LECITHIN CHOLESTEROL ACYLTRANSFERASE ACTIVITY IS DECREASED IN TYPE 2 DIABETES MELLITUS

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Abstract- Lecithin cholesterol acyltransferase (LCAT) plays a major role in the removal of free cholesterol from tissues via assisting HDL-C maturation, and its activity has been proposed as the main indicator of HDL-C function. The aim of the study was to measure LCAT activity in type 2 diabetic patients and to elucidate whether LCAT is associated with metabolic control, and insulin resistance. A case-control study was conducted, recruiting 45 type 2 diabetes mellitus patients and 45 healthy subjects. Cases and controls were matched regarding gender, age and body mass index (BMI). FBS, lipid profile, LCAT activity, HbA_{1C}, insulin were measured and insulin resistance (HOMA-IR) was calculated for both patients and controls. The studied variables were then compared between the two groups, and the association of LCAT activity with any of the variables was examined. Twenty-five subjects were female and 20 male both among patients and controls. Mean age of diabetics was 49.9 yrs and of controls 51.1 yrs. FBS, HbA_{1C}, HOMA-IR and TG in patients were significantly higher than controls, and HDL-C in controls was significantly higher than patients. LCAT activity of patients (73 ± 9.1 μmol/L/h) was significantly lower than that in controls (88 ± 4.5 μmol/L/h) ($P < 0.001$). LCAT activity had significant inverse correlations with HbA_{1C} and duration of diabetes. After multilinear regression analysis in patients, LCAT activity was only correlated with HbA_{1C} level (ß= –0.9, $P < 0.001$). LCAT activity had no significant association with HDL-C and HOMA-IR in any of the groups. LCAT activity is significantly decreased in patients with type 2 diabetes compared with healthy controls, and has an inverse correlation with the magnitude of hyperglycemia.

Acta Medica Iranica, 45(6): 481-486; 2007

Key words: LCAT, HDL-C, diabetes mellitus

INTRODUCTION

Dyslipidemia is a prominent feature of type 2 diabetes mellitus (T2DM), which mainly presents as decreased high-density lipoprotein cholesterol (HDL-C), high triglyceride (TG), and small dense low-density lipoprotein cholesterol (LDL-C) which poses a greater risk for atherosclerosis (1). Low HDL-C is a strong risk factor for the development of cardiovascular disease (CVD). It is thought that the cardio-protective role of HDL is at least in part related to its role in reverse cholesterol transport (RCT). RCT is a multi-stage process which includes removal of free cholesterol by HDL from peripheral cells, esterification of free cholesterol by lecithin cholesterol acyltransferase (LCAT) to cholesteryl-ester, and the ultimate delivery of HDL-cholesteryl ester into the liver for metabolism and excretion in the bile or its transfer to apoB-containing lipoproteins (apoB-LP) (2).

LCAT is mainly produced by the liver. This enzyme forms cholesteryl esters on the surface of HDL-C particles, which creates a gradient of unesterified cholesterol from the cell membrane toward the HDL-C particles and thus formation of

Received: 14 May 2007, Revised: 4 Jun. 2007, Accepted: 27 Jul. 2007

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big spherical HDL particles that can be easily recognized by their receptors (2). Hence, the process of formation and maturation of HDL-C particles is dynamic. In this respect, a pure measurement of HDL level in the plasma is not an appropriate method to assess the dynamic process of cholesterol transfer from the peripheral tissues to the liver, neither is it a good indicator of the efficiency of RCT system in the prevention of atherosclerosis (3,4). Instead, the best indicators of HDL-C metabolism and its role in RCT system are the related enzymes, namely, LCAT and paraoxonase (4).

There is bulk of evidence that in diabetic patients, not only HDL-C level is decreased but also its role in RCT system is reduced which is in part due to glycosylation – oxidation – and desialylation of LCAT as a result of chronic hyperglycemia (2,3). Given the pivotal role of LCAT in the removal of cholesterol from peripheral cells, evaluation of its function as a marker of HLD-C competence is of great importance in diabetic subjects. In this study, we measured the activity of LCAT and explored the associated factors in patients with T2DM.

MATERIALS AND METHODS

A case-control study was performed in Diabetes Clinic of Imam Khomeini Hospital (Tehran, Iran) during 2006. Cases were selected from adult clients who had documented FBS levels of 126 mg/dl or above in at least two measurements, and/or who had previous history of T2DM. Controls were selected from healthy adult volunteers with established levels of FBS<126 mg/dl. Informed written consent was taken from each participant.

Initially, all subjects were interviewed for their medical history and were examined. Both in cases and controls, exclusion criteria were set as having: renal failure (Creatinine (Cr) >1.5 mg/dl or GFR < 70 ml/min), proteinuria, history of cigarette smoking, history of hyper- or hypothyroidism, an account of malignancy, liver disease, and use of aspirin, metformin, insulin, lipid lowering drugs, ACE inhibitors or antioxidants (vitamin E or C). A total of 45 subjects with T2DM and 45 healthy persons were enrolled. The patients were under treatment with glibenclamide or diet.

Demographic data, weight, height, waist girth, duration of diabetes (in patients), vital signs including systolic and diastolic blood pressure (measured by a digital set) were recorded for each subject. Patients and controls were matched for gender, age and body mass index (BMI) (P > 0.05).

10 ml venous blood sample was taken from each person following a fasting period of 10-12 hours. After centrifugation, serum samples were stored at −70ºC until analysis. The following biochemical factors were measured for each participant: plasma insulin using IRMA (immunotech, Czchec), FBS, total cholesterol and TG by means of enzymatic colorimetry (Pars Azmoon, Iran), LDL-C via direct enzymatic colorimetry, and Cr using Jaffe reaction (Pars Azmoon, Iran). LCAT activity was measured by fluorescent LCAT assay kit (Roar Biomedical, USA). In this method, the fluorescent labeled LCAT substrate is exposed to and incubated with human serum at 37º C for 4-8 hours. LCAT catalyzes the transfer of a fatty acyl chain to plasma cholesterol. The emission intensities of 390 nm represent the emission of the hydrolyzed substrate (monomer) and those of 470 nm represent the emission of the not hydrolyzed substrate (intact). LCAT activity is measured from the ratio of the two emission intensities (470/390). HbA_{1C} was checked by HPLC(high performance liquid chromatography) method. To determine insulin resistance (IR), Homeostasis Model Assessment (HOMA) index was used as follows: HOMA-IR= FBS (mg/dl) × insulin (mu/L)/405.

SPSS version 12.0 for Windows was applied to perform statistical analyses. Continuous variables were expressed as mean ± standard deviation (SD). As quantitative variables had a normal distribution, Student's t-test was employed to compare mean levels of variables between groups. Chi-squared test was applied to compare categorical variables between patients and controls. The associations between LCAT activity and independent variables were examined using Pearson's correlation test. The overall association of variables with LCAT activity was examined through multilinear regression analysis. P value less than 0.05 was considered significant.

Table 1. Characteristics of studied patients and controls; Quantitative variables are expressed as mean ± SD

Variable		Cases	Controls	*P*
gender	Male	20	20	
	Female	25	25	
Age (yrs)		49.9 ±6.9	51.1 ±6.8	0.9
BMI (kg/m^2)		27.4 ±2.8	26.3 ±2.7	0.6
Systolic BP (mmHg)		125.7 ±13.6	125.2 ±10.4	0.8
Diastolic BP (mmHg)		78.6 ±9.9	78.4 ±7.8	0.9
Waist girth (cm)		100 ±8.6	92 ±8.2	<0.001

RESULTS

Table 1 displays the demographic and the clinical characteristics of patients and controls. Laboratory findings of cases and controls are represented in table 2. Table 3 shows the correlation between LCAT activity and independent variables. In patients, LCAT activity had a significant strong inverse correlation with HbA_{1C} (r= –0.95, $P < 0.001$, Fig. 1), and moderate correlation with duration of diabetes (r= –0.46, $P < 0.001$, Figure 2). In controls, LCAT activity had a significant inverse correlation with TG, total cholesterol and diastolic blood pressure (r= –0.36, –0.47, –0.45, respectively, p<0.001). LCAT activity had no association with HOMA-IR in any of the groups. After multilinear regression analysis, only the association of LCAT activity and HbA_{1C} remained significant in patients (β=–0.9, p<0.001); and in controls, only the relations of LCAT activity and total cholesterol and diastolic blood pressure remained significant (β= –0.31, p=0.05; β= –0.33, p=0.01, respectively).

Table 2. Biochemical data of patients and controls; values expressed as mean ±SD

Variable	Cases	Controls	*P* value
FBS (mg/dl)	181 ±58	88 ±7.1	<0.001
TG (mg/dl)	178 ±101	105 ±37	<0.001
Total chol (mg/dl)	197 ±51	205 ±22.6	0.3
HDL-C (mg/dl)	40 ±8.3	52 ±11	<0.001
LDL-C (mg/dl)	110 ±27	113 ±11.4	0.6
Cr (mg/dl)	0.8 ±0.1	0.95 ±0.1	<0.001
HbA_{1C} (%)	9.5 ±2	4.8 ±0.4	<0.001
Insulin (mu/L)	6.7 ±5	6.3 ±3.7	0.6
HOMA-IR	2.9 ±2.2	1.4 ±0.87	<0.001
LCAT (µmol/L/h)	73 ±9.1	88 ±4.5	<0.001

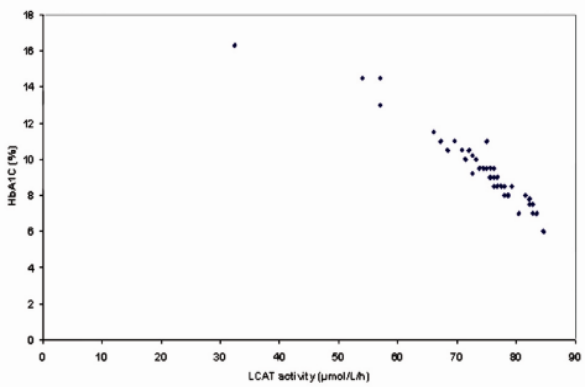

Fig. 1. Correlation of LCAT activity and HbA_{1C} in diabetic patients (r= -0.95, P< 0.001).

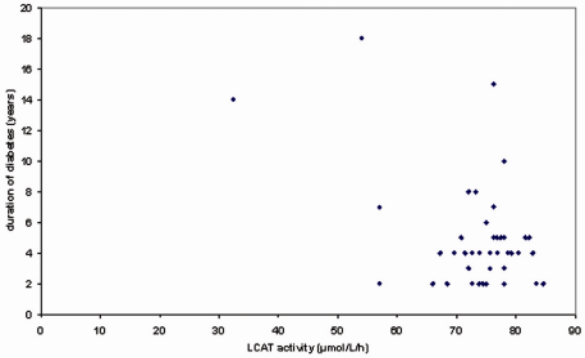

Fig. 2. Correlation of LCAT activity and duration of diabetes in patients (r= -0.46, p= 0.001).

Table 3. Pearson coefficients of correlations between LCAT activity and independent variables and their significance in patients and controls

Variable	Cases		Controls	
	r	*P* value	r	*P* value
Age	– 0.23	0.12	– 0.2	0.17
Gender	–	0.4	–	0.3
BMI	– 0.13	0.38	– 0.25	0.08
Duration of diabetes	– 0.46	0.001	–	–
HbA_{1C}	– 0.95	<0.001	– 0.2	0.15
Diastolic BP	– 0.2	0.18	– 0.45	0.002
Systolic BP	– 0.18	0.23	– 0.26	0.08
FBS	– 0.18	0.21	– 0.11	0.46
TG	– 0.17	0.24	– 0.36	0.01
Total cholesterol	– 0.04	0.78	– 0.47	0.001
HDL-C	0.06	0.65	0.12	0.4
LDL-C	0.03	0.8	– 0.13	0.37
Cr	– 0.04	0.76	– 0.05	0.7
Insulin	0.08	0.6	– 0.01	0.9
HOMA-IR	0.02	0.85	– 0.04	0.77

DISCUSSION

This study shows that LCAT activity in T2DM patients is significantly decreased compared with healthy controls. To the best of the authors' knowledge, the current study is the first research from Iran that has studied LCAT activity in diabetics. Although results of our study are supported by some previous reports (7, 8), there are not many studies on this topic in medical literature – with some in vitro studies (5, 9), animal experiments (6) and human researches (7, 8, 10, 11).

In 1999, Brite and colleagues showed that when they altered chemical structure of HDL particles in a hyperglycemic environment, the capacity of HDL to attach to cell surface and remove cholesterol would diminish (5). In the same year, Passarelli et al documented a decreased rate of mouse peritoneal macrophage cholesterol efflux by HDL-C drawn from diabetic subjects with unsatisfactory control of blood glucose (6). Dürücan and coworkers (2003) compared LCAT activity between 42 patients with T2DM and 29 sex, age and BMI matched non-diabetic controls and, similar to this study, showed significantly lower LCAT activity in diabetics (7). Their study was also comparable to this work in that their patients were only receiving one type of oral hypoglycemic agent or being treated with diet, and none were receiving any medications that can affect the lipid metabolism. Studying 10 diabetics with a BMI>25 kg/m^2 and 9 with a BMI<25 kg/m^2, Akanji and Agdebana exhibited that LCAT activity is lower in those diabetics with values of BMI>25 kg/m^2 (8). Besides, when oral hypoglycemic treatment was discontinued for 1 week in 10 diabetic (5 obese) patients and they went through hyperglycemia, authors noted further reduced fasting levels of LCAT activity.

On the contrary, some studies have reported a similar state of LCAT activity in diabetics and non-diabetics. Passarelli et al study in 1997 showed that the capacity of plasma lipoproteins drawn from diabetic subjects in the esterification of cholesterol in an in vitro setting was similar to that in normal subjects (9). Weight and coworkers (1993) did not appreciate any significant difference regarding LCAT activity between ten patients with type 1 diabetes mellitus and ten healthy controls (10). However, they showed that after treatment and improving glycemic control, LCAT activity increased in diabetics considerably. Likewise, Vardareli and colleagues (1998) did not find any significant difference when comparing T2DM patients with healthy non-diabetics. Of interest, they also did not detect any effect of treatment type on LCAT activity in their diabetic patients (11).

With respect to the etiology of decreased LCAT activity in diabetes, several explanations have been proposed, of which the most important one is glycation of LCAT or its cofactor – apoA1 (2). Glycated apoA1 associates weakly with HDL-C and, thus, is removed by the kidney at a faster rate. In addition, glycation of apoA1 – which is carried within discoid pre-ßHDL particles – could disrupt the interaction of pre- ßHDL with peripheral cells and with LCAT enzyme, for the removal of excess cholesterol (2). Some other possible reasons of decreased LCAT activity in diabetic subjects have been reported as a state of oxidative stress and formation of minimally modified LDL (MM-LDL) (12), increased urinary excretion of LCAT (13), increased uptake by LDL-receptor related protein (LRP) and intracellular sequestration (14) and an augmented state of inflammatory processes (15). The strong inverse correlation of LCAT activity and HbA_{1C} in our patients suggests that the main cause of decreased LCAT activity is probably its own or its cofactor's (apoA1) glycation in a hyperglycemic milieu as mentioned (7, 8). Thus, it can be assumed that the higher the extent of hyperglycemia, the lower the LCAT activity.

The diversity of results on LCAT activity in different studies might be related to the intensity of blood glucose control in patients. In addition, it appears that HDL-C particles in diabetic patients are subject to complex chemical and structural modifications which can affect LCAT activity (3). In this research, we tried to idealize study conditions as best as we could by selecting an adequate sample size and removing the effect of some potential confounding factors – such as gender, age and BMI – while recruiting the control group. We also minimized the possible impact of pharmacological agents, as 43 of 45 diabetic subjects were only receiving glibenclamide.

Although LCAT activity showed an inverse correlation with duration of diabetes in bivariate correlation analysis, the association lost significance in multilinear regression analysis. That is to say, decreased LCAT activity occurs even in the setting of short durations of hyperglycemia. Studies by Akanji et al (8) and Weight et al (10) are in line with our finding in this respect. Despite the significant negative correlation between BMI and LCAT activity in Akanji et al study, we did not appreciate any association of these variables in our study. It is possible that the relatively small sample size of their study had affected the significance of their results.

We could not draw any significant association between LCAT activity and HDL level either in patients or controls. Here, it is essential to mention that LCAT activity is an appropriate indicator of HDL-C function, but this function is not directly correlated with plasma levels of HDL-C, as we mentioned before. Instead, LCAT activity might directly be correlated with the levels of HDL2 – bigger particles full of esterified cholesterol (3), however we did not measured HDL subfractions.

We did not detect any significant relationship between HOMA-IR and LCAT activity. Intriguingly, an experimental study on LCAT deficient mice showed a higher level of insulin sensitivity in such gene targeted mice, which is because of the decreased expression of a number of genes known to modulate hepatic gluconeogenesis, including the rate-limiting enzyme phosphoenolpyruvate carboxykinase (16). To the best of our knowledge, no study has ever reported such an association between these factors.

Among our controls, LCAT activity had a significant inverse correlation with TG level. In this respect, one study has shown that subjects with heterozygote LCAT gene defects have about a 22% increase in TG levels, and those with homozygote defects about a 337% increase (17). In experiments on LCAT deficient mice, this has been attributed to reduced lipoprotein lipase activity and hepatic overproduction of VLDL (16,17). It can be expected that diminished LCAT activity lead to decreased esterification of cholesterol both from cell membranes and plasma lipoproteins, and thereby result in elevated serum cholesterol. This was the case in healthy controls in our study, as LCAT activity had a significant inverse correlation with total cholesterol level. In conclusion, overall, it is concluded that LCAT activity is considerably lower in diabetics compared with non-diabetics. Also, insufficient control of blood glucose– regardless of diabetes duration–appears to be an important determinant of LCAT activity.

Conflict of interests

The authors declare that they have no competing interests.

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