with study of Stamouli M et al. Stamouli M et al 2014 found 70.0% of diabetic patients presented at least one lipid abnormality. Elevated LDL-C, elevated Total Cholesterol, elevated TG, and reduced HDL-C levels were noted in 28.37%, 36.37%, 39.01%, and 30.12% of the patients, respectively. The combination of elevated TG and reduced HDL-C was the most prevalent of the combined lipid abnormalities. [23]

In Present study elevated levels of TG, cholesterol, LDL & reduced levels of HDL. This indicates the influence of Type-2 DM patients with lipid abnormalities might cause CVD risk in future.

Our study shows the level of Apolipoprotein AI is highly increases in type 2 diabetic group as compare to control group. (Table 1 and Graph 2)(p<0.001)

Our results are concurrent with Doddamani S et al, Mallick A et al & Gugliucci et al. Doddamani S et al 2015 found that the levels of Apo A-I were significantly decreased (p<0.001) in newly detected type -2 diabetes mellitus in comparison with the control group. [24] Mallick A et al 2011 studies found that decrease in Apo A-I and HDL-C level were observed in poorly controlled diabetic group compared to well controlled diabetic group. This suggests that though the Apo A-I and
HDL-C levels were significantly reduced in diabetics, improvement of glycemic control raises the Apo A-I levels. Gugliucci et al concluded that Apo A-I levels are decreased in type 2 DM. [25] CALVO et al also showed reduced levels of Apo A-I in DM. Thus in newly detected type 2 diabetes mellitus levels of Apo A-I might be reduced. [26]

Apolipoprotein A1 is one of the major protein components of high density lipoprotein (HDL) in plasma. Chylomicrons secreted from the intestinal enterocyte also contain apo A1, but it is quickly transferred to HDL in the bloodstream. The protein promotes fat efflux, including cholesterol, from tissues to the liver for excretion. It is a cofactor for lecithin cholesterol acyl transferase (LCAT) which is responsible for the formation of most plasma cholesterol ester. In present study low levels of Apo A-I in DM might be due to decrease insulin action and protein synthesis that may reduce Apo A-I biosynthesis. [27]

Present shows the level of LCAT activity is significantly decreased in type 2 diabetic group as compare to control group (Table 1 and Graph 1)(p≤0.001). Our results for LCAT are concurrent with the study of Hovingh G et al & Nikam S et al and Hovingh G et al 2005 provide evidence that heterozygotes for LCAT gene defects, who present with an average 36% decrease in HDL-C levels, exhibit an increased risk for atherosclerosis as assessed by intima media thickness. [12]

In type 2 diabetes mellitus there is a glycation of LCAT, due to this structural changes occurs in HDL. These structural changes may reduce the functional capacity of HDL in type 2 diabetes mellitus patients. Thus, the decrease levels of Apo A-I reduce the biosynthesis of HDL cholesterol and glycated LCAT reduce the functional capacity of HDL in type 2 diabetes mellitus. This lowered HDL levels lead to reduced esterification of cholesterol. This lowered esterification of cholesterol might be responsible for higher free cholesterol levels in newly detected type 2 diabetes mellitus patients.

Nikam S et al 2014 found that the correlation between LCAT and Apo A-I was 0.626 in controls and 0.166 in diabetes. It shows that both the variables are positively correlated. They further suggested that reduced APO A-I and LCAT activity is involved in pathogenesis of atherosclerosis in type 2 Diabetes Mellitus. [28]

In type 2 diabetes mellitus, there is structural changes occurs in HDL with decreasing its levels therefore its role in reverse cholesterol transport system is reduced which is in part due to glycosylation-oxidation and desialylation of LCAT, as a result of chronic glycemia. We found low level of LCAT in DM patients that may be due to glycosylation oxidation & desialylation of LCAT due to hyperglycemia that affects the reverse cholesterol transport system. Secondly APO A-I is an activated of LCAT as APO A-I level is decreased that leads to decrease LCAT.

We found LCAT is positively correlated with APO A-I & HDL that indicates hyperglycemia reduces the activity of LCAT, APO A-I, HDL level. LCAT is negatively correlated with Cholesterol, TG, LDL & HbA1c that suggests poor glycemic control causes the elevation of TG, LDL & Cholesterol and reduces the LCAT activity. (Graph 3& 4)

5. CONCLUSION

In our study, we found strong correlation of LCAT activity and Apolipoprotein A1 with type 2 diabetes mellitus. Present study shows that there is increased Total Cholesterol, Triglycerides & LDL levels as well as decreases the HDL Level in type 2 diabetes mellitus as compared to control subjects. Present study shows that there was significantly decreased LCAT activity in type 2 diabetes subjects as compared to control subjects. Thus decreased concentration of LCAT activity may be due to glycation of LCAT, due to this, undesirable structural changes occurs
in HDL. This may reduce the functional capacity of HDL in type 2 diabetes mellitus patients.

Present study also shows significantly decreased level of Apolipoprotein A-I (APO A-I) levels in type 2 diabetes mellitus as compared to control subjects. Thus decreased concentration of APO A-I level may be due to decrease in protein biosynthesis that may reduces APO A-I level.

6. ACKNOWLEDGMENTS
The authors would like to express their sincere gratitude to all the patients who agreed to participate in this study.

7. Competing Interests
The authors declare that we have no conflict of interests to declare.

REFERENCES
15. DANIEL J. Disorder of lipoprotein metabolism, Harrison’s Internal medicine ;17th edition , 2416-17.


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