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Activity of Lecithin Cholesterol Acyl Transferase and Apolipoprotein A-I in Newly Detected Type 2 *Diabetes mellitus*

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Authors' contributions

Author SD performed the practical work, and wrote the first draft of the manuscript. Author SN designed the study and wrote the protocol, author PN managed the analyses of the study. Author AD helped in providing the cases. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: 1. To study the levels of Apolipoprotein A-I and activity of Lecithin cholesterol acyl transferase (LCAT) in newly detected type 2 Diabetes Mellitus.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Biochemistry and Department of Medicine, Belgaum Institute of Medical Sciences (BIMS), Belgaum, Karnataka, India, between November 2011 and June 2013.
Methods: Study included 100 patients (50 men, 50 women, age range 30-60 years) with newly detected type 2 Diabetes Mellitus and 100 age and sex matched healthy participants. 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 immunoturbidemetric method using semi auto analyzer. HDL cholesterol level was measured by CHOD-POD method.

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Results: The mean±SD value of various parameters in newly detected type 2 Diabetes Mellitus was HDL cholesterol(33.37±4.44mg/dl), Apolipoprotein A-I(133.10±24.22mg/dl), and LCAT activity(59±9.86 IU/L), versus HDL cholesterol(48.76±16.84mg/dl), Apolipoprotein A-I(188.72±19.49mg/dl) and LCAT activity (91.74±6.50IU/L) in controls. LCAT activity, Apolipoprotein A-I and HDL levels were significantly ($p < 0.01$) decreased in patients with newly detected type 2 Diabetes Mellitus when compared with healthy participants.
Conclusion: The reduced LCAT activity, Apolipoprotein-A-I and HDL cholesterol may be associated with a reduction in Reverse cholesterol transport(RCT) and contribute to the development of atherosclerosis in newly detected type 2 Diabetes Mellitus.

Keywords: Diabetes mellitus; LCAT; apolipoprotein A-I; HDL.

1. INTRODUCTION

Diabetes mellitus (DM) is a global problem. DM refers to a group of common metabolic disorders that share the phenotype of hyperglycemia [1]. The world wide prevalence of Diabetes Mellitus has risen dramatically over past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. Based on current trends, the International Diabetes Federation project that 438 million individuals will have Diabetes by the year 2030. Although the prevalence of both type 1 and type 2 Diabetes Mellitus is increasing worldwide, the prevalence of type 2 Diabetes Mellitus is rising much more rapidly [2].

One of the dominant characteristics of Diabetes Mellitus is the change in lipoprotein metabolism. In type 2 Diabetes Mellitus, the changes in lipoprotein metabolism play a role in the development of macrovascular complication and are important factors leading to an increase in mortality and morbidity [3].

HDL plays a central role in RCT 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 [4]. Human LCAT is a 416 amino acid glycoprotein circulating in plasma associated with lipids and apolipoproteins in the HDL fraction [5]. LCAT is the enzyme that generates almost all of the cholesterol esters in plasma. It plays a key role in reverse cholesterol transport and is activated by the Apolipoprotein A-I in HDL [6]. It promotes reverse cholesterol transport by maintaining a free cholesterol gradient between HDL and peripheral tissues [7]. There is ample evidence to show that in diabetic patients, not only HDL cholesterol level is decreased but also its role in RCT system is reduced [8]. Hence we planned to study the levels of Apolipoprotein A-I and activity of Lecithin cholesterol acyl transferase (LCAT) in newly detected type 2 Diabetes Mellitus which will give an account of RCT system.

2. MATERIALS AND METHODS

2.1 Source of the Data

The study group was comprised of 100 newly detected type 2 diabetic patients in the age group of 30-60 years visiting medicine Out Patient Department of BIMS Hospital, Belgaum. The diagnosis of Diabetes Mellitus was done by senior physician The diagnosis of type 2 DM was confirmed by measuring FBS (>126mg/dl) and 2hour OGTT (>200mg/dl) values on two occasions as per American Diabetic Association's revised criteria. 100 age and sex matched

healthy participants were taken as control group. All authors hereby declare that the experiments have been examined and approved by institutional ethical committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

2.2 Sample Collection

After obtaining informed written consent, 5ml of blood sample was collected from diabetic patients and the control group under all aseptic conditions. The blood samples were centrifuged and serum samples were used for measuring various parameters. Fresh samples were used for the study, hence there is no time for esterification of cholesterol.

2.3 Methods

LCAT activity was assessed by measuring the difference between esterified and free cholesterol [9]. And in plasma there is no other enzyme for esterification of cholesterol. Hence as per above mentioned reference we concluded that the difference is indirectly proportional to LCAT activity. Determination of free and ester cholesterol was done by using digitonin precipitation method [9]. Apolipoprotein A-I was measured by immunoturbidemetric method using semiautoanalyzer [10]. HDL cholesterol level [11] and Total cholesterol was measured by CHOD-POD method [11]. Triglyceride estimation was done by GPO-PAP method [11]. VLDL and LDL cholesterol was calculated by formula [11]. Fasting blood glucose was measured by Glucose Oxidase Peroxidase method [12].

2.4 Exclusion Criteria

Patients on hypolipidemic drugs, steroids and oral contraceptives were excluded. Known cases of Hypothyroidism, Hyperthyroidism, Cushing's syndrome, kidney diseases, Hepatic diseases and patients with Type 1 Diabetes Mellitus were also excluded.

2.5 Limitations

The duration of diabetes before the formal diagnosis was unknown.

Because of limited resources the direct methods available for measuring LCAT activity could not be used. The LCAT activity was indirectly measured as the difference between esterified cholesterol and free cholesterol.

2.6 Statistical Analysis

The results expressed as mean±SD. The results were further subjected to students't' test for comparisons and differences between means were considered significant at $p< 0.05$.

3. RESULTS AND DISCUSSION

One of the dominant characteristics of Diabetes Mellitus is the change in lipoprotein metabolism. Association of type 2 DM with an increased premature atherosclerosis risk is not clear, but it has been partly attributed to disturbances in RCT. HDL plays a central role in RCT because it not only promotes the efflux of cholesterol from peripheral tissues but is also the major site for esterification of cholesterol by LCAT [13]. LCAT plays a central role in

intravascular HDL metabolism and in RCT. A defect in LCAT function is thus expected to enhance atherosclerosis by interfering with these processes [14].

Present study found that in newly detected type 2 diabetes patients the activity of LCAT was significantly reduced ($p<0.01$) on comparison with the control group (Table 1). Durucan and coworkers found significantly lower LCAT activity in diabetics [15]. A. Ghanei concluded that LCAT activity is considerably lower in diabetics compared with non-diabetics [16]. There is nonenzymatic glycation of LCAT in type 2 diabetes mellitus, concluded by S. Nikam and Suman D [17].

Present study found that on comparison of male and female cases with male and female controls there were significant differences in the lipid parameters (Table 2 and Table 3), LCAT activity, HDL cholesterol levels and apo A-I levels were significantly reduced in diabetic males and females compared to males and females in the control group.

Table 1. Serum parameters in patients and control participants

Sl. no		Newly detected type 2 DM (n = 100)	Controls (n = 100)
1	LCAT (IU/L)	59±9.86*	91.74±6.50*
2	Apolipoprotein-A-I (mg/dL)	133.10±24.32*	188.72±19.49*
3	HDL (mg/dL)	33.37±4.44*	48.76±16.84*
4	LDL (mg/dL)	130.57±36.04*	95.98±39.16*
5	VLDL (mg/dL)	40.38±17.12*	29.74±19.70*
6	Total Cholesterol (mg/dL)	205.43±35.70*	175.07±39.88*
7	Triglycerides (mg/dL)	227.36±106.01*	155.56±107.22*
8	FBS (mg/dL)	156.05±41.14*	74.34±15.09*

*$p < 0.0001$ = Significant, n = Number of subjects

Table 2. Comparison of various serum parameters in male cases and male controls

Sl. no		Newly detected type 2 DM (n = 50)	Controls (n = 50)
1	LCAT (IU/L)	49.61±7.84*	93.00±7.82*
2	Apolipoprotein-A-I (mg/dL)	126.05±21.83*	196.94±21.25*
3	HDL (mg/dL)	28.15±3.75*	42.25±16.42*
4	LDL (mg/dL)	155.68±34.43*	95.62±35.40*
5	VLDL (mg/dL)	48.96±16.10*	34.87±22.57*
6	Total Cholesterol (mg/dL)	238.06±35.45*	171.85±37.08*
7	Triglycerides (mg/dL)	236.72±113.69*	171.09±114.80*

*$p < 0.0001$ = Significant, n = Number of subjects

Present study found that on comparison of female and male cases there were significant differences in the lipid parameters, LCAT activity, HDL cholesterol levels and apo A-I levels were significantly reduced in males compared to females which shows that risk for atherosclerosis complications is more in males compared to female diabetic patients (Table 4).

Table 3. Comparison of various serum parameters in female cases and female controls

Sl. no		Newly detected type 2 DM (n = 50)	Controls (n =50)
1	LCAT (IU/L)	58.38±11.59*	90.19±3.93*
2	Apolipoprotein-A-I (mg/dL)	136.65±26.16*	178.78±10.73*
3	HDL (mg/dL)	34.58±5.06*	56.71±13.75*
4	LDL (mg/dL)	130.47±37.29*	96.43±43.72*
5	VLDL (mg/dL)	38.81±18.13*	23.47±13.23*
6	Total Cholesterol (mg/dL)	209.80±35.76*	179.00±43.16*
7	Triglycerides (mg/dL)	218.00±97.99*	136.58±94.97*

*$p < 0.0001$ = Significant, n = Number of subjects

Table 4. Comparison of various serum parameters in male cases and female cases

Sl. no		Males (n = 50)	Females (n = 50)
1	LCAT (IU/L)	49.61±7.84*	58.38±11.59*
2	Apolipoprotein-A-I (mg/dL)	126.05±21.83*	136.65±26.16*
3	HDL (mg/dL)	28.15±3.75*	34.58±5.06*
4	LDL (mg/dL)	155.68±34.43*	130.47±37.29*
5	VLDL (mg/dL)	48.96±16.10*	38.81±18.13*
6	Total Cholesterol (mg/dL)	238.06±35.45*	209.80±35.76*
7	Triglycerides (mg/dL)	236.72±113.69*	218.00±97.99*

*$p < 0.05$ = Significant, n = Number of subjects

In present study the levels of apo-A-I and HDL cholesterol were significantly decreased ($p<0.01$) in newly detected type 2 Diabetes Mellitus in comparison with the control group (Table 1) Sapna Smith et at. [18] concluded that HDL cholesterol was significantly lower in diabetic subjects as compared to controls. Study by Mall, Vancouver showed that Insulin rapidly activates protein synthesis by activating components of the translational machinery [19]. In Diabetes Mellitus there is decreased insulin action and protein synthesis [20]. These decreased insulin action and protein biosynthesis may reduce apo A-I biosynthesis. Thus in type 2 diabetes mellitus levels of apo A-I are reduced.

In type 2 Diabetes Mellitus there is glycation of LCAT, due to this structural change occurs in HDL [21]. These structural changes may reduce the functional capacity of HDL in type 2 diabetes mellitus patients. Thus the study hypothesized that, the decreased 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 leads 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.

Elevated levels of LDL and VLDL in newly detected type 2 diabetes may be due to decreased levels and functional capacity of HDL Cholesterol (Table 1).

Present study also found that correlation between LCAT and apo A-I was 0.626 in controls (Fig. 1) and 0.166 in diabetics. It shows that both the variables are positively correlated (Fig. 2). Present study suggested that reduced apo-A-I and LCAT activity is involved in pathogenesis of atherosclerosis in type 2 Diabetes Mellitus.

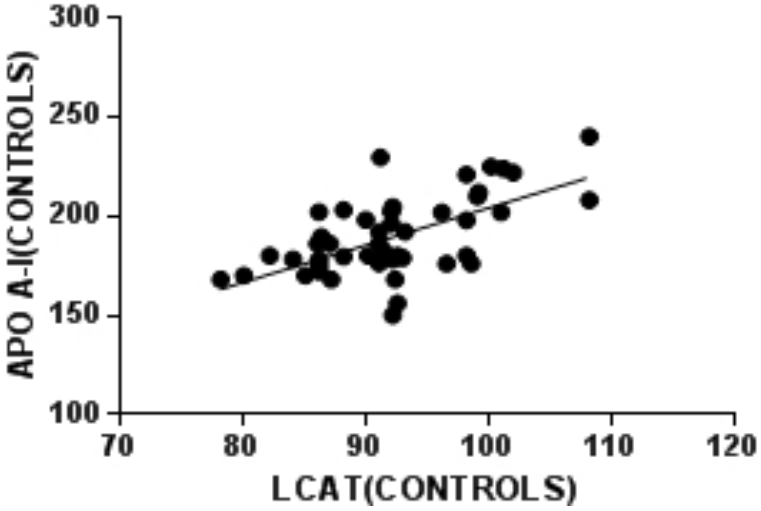

Fig. 1. Correlation between LCAT Activity and Apo A-I in control group

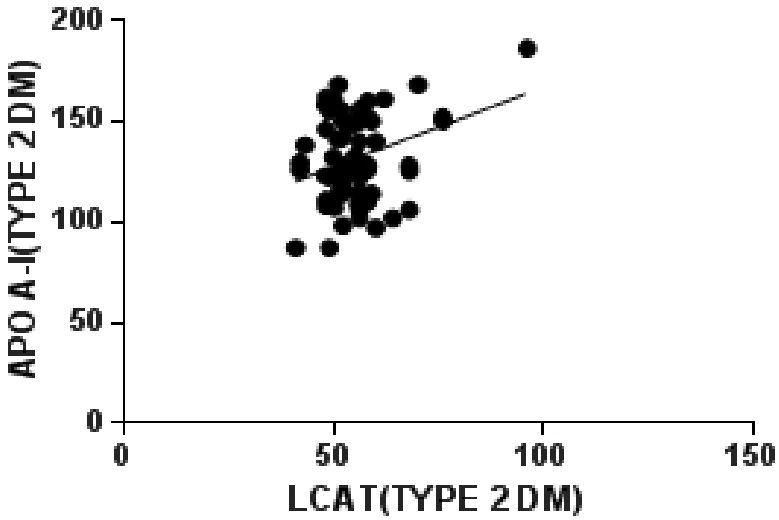

Fig. 2. Correlation between LCAT Activity and Apo A-I in Type 2 DM

5. CONCLUSION

The decreased LCAT activity, Apolipoprotein-A-I levels and HDL may be associated with a reduction in RCT and contribute to the development of atherosclerosis in newly detected type 2 Diabetes Mellitus patients.

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COMPETING INTERESTS

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

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