Oxidized LDL is Correlated with Urine Albumin in Type 2 Diabetes Mellitus: A Case-Control Study

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Abstract

Objective: In patients with diabetes mellitus, increased rate of lipoprotein oxidation and oxidative stress have important role in diabetic angiopathy, including nephropathy. We designed a study for comparing the level of Ox-LDL in diabetic patients with Macro-albuminuria, Micro-albuminuria, versus Normo-albuminuric.

Materials and Methods: One hundred and thirty five patients with type 2 diabetes mellitus who had been referred to the Yazd Diabetes Clinic, from 1391 through 1392, were enrolled to our study. The 24 hours urinary albumin excretion was measured and Macro-albuminuria defined as albumin excretion of greater than 300mg per day and below 30mg per day in normo-albuminuric group and between 30-300mg defined as Micro-albuminuria group. Plasma level of OX-LDL was measured.

Results: The plasma OX-LDL level in patients with Macro-albuminuria was higher than those in Normo-albuminuric group, (16.1±3.6 U/ml versus 8.6±1.7 U/ml). There was a significant correlation between the OX-LDL and urine albumin in Macro-albuminuria and Micro-albuminuria groups. There was no significant correlation between the OX-LDL and HbA1C level and diabetes duration.

Conclusion: Significantly elevated plasma OX-LDL in patients with Macro-albuminuria suggests that OX-LDL may play an important role in the progression of diabetic nephropathy.

Keywords: Diabetic mellitus, Oxidized LDL, Nephropathy.

Introduction

Oxidative change of LDL and conversion to Ox-LDL is non-enzymatic post-translational reaction. The Ox-LDL plays important role in starting and progressing of atherosclerotic process via induction of foam cells and inflammatory changes (1,2). This particle can be a cardiovascular risk factor, diagnostic and prognostic marker for vascular diseases (3).

Hyperglycemic status of diabetic mellitus (DM) is an oxidative stress status. Hyperglycemic status induces upregulation of

lipoxygenase (4,5,6), and prolonged half-life of small dense LDL contribute to prolonged duration time for exposing to oxidative stress reactions (1).

Regarding the similarity between nephrosclerosis and atherosclerosis pathogenesis and the important role of hyperlipidemia as a risk factor of diabetic nephropathy, therefore, the oxidation of LDL can explain the function of hyperlipidemia in pathogenesis of vascular and renal Injuries. (4)

Removing of Ox-LDL by scavenger receptors is not adjustable and Ox-LDL are resistant to lysosomal hydrolases, therefore, afflux of cholesterol from Ox-LDL to HDL in reverse cholesterol transport (RCT) cycle cannot be done (7). Recently, existence of these receptors on membrane of glomerular mesangial cells was proved.

Ox-LDL may be taken up by scavenger receptors on mesangial cells and monocyte-macrophages, resulting in foam cell formation. Alteration in renal hemodynamic via arachidonic acid metabolites, and induction of macrophage infiltration (4,5).

Diabetes is one of the most important cause of end stage renal disease in Iran and worldwide (18). This present study, we designed a study for comparing of level of Ox-LDL in diabetic patients with Macro-albuminuria, Micro-albuminuria, versus Normo-albuminuric .

Materials and Methods

In this case- control study 135 patients with well-documented type 2 DM (60 men and 75 women) who were referred to the Yazd Diabetes clinic, from 1391 through 1392, were enrolled.

All patients gave their written informed consent. Exclusion criteria were: plasma creatinine ≥ 2mg/dl, or GFR≤50, history of renal disease before the onset of diabetes, smoking, history of liver and thyroid disorders, history of congestive heart failure and vitamin E consumption.

Demographic data, weight, height, duration of diabetes and 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). About 10 ml venous blood sample was taken from each patients following a fasting period of 10-12 hours. After centrifugation, serum samples were stored at -70ºC until analysis. The following biochemical factors were measured: plasma FBS was performed using the glucose oxidase method. Cholesterol, triglycerides, HDL-C and LDL-C were determined by enzymatic methods (Pars Azmoon, Karaj, Iran), and Creatinine (Cr) by Jaffe reaction (Pars Azmoon, Iran). HbA1c was measured by high-pressure liquid chromatography method. Ox-LDL was measured using commercially available sandwich enzyme-linked immunosorbent assay (enzyme-linked immunosorbent assay; Mercodia, Uppsala, Sweden). The intra and inter assay CV for the assay ranged between 5.4% and 8.3%. The detection limit was 0.03 U/L.

The 24 hours urinary albumin excretion with Macro-albuminuria defined as albumin excretion of greater than 300mg per day and below 30mg per day in Normo-albuminuric group and albumin excretion between 30-300mg defined as Micro-albuminuria group .Urine albumin analysis was repeated and if the same results were detected the patient was enrolled.

Data were analyzed with SPSS statistical program (version 18). The continuous variables were tested by variance analysis. All variables were expressed as means ± standard deviation (SD). Pearson correlation

Table 1. Demographic and biochemical data of patients

Variable	Group A Macro-albuminuria n=45	Group B Micro-albuminuria n=45	Group C Normo-albuminuric n=45	*P*-value
Age (years, mean ±SD)	58.09±6.29	58.07±6.32	58.38±5.87	0.96
Male Population(%)	42	47	45	--
Diabetic Duration (years, mean ±SD)	9.11±3.14	6.96±2.8	5.3±2.1	0.001
BMI (kg/m2, mean ±SD)	28.02±1.79	27.75±1.81	28.4±2.65	0.35
SBP (mmHg, mean ±SD)	145.33±13.7	142.7±13.46	144.5±10.21	0.61
DBP (mmHg, mean ±SD)	89.7±10.6	87.56±6.8	86.7±6.9	0.21
Cr SD± (mg/dl, mean)	1.1±0.2	0.91±0.17	0.98±0.16	0.001
Urine-Albumin (mg/24h, mean ±SD)	1027±378.2	184.9±68.8	17.24±6.14	0.001
FBS (mg/dl, mean ±SD)	178.91±50.93	172.2±50.1	181.8±49.4	0.646
HbA1C (%, mean ±SD)	9.27±1.37	8.61±1.44	8.27±1.24	0.002
Chol (mg/dl, mean ±SD)	194.6±49.8	203.2±36.6	205.8±21.96	0.34
HDL (mg/dl, mean ±SD)	41.73±7.5	43.1±7.16	45.4±7	0.05
TG (mg/dl, mean ±SD)	240.64±153.4	226.29±82.6	235.5±93.66	0.83
OX-LDL (mu/L, mean ±SD)	16.12±3.62	11.83±2.67	8.65±1.78	0.001

Table2. Pearson coefficients of correlations between Ox-LDL and independent variables

Variable	Group A Macro-albuminuria		Group B Micro-albuminuria		Group C Normo-albuminuric	
	r	***P*-value**	**r**	***P*-value**	**r**	***P*-value**
FBS	0.080	0.300	0.160	0.147	0.047	0.379
HbA1C	0.48	0.376	0.169	0.134	-0.071	0.321
Cr	0.283	0.030	0.061	0.345	0.027	0.429
Urine Albumin	0.772	0.001	0.853	0.001	0.228	0.066
Diabetic Duration	0.104	0.247	-0.036	0.407	-0.107	0.243

Coefficients between Ox-LDL and other continuous variables were calculated.

P-values of less than 0.05 were considered as statistical significance.

Results

The clinical characteristics of the patients classified according to urinary albumin excretion rate and those of the normal controls were shown in Table 1. There were no significant differences between the groups for age (*P*=0.96), sex (*P*=0.96), BMI (*P*=0.35), systolic blood pressure (*P*=0.61), diastolic blood pressure (*P*=0.21), FBS (*P*=0.64), total Cholesterol (*P*=0.34), LDL (*P*=0.102), and TG (*P*=0.83).

The duration of diabetes mellitus was significantly longer in the macro- group (9.1±3.1 years) than in the normo- group (5.3±2.1 years) and the micro-group (6.9±2.8 years) (*P*=0.001). HbA1C levels were similar in the normo-group (8.2±1.2%) and micro-group (8.6±1.4%), but significantly lower than macro-group (9.2±1.3%) (*P*=0.002).

The plasma Ox-LDL level was significantly higher in the macro- group (16.1±3.6 U/ml)

than in the normo- group (8.6±1.7 U/ml), micro- group (11.8±2.6 U/ml) (*P*=0.001).

Table 2 showed the correlation between Ox-LDL and independent variables. In macro and micro-groups a significant correlation with urine albumin (*P*<0.001) was seen. Ox -LDL had no significant correlation with duration of diabetes, HbA1c, FBS, total Chol, TG, and HDL.

Discussion

Oxidative reaction is known as a common pathway in pathogenesis and complications of diabetes (8). Ox-LDL is resulted from function of LDL in oxidative stress and converted form to aggregated particles with different structure. Ox-LDL is not identifiable by LDL-receptors which are in blood and get cleaned via scavenger receptors on membrane of macrophages (1,2).

Until recent years, sensitive and specific assays techniques for the measurement of Ox-LDL in the plasma were unavailable (4).

Nowadays, with sensitive and accurate techniques, there is ability for measurement of it via ELISA method. There are evidences about increase LDL oxidation in diabetic patients in previous studies (4,9,10,11,12). However, several published papers did not found increased susceptibility of LDL to oxidation in diabetic patients. (13,14,15) Type 2 diabetes have small and dense LDL, which is more prone to oxidation than large buoyant LDL (16).

Our study showed that the Ox-LDL level was significantly higher in the macro- group than in the micro- group, and Normo-albuminuric group, suggesting that Ox-LDL may play an important role in diabetic nephropathy. The results of the present study along with those obtained by Noriko ujihara (4), Sac hie T Suzura (11), Wang H (12), D. Atchley (17) and Nakhjavani (18).

Lipid oxidation is increased in diabetes, especially in patients with vascular complications (4). The pathogenesis of nephrosclerosis in DM remains unclear, but recent work provides strong evidence for the existence of scavenger receptors on mesangial cells (19). Available evidence suggests that Ox-LDL is involved in nephrosclerosis as well as atherosclerosis. Ox-LDL may be taken up by scavenger receptors on mesangial cells and monocyte-macrophages, resulting in foam cell formation. Alteration in renal hemodynamic via arachidonic acid metabolites (20) and induction of macrophage infiltration (19). These events may contribute to renal damage. Previous studies have demonstrated that elevated glucose levels increase oxidative stress and augment lipid peroxidation in glomeruli (22). In support of this view, the presence of Ox-LDL has been demonstrated in the glomeruli of rats with focal segmental glomerulo-sclerosis (4). Moro and et al. measured plasma levels of electronegative LDL (LDL^-), an indicator of lipid oxidation, in 24 patients with type 2 DM and reported high LDL^- levels in patients with Micro-albuminuria (21). In our study there was higher HbA1c in macro-group but no significant correlation between HbA1c and Ox-LDL.

Also, no significant correlation was seen between Ox-LDL and FBS, in three groups. Therefore, the high Ox-LDL level most likely was not directly caused by present hyperglycemic state in our study. Our results taken together with the findings of previous studies thus suggest that continued impaired glucose metabolism increases oxidative stress and it may be involved in the initiation and

progression of the glomerular and mesangial cell damage found in diabetic nephropathy.

Conclusion

By considering the results, suggesting a link between high level Ox-LDL and diabetic nephropathy, more clinical trials that evaluate effects of antioxidants on modulating oxidative stress, Ox-LDL and amount of proteinuria will be valuable.

References

1. Mertens A, Holvoet P. Oxidized LDL and HDL: antagonist in atherothrombosis. FASEB. 2001;15:2073-84.
2. Itabe H. Oxidized low-density lipoproteins: what is understood and what remains to be clarified. Biological and Pharmaceutical Bulletin 2003;26(1):1-9.
3. Itab H, Ueda M. Measurement of Plasma Oxidized Low-Density Lipoprotein and its Clinical Implications. Journal Atheroo & thrombosis. 2006;14(1):1-11.
4. Ujihara N, Sakka Y, Takeda M, Hirayama M, Ishii A, Tomonaga O, et al. Association between plasma diabetic nephropathy and Oxidized LDL. Diabetes Research and clinical practice. 2002;58:109-14.
5. Kopprasch S, Pietzsch J, Kuhlisch E, Fuecker K, Temelkova-Kurktschiev T, Hanefeld M, et al. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. Diabetes. 2002;51:3102-6.
6. Li C, Gu Q. Protective effect of paraoxonase 1 of high-density lipoprotein in type 2 diabetic patients with nephropathy. Nephrology 2009;14(5):514-20.
7. Dhaliwal BS, Steinbrecher UP. Cholesterol delivered to macrophages by oxidized low density lipoprotein is sequestered in lysosomes and fails to efflux normally. Journal of Lipid Research. 2000;41:1658-1669.
8. Vessby J, Basu S, Mohsen R, Berne C, Vessby B. Oxidative stress and antioxidant status in type 1 diabetes mellitus. Journal Intern Med 2002; 251(1):69-76.
9. Gokulakrishnan K, Deepa R, Velmurugan K, Ravikumar R, Karkuzhali K, Mohan V. Oxidized low-density lipoprotein and intimal medial thickness in subjects with glucose intolerance: the Chennai Urban Rural Epidemiology Study-25. Metabolism 2007;56(2):245-50.
10. Kostić N, Caparević Z, Ilić S. Antioxidant status in type II diabetes mellitus patients with or without microvascular complications. Srp Arh Celok Lek 2007;135(3-4):143-6.
11. Tsuzura S, Ikeda Y, Suehiro T, Ota K, Osaki F, Arii K, et al. Correlation of plasma oxidized low-density lipoprotein levels to vascular complications and human serum paraoxonase in patients with type 2 diabetes. Metabolism 2004;53(3):297-302.
12. Wang H, Deng H, Liu W. The effects of paraoxonase-1 and oxidized low density lipoprotein on nephropathy in type-2 diabetes mellitus. Zhonghua Nei Ke Za Zhi 2002;41(3):179-82.
13. Oranje WA, Wolffenbuttel BH. Lipid peroxidation and atherosclerosis in type II diabetes. J Lab Clin Med 1999;134(1):19-32.
14. Schwenke DC, D'Agostino RB Jr, Goff DC Jr, Karter AJ, Rewers MJ, Wagenknecht LE; Insulin resistance atherosclerosis study. Differences in LDL oxidizability by glycemic status: the insulin resistance atherosclerosis study.Diabetes Care 2003;26(5):1449-55.
15. Oranje WA, Rondas-Colbers GJ, Swennen GN, Wolffenbuttel BH. Lipid peroxidation in type 2 diabetes: relationship with macrovascular disease? Neth J Med 1998;53(2):61-8.
16. Lamarche B, Lemieux I, Després JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, patho-physiology and therapeutic aspects. Diabetes Metab 1999;25(3):199-211.
17. Atchley DH, Lopes-Virella MF, Zheng D, Kenny D, Virella G. Oxidized LDL-anti-oxidized LDL immune complexes and diabetic nephropathy. Diabetologia 2002;45(11):1562-71.
18. Nakhjavani M, Asgarani F, Esfahanian F, Esteghamati A. Plasma Level of Oxidized Low-Density Lipoprotein in Macroalbuminuric Type 2 Diabetic Patients versus Normoalbuminuric Group. Acta medica iranica 2009;47(5):349-52
19. Schlondorff D. Cellular mechanisms of lipid injury in the glomerulus. Am J Kidney Dis 1993;22(1):72-82
20. Kaplan R, Aynedjian HS, Schlondorff D, Bank N, Renal vasoconstriction caused by short-term cholesterol feeding is corrected by thromboxane antagonist or probucol, J. Clin. Invest 1990;86:1707-14.
21. Moro E, Zambon C, Pianetti S, Cazzolato G, Pais M, Bon GB. Electronegative low density lipoprotein subform (LDL-) is increased in type 2 (non-insulin-dependent) microalbuminuric diabetic patients and closely associated with LDL

susceptibility to oxidation, Acta Diabetol 1998;35:161-4.

22. Ha H, Yoon SJ, Kim KH. High glucose can induce lipid peroxidation in the isolated rat glomeruli, Kidney Int. 1994;46:1620-6.