

Plasma Level of Oxidized Low- Density Lipoprotein in Macroalbuminuric Type 2 Diabetic Patients versus Normoalbuminuric Group

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Received: 1 May 2008 ; Received in revised form: 6 Jul. 2008; Accepted: 20 Jul. 2008

Abstract- Accumulating evidence suggest that in patients with diabetes mellitus, increased rate of lipoprotein oxidation and oxidative stress have important role in diabetic angiopathy, including nephropathy. To evaluate the association of oxidized low- density lipoprotein (ox-LDL) with the development of diabetic nephropathy, plasma level of ox-LDL were measured in 70 diabetic patients with macro and 63 patients with normoalbuminuria. The plasma oxidized- LDL level in patients with macroalbuminuria was higher than those in normoalbuminuric group, (85.72 ± 32.92 $\mu\text{g/L}$ versus 75.07 ± 26.46 $\mu\text{g/L}$, $P= 0.041$). Hemoglobin A1C (HbA1C) levels were similar in diabetic patients with macro ($9.0 \pm 1.80\%$) and normoalbuminuria ($8.52 \pm 1.7\%$, $P= 0.098$). There was no significant correlation between the ox-LDL and HbA1C level. The significantly elevated plasma oxidized- LDL in patients with macroalbuminuria suggests that ox-LDL may play an important role in the progression of diabetic nephropathy.

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Acta Medica Iranica 2009; 47(5): 349-352.

Key words: Diabetic nephropathy, oxidized low- density lipoprotein

Introduction

Superoxide (O_2^-) or other reactive oxygen species (ROS) are known to be involved in the mediation of renal injury (1). Studies have suggested that oxidized-LDL (ox-LDL) may be implicated in the pathogenesis of progressive glomerulosclerosis (2). Uptake of oxidized low density lipoprotein (ox-LDL) via scavenger receptor is considered an important step in the development of early atherosclerotic lesions (3, 4). Glomerular mesangial cells have a stronger affinity for ox-LDL than for native LDL, suggesting the presence of scavenger receptor on these cells and the potential involvement of ox-LDL in glomerulosclerosis (5). In the present study we measured plasma oxidized- LDL level in macroalbuminuric type 2 diabetic patient and compared with normoalbuminuric group to determine whether the plasma oxidized- LDL is related to the presence of diabetic nephropathy.

Patients and Methods

A total of 70 consecutive patients with type 2 diabetes and macroalbuminuria (group A) and 63 patients with type 2 diabetes but without proteinuria (group B), who

referred to our diabetes clinic between October 2006 and November 2007, were enrolled into our cross sectional study.

All patients gave their written informed consent and the local ethics committee at Tehran University of Medical Sciences approved the study protocol.

Exclusion criteria were plasma creatinine $\geq 2\text{mg/dl}$, history of renal disease before the onset of diabetes, smoking, previous treatment with statins, presence of any sign or symptoms of inflammatory renal disease and congestive heart failure.

In addition to a thorough physical examination at referral, we examined the following variable: age, sex, body mass index (BMI), diabetes duration and medication (oral agents, insulin or both), history of CCU admission and coronary heart disease, blood pressure, FBS, HbA1C, total cholesterol, LDL, HDL, TG, creatinin and GFR. Oxidized- LDL was measured by Sandwich ELISA (Mecrodia, Sweden) using the mouse monoclonal antibody.

24 hours urinary albumin excretion with macroalbuminuria defined as albumin excretion of greater than 300mg per day and below 30mg per day in normoalbuminuric group. Data were analyzed with SPSS statistical program (version 11.5). Significance of

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differences between group means was tested by student's t- test. Results were presented as mean \pm SD and $P < 0.05$ was considered statistically significant.

Results

Table 1 illustrates clinical and biomedical characteristics of both groups. Among 133 individuals included in the study, 59 (44.4%) were men and 74 (55.6%) were women, with a median age of 58.79 ± 8.11 and median duration of diabetes, 137.90 ± 64.84 months. There were

no significant differences between the two groups (A and B) for age ($P = 0.689$), duration of diabetes ($P = 0.997$), CCU admission ($P = 0.856$), BMI ($P = 0.154$), systolic blood pressure ($P = 0.140$), diastolic blood pressure ($P = 0.351$), FBS ($P = 0.957$), HbA1C ($P = 0.097$), total Chol ($P = 0.305$), LDL ($P = 0.102$), HDL ($P = 0.403$) and TG ($P = 0.320$).

Creatinin was higher and glomerular filtration rate was lower in macroalbuminuric group ($P < 0.001$ and $P < 0.003$, respectively).

Table 1. Clinical and biomedical characteristics of the two groups

Parameter	Group A (normoalbuminuric, n=63)	Group B (macroalbuminuric, n=70)	P
Age (years, mean \pm SD)	58.79 \pm 8.11	59.37 \pm 8.44	0.689
Percent of male (%)	33	54.3	* 0.015
Diabetes duration (months, mean \pm SD)	137.90 \pm 64.84	137.94 \pm 67.31	0.997
History of CCU admission (%)	4	5	0.856
BMI (kg/m ² , mean \pm SD)	26.89 \pm 5.77	25.62 \pm 4.21	0.154
Systolic BP (mmHg, mean \pm SD)	134.11 \pm 16.75	138.38 \pm 16.33	0.139
Diastolic BP (mmHg, mean \pm SD)	83.19 \pm 9.98	84.77 \pm 9.44	0.350
Creatinin (mg/dl, mean \pm SD)	0.91 \pm 0.23	1.11 \pm 0.29	*** <0.0001
GFR (cc/min, mean \pm SD)	80.01 \pm 23.46	68.09 \pm 22.46	** < 0.003
Albumin excretion rate (mg/24h)	9.39 \pm 4.10	855.48 \pm 413.01	*** <0.0001
FBS (mg/dl, mean \pm SD)	171.26 \pm 71.08	171.85 \pm 52.63	0.957
HbA1C (% , mean \pm SD)	8.52 \pm 1.70	9.03 \pm 1.80	0.098
Chol (mg/dl, mean \pm SD)	188.30 \pm 50.27	197.34 \pm 50.82	0.305
LDL (mg/dl, mean \pm SD)	83.30 \pm 28.04	91.27 \pm 27.66	0.102
HDL (mg/dl, mean \pm SD)	32.26 \pm 7.77	31.31 \pm 5.21	0.403
TG (mg/dl, mean \pm SD)	183.190 \pm 80.35	196.07 \pm 66.75	0.315
Ox-LDL(mu/L, mean \pm SD)	75.07 \pm 26.46	85.72 \pm 32.92	* 0.041

*P value <0.05 is significant

** P value < 0.01

*** P value <0.001

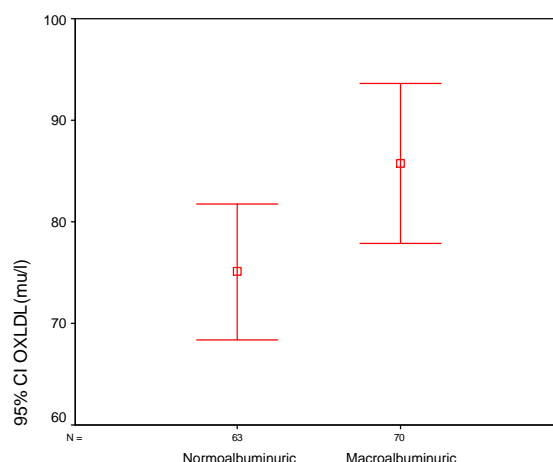


Figure 1. Comparison of serum oxidized-LDL level in macro and normoalbuminuric group.

The plasma oxidized- LDL was significantly higher in the macroalbuminuric than in the normoalbuminuric group, ($P=0.041$).

The plasma oxidized- LDL was higher in the macroalbuminuric than in the normoalbuminuric group (85.72 ± 32.92 $\mu\text{g/L}$ versus 75.07 ± 26.46 $\mu\text{g/L}$, $P = 0.041$), (Figure and Table 1).

There was no significant correlation between oxidized- LDL level and duration of diabetes, CCU admission, blood pressure, BMI, creatinin and lipid profile.

Discussion

Oxidative stress has been suggested to be a common pathway linking diverse mechanism for the pathogenesis of complication in diabetes (6). Superoxide (O_2^-) or other reactive oxygen species (ROS) are known to be involved in the mediation of renal injury (7,8). Elevated plasma levels of lipid peroxides are found in poorly controlled diabetic patients with micro and macro-angiopathy (9,10). Available evidence suggests that oxidized LDL is involved in nephrosclerosis as well as atherosclerosis (11). Our aim was to determine whether the amount of oxidized- LDL correlates with the degree of albuminuria in type 2 diabetic patients.

While some studies have not found increased susceptibility of LDL to oxidation in diabetic patient (12-14), evidence for increased LDL oxidizability in diabetic subjects has been shown in another studies (3,15-18). However type 2 diabetes has small, dense LDL, which is more prone to oxidation than large buoyant LDL (19).

Our study showed that oxidized- LDL level was significantly higher in macroalbuminuric type 2 diabetic patient than in the normoalbuminuric group, suggesting that oxidized-LDL may play an important role in diabetic nephropathy.

The results of the present study along with those obtained by Noriko Ujihara (3), Sachie T Suzura (17), Wang H (18) and D. Atchley (20).

Studies have suggested that oxidized LDL (ox-LDL) may be implicated in the pathogenesis of progressive glomerulosclerosis (21). In support of this view, the presence of ox-LDL has been demonstrated in the glomeruli of rats with focal segmental glomerulosclerosis (FSGS) (22,23).

Glomerular mesangial cells have a stronger affinity for ox-LDL than for native LDL, suggesting the presence of Scavenger receptor on these cells and the potential involvement of ox-LDL in glomerulosclerosis (24,25).

In support of this view, the presence of oxidized-LDL has been demonstrated in the glomeruli of rats with focal

segmental glomerulosclerosis (22,23). Hyun Soon Lee and et al, also suggest that patients with heavy oxidized-LDL accumulation in the sclerotic segments of glomeruli have more advanced renal disease than those with mesangial ox-LDL (11).

Moro et al. Measured plasma levels of electronegative LDL (LDL^-), an indicator of lipid oxidation, in 24 patients with type 2 diabetes mellitus and reported high LDL^- levels in patients with microalbuminuria in comparison to normoalbuminuric group (26).

However, they also found significantly higher HbA1C levels in these patients and concluded that hyperglycemia was largely responsible for the higher LDL^- values. In our study, there was no significant difference in HbA1C levels among the macro and normoalbuminuric groups. Furthermore no significant correlation was seen between oxidized LDL and FBS, HbA1C in two groups.

Therefore, the high oxidized- LDL in macroalbuminuric groups, most likely were not directly caused by present hyperglycemic state in our study. The higher percentage of men in macroalbuminuric group without primary selection, indicates the role of sex male in severity of diabetic nephropathy. By considering the results, suggesting a link between high level oxidized-LDL and diabetic nephropathy, more clinical trials that evaluate effects of antioxidants on modulating oxidative stress, oxidized-LDL and amount of proteinuria will be valuable.

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