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Correlation between oxidized-LDL and interleukin-6 in type 2 diabetic patients

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Abstract

Background: Studies have suggested that oxidative stress is a common pathway of different leading mechanisms to diabetes complications. Oxidative stress play a crucial role in atherogenesis and cause oxidation of low density lipoprotein. Evidence has been shown that oxidized LDL in diabetic patients is higher than nondiabetic individuals. Regarding to known role of oxidative stress in developing of micro and macrovascular complications of diabetes and recent evidences about importance of IL-6 in initiating of inflammatory processes in atherosclerotic plaques formation and reports that shown the effects of Ox-LDL upon IL-6 release, in this study evaluation of serum levels of IL-6 and correlation of these two agents in diabetic patients in comparison with healthy persons was performed.

Methods: This stratified cross-sectional study was conducted in diabetic clinic of Imam khomeini Hospital, Tehran University of Medical Sciences during 2009-2010, recruiting 40 type2 diabetic (T2DM) patients as cases and 40 healthy subjects as controls. FBS, lipid profile, HbA1c, oxidized-LDL and IL-6 levels were measured for both patients and controls after 12 hours fasting state.

Results: The mean of Ox-LDL/LDL ratio in T2DM group (0.65±0.14) were significantly higher than control group (0.5±0.15) ($p<0.001$). The mean level of IL-6 in T2DM group were 2.6±1.8 pg/ml that was higher than control group (1.9± 0.8pg/ml) ($p=0.05$). BMI, systolic and diastolic blood pressures revealed significant correlation with IL- 6 level in diabetic group. There was no correlation between diabetes duration and IL-6 level.

Conclusion: We concluded that diabetes, as an independent factor, is responsible for increased IL-6 in T2DM.

Keywords: Interleukin-6, Diabetes mellitus, Oxidized LDL

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Introduction

Diabetes is a chronic metabolic disorder that its prevalence is increasing and becomes a major worldwide health problem. Microvascular and macrovascular complications of diabetes decrease quality of life and life expectancy, and also imposes heavy economic and human burden on the community. Extensive studies have been accomplished to understanding the pathogenesis and preventive ways of diabetes complications. Studies have suggested that oxidative stress is common pathway of variant mechanism for diabetic complication's pathogenesis (1-2) . Oxidative stress play a crucial role in atherogenesis and cause oxidation of low density lipoprotein. Evidence has been shown that oxidized LDL in diabetic patients is higher than nondiabetic individuals (3-5). Study of Galland et al. showed increased Ox-LDL/LDL ratio in T2DM patients (6). In diabetic patients with macrovascular disease, this ratio is higher than patients without macrovascular disease (7) .

There are evidences that insulin resistance and type 2 diabetes are related to a chronic low grade inflammatory state. Cross sectional studies have reported that proinflammatory cytokines and acute phase markers are elevated in subjects with T2DM (8-12). IL-6 is a major cytokine that acts on the liver to stimulate the production of CRP (8). There is an increased risk for coronary artery disease in T2DM. It has been shown that subclinical (early) atherosclerosis increases with increasing degrees of glucose intolerance. Several mechanisms have been proposed to explain the accelerated and premature atherosclerotic changes seen with T2DM. This mechanisms includes inflammation (13-14), increased platelet activation (15-16) and oxidative stress (17). Studies have been shown the ability of oxidized-LDL to induce IL-6 secretion from monocytes (18), hepatocytes (19) and mesangial cells (20) in cell culture. In addition, IL-6 have found in atherosclerotic plaques. Given known role of oxidative stress in developing of micro and macrovascular complications of diabetes (21) and recent evidences regarding the role of IL-6 in initiating of inflammatory processes in atherosclerotic plaques formation and studies that shown the effects of ox-LDL on IL-6 release, in this study evaluation of serum level of IL-6 and correlation of these two agents in diabetic patients comparison with healthy persons is accomplished.

Methods

This stratified cross-sectional study was conducted in diabetic clinic of Imam khomeini Hospital, Tehran University of Medical Sciences during 2009-2010, recruiting 40 type2 diabetic (T2DM) patients as cases and 40 healthy subjects as controls. Cases and controls were matched regarding age and gender and BMI. Smokers, those with creatinine levels equal or more than 1/5mg/dl, taking NSAIDS, Statins, Vitamins, Glucocorticoids; presence of heart failure class III and IV, infections and inflammatory disease and pregnant women were excluded from the study. The research was carried out according to the principles of the Declaration of Helsinki. The local ethics review committee of Tehran University of Medical Sciences approved the study protocol. All participants gave written informed consent before sampling. FBS, lipid profile, HbA1c, Oxidized-LDL and IL-6 levels were measured for both patients and controls after 12 hours fasting state. Serum oxidized-LDL and Il-6 were detected by ELISA assays.

Statistical Analysis

Data were analyzed using SPSS software (version 13.0; SPSS Inc; Chicago, USA). Continuous variables are expressed as Means±standard deviation (SD). Comparisons between patients and controls were performed by Student's T-test for quantitative variables. We used Pearson correlation for quantitative variables. Regression analysis used to calculate IL-6 level . $P \leq 0.05$ was considered as statistically significant.

Results

A total of 40 diabetic patients and 40 age, sex and BMI matched healthy adult volunteers were recruited in this cross sectional study. Demographic and biochemical characteristics of patients and controls are expressed in Table 1. There was no significant difference between groups with respect to age, sex, BMI, systolic and diastolic blood pressures, total cholesterol,

triglyceride and OX-LDL levels. HDL-C and LDL-C levels revealed significant difference between groups.

The mean levels of Ox-LDL/LDL ratio in T2DM group (0.65±0.14) were significantly higher than control group (0.5±0.15)($p<0.001$)(Figure 1). The mean level of IL-6 in T2DM group were 2.6±1.8 pg/ml that was higher than control group (1.9±0.8pg/ml)(P=0.05)(Figure 2). According Pearson correlation, as presented in Table 2, IL-6 significantly ($p\leq0.05$) correlated with BMI (r=0.32), systolic blood pressure (r=0.32) and diastolic blood pressure (r=0.35) in diabetic patients. In healthy controls, IL-6 was correlated with BMI (r=0.34), OX-LDL (r=0.35) and Ox-LDL/LDL(r=0.47) (Table 3). In diabetic patients, IL-6 was not correlated with OX-LDL and Ox-LDL/LDL. Regression analysis used for determining IL-6 level. By using BMI, LDL cholesterol and OX-LDL, in control group, this equation was statistically correlated with $R^2= 0.26$, r=0.51; P=0.015. It was not significant in diabetic patients.

Table 1. Characteristics of patients and control participants

Variable	Diabetic patients (n=40)	Healthy controls (n=40)
Age (year)	49±10	49±9
BMI (kg/m2)	27.7±4.3	28±3.6
Systolic Blood Pressure (mmHg)	118.4±14.6	115.25±10
Diastolic Blood pressure (mmHg)	75±10.19	76.87±6.47
Diabetes duration (years)	6±5.7	-
FBS (mg/dl)*	156.2±64.1	89.7±10.6
HbA1c (%)*	9.19±2.6	5.24±0.6
Triglyceride (mg/dl)	162.6±60.5	158.2±79.4
Total cholesterol (mg/dl)	198.3±36.4	214.3±44.2
HDL-c (mg/dl)*	38.5±10.5	48.6±12.8
LDL-c (mg/dl)*	105.1±20	135.2±38
IL-6 (pg/ml)**	2.6±1.8	1.9±0.8
OX-LDL (u/l)	68.4±18.9	66±20.7
OX-LDL/LDL*	0.65±0.14	0.5±0.15

*: P<0.001, **: p=0.05
Cross sectional study
T test analysis
Variables are expressed as Mean ± SD

Table 2. Correlation coefficients among IL-6 and other variables in diabetic group

Variable	R
Age	0.02
BMI *	0.32
Systolic Blood Pressure *	0.32
Diastolic Blood pressure *	0.35
Diabetes duration	0.001
FBS	_0.01
HbA1c	_0.003
Triglyceride	0.07
Total cholesterol	_0.23
HDL-c	_0.11
LDL-c	_0.21
OX-LDL	_0.02
OX-LDL/LDL	0.19

Number of diabetic patients: 40
Cross sectional study
*p ≤ 0.05, Pearson correlation

Table 3. Correlation coefficients among IL-6 and other variables in control group

Variable	R
Age	0.11
BMI*	0.34
Systolic Blood Pressure	0.16
Diastolic Blood pressure (mmHg)	0.1
FBS	0.16
Triglyceride	0.17
Total cholesterol	_0.05
HDL-c	_0.18
LDL-c	_0.13
OX-LDL*	0.35
OX-LDL/LDL*	0.47

Number of control group: 40
Cross sectional study
*p ≤ 0.05, Pearson correlation

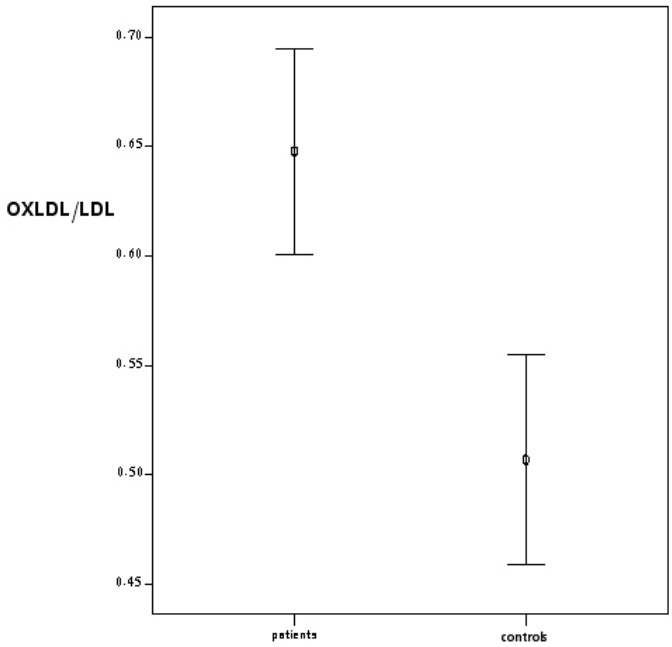

Figure 1. Comparison of OX-LDL/LDL in two groups (p<0.001)

Number of diabetic patients: 40, Number of control group: 40; Cross sectional study

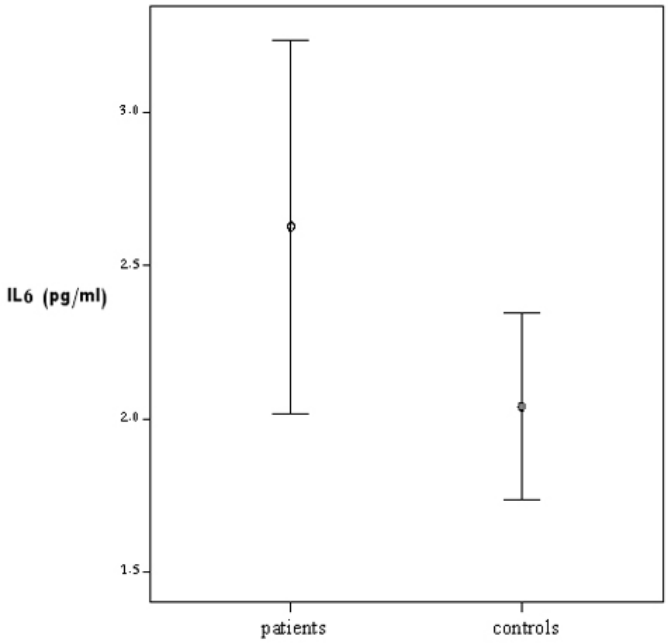

Figure 2. Comparison of IL-6 in two groups (p=0.05)

Number of diabetic patients: 40, Number of control group: 40
Cross sectional study

Discussion

In this study, we demonstrated that Ox-LDL/LDL increased in type 2 diabetic patients. Our data are consistent with study of Galland in diabetic patients (6). In diabetic patients with macrovascular disease, this ratio is higher than patients without macrovascular disease (7). This study also showed that if we have optimized level of LDL cholesterol (22), Ox-LDL does not decrease. In the other hand, oxidation reaction is a continuous process. Our data are generally consistent with previous reports (23-25). In our study, there was no difference between groups with respect to OX-LDL. In the present study, the mean duration of diabetes was 6 years. Previous studies demonstrated association of serum oxidized-LDL with diabetes duration independent of maintaining optimized levels of LDL cholesterol (23). These findings may suggest short duration of diabetes in our study. Also mean level of IL-6 in T2DM group was higher than control group. This finding is consistent with previous studies (8-12). In diabetic patients, Il-6 was correlated with BMI, systolic and diastolic blood pressures that was in line with Cardellini report (8). In Doo study, there was no correlation between OX-LDL antibody level and IL-6 in patient with unstable angina but there was association between OX-LDL antibody level with CRP and ICAM-1 in these patients (26). Hulthe showed no association between OX-LDL level and IL-6 in healthy adult volunteers (14).

In-vitro studies have been shown that oxidized-LDL can induce IL-6 secretion from monocytes (18), hepatocytes (19) and mesangial cells (20) in cell cultures. Alloxan-induced type 1 diabetic rats revealed higher levels of IL-6 and oxidative stress markers in Gumleniczek et al. report (27). Treatment of rats with antioxidant drugs decreased these markers. In one study, acute induced hyperglycemia in healthy adult volunteers increased IL-6 level but was not seen with simultaneous injection of glutathione (as antioxidant) (28).

In this study, Il-6 was not correlated with OX-LDL and Ox-LDL/LDL in diabetic group. Diabetes is a state of chronic hyperglycemia that may be differing from acute induced hyperglycemia. It is possible that complex of product of oxidative stress may be responsible for high level of IL-6 in diabetic patients and OX-LDL is one part of this complex. Further studies are necessary to confirm this hypothesis or find other possible mechanisms.

In this study, IL-6 had positive correlation with OX-LDL and Ox-LDL/LDL ratio in healthy group. By using BMI, LDL-

cholesterol and OX-LDL, it is possible to determine IL-6 level in healthy adults. It is not true in diabetic patients. In our study two groups were matched regarding BMI but diabetic patients had higher IL-6 levels than controls. This finding is consistent with recent performed study (10). Morohoshi showed in-vitro production of glucose dependent Interleukin-6 by human peripheral blood monocytes (29). This data verified that increased IL-6 in T2DM is independent of insulin resistance and obesity. In conclusion, it seems that diabetes can be considered as an independent contributor to development of inflammatory cytokines such as IL-6.

Nonetheless, our study does not support the hypothesis that Oxidized-LDL can influence IL-6 level in diabetics. Our results suggest that diabetes is an independent factor for releasing of inflammatory cytokines.

References

1. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine and Pharmacotherapy* 2005; 59: 365-373.
2. Mehta JL. The role of Lox-1, a novel lectin- like receptor for oxidized low density lipoprotein in atherosclerosis. *Can J Cardiol* 2004; 20(suppl B): 32B-36B.
3. EL-Bassiouni EA, Helmy MH, EL-Zoghby S M, Nabi EL, Kamel MA, Hosny RM. Relationship between level of circulating modified LDL and the extent of coronary artery disease in type 2 diabetic patietns. *Br J Biomed Sci* 2007; 64(3): 109-116.
4. Caparevic Z, kostic N, Ilic S, Stojanovic D, Ivanovic AM. Oxidized LDL and C-reactive protein as markers for detection of accelerated atherosclerosis in type 2 diabetes. *Med pregl* 2006; 59(3-4):160-164.
5. 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: 245–250.
6. Galland F, Duvillard L, Petit JM, Lagrost L, Vaillant G, Bran JM, Gambert P, Verges B. Effect of Insulin treatment on plasma oxidized LDL/LDL-cholesterol ratio in type2 diabetic patients. *Diabetes Metab* 2006; 32:625–631.
7. 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; 53:297-302.
8. Cardellini M, Andreozzi F, Larratta E, Marini MA, Lauro R, Hribal ML, Perticone F, Sesti G. Plasma Interleukin-6 levels are increased in subjects with impaired glucose tolerance but not in those with impaired fasting glucose in a cohort of Italian Caucasians. *Diabetes Metab Res Rev* 2007; 23:141-145.
9. Pickup JC, Chusney GD, Thomas SM. Plasma interleukin-6, tumor necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 2000; 67:291–300.
10. Arnalich F, Hernanz A, Lopez M , Anderuelo D. Enhanced acute-phase response and oxidative stress in older adults with type II diabetes. Horm Metab Res 2000; 32:407–412.
11. Kado S, Nagase T, Nagata N. Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. *Acta Diabetol* 1999;36:67–72.
12. Shikano M, Sobajima H, Yoshikawa H. Usefulness of a highly sensitive urinary and serum IL-6 assay in patients with diabetic nephropathy. *Nephron* 2000; 85:81–85.
13. Mohan V, Deepa R, Velmurugan K, Premalatha G. Association of C-reactive protein with body fat, diabetes and coronary artery disease in Asian Indians-The Chennai Urban Rural Epidemiology Study (CURES-6). *Diabet Med* 2005; 22:863 - 870.
14. Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arterioscler Thromb Vasc Biol* 2002; 22:1162-1167.
15. Gokulakrishnan K, Deepa R, Mohan V, Gross MD. Soluble P-selectin and CD40L levels in subjects with pre-diabetes, diabetes mellitus and metabolic syndrome-The Chennai Urban Rural Epidemiology Study (CURES-16). *Metabolism* 2006; 55:237-242.
16. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. *Circulation* 2001; 103:491-495.
17. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 2004; 24:816-823.
18. Shanmugan N, Figarola JL, Li Y, Swiderski PM, Rahbar S, Natarajan R. Proinflammatory Effects

of Advanced Lipoxidation End product in Monocytes. *Diabetes* 2008; 57:879-888.
19. Van Lenten BJ, Wagner AC, Navab M, Fogelman AM. Oxidized phospholipids Induce changes in Hepatic paraoxonase and Apo-J but Not Monocyte chemoattractant protein-I via Interleukin-6. *The journal of Biological chemistry* 2001; 276:1923-1929.
20. Massy ZA, Kim Y, Guijarro C, Kasiske BL, Keane WF, O'Donnell MP. Low density Lipoprotein induced expression of interleukin-6, a marker of human mesangial cell inflammation: effects of oxidation and modulation by Lovastatin. *Biochem Biophys Res Commun* 2000; 267:536-540.
21. Kuroki T, Isshiki K, King GL. Oxidative stress: The lead or supporting actor in the pathogenesis of diabetic complications. *American journal of kidney disease* 2000; 16: 134-139.
22. Silverstein RL. Executive summary of the third report of National cholesterol Education program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment panel III). *JAMA* 2001; 285:2486-2497.
23. Nakhjavani M, Khalilzadeh O, Khajehali L, Esteghamati A, Morteza A, Jamali A, Dadkhahipour S. Serum oxidized LDL is associated with Diabetes duration independent of maintaining optimized levels of LDL cholesterol. *Lipids* 2010; 45:321-327.
24. Holvoet P. Relation between metabolic syndrome, oxidative stress and inflammation and cardiovascular disease. *Verh K Acad Geneeskd Belg* 2008; 70:193–219.
25. Lee IT, Chan YC, Lin CW, Lee WJ, Sheu WH. Effect of cranberry extracts on lipid profiles in subjects with type 2 diabetes. *Diabet Med* 2008; 25:1473–1477.
26. Doo YC , Ham SJ, Lee JH, Cho Gy, Hong KS, Han KR . Association Among oxidized low Density Lipoprotein Antibody, C-Reactive protein , Interleukin-6 and circulating cell Adhesion Molecules in patients with unstable Angina pectoris. *Am J Cardiol* 2004;93:554-558.
27. Gumleniczek A, Hopkata H, Rolinski J, Bojarska-Junak A. Interleukin-6 and oxidative stress in plasma of Alloxan-Induced diabetic rabbits after Pioglitazone treatment. *Immunopharmcology and Immunotoxicology* 2006; 28:81-91.
28. Esposito K, Nappo F, Marfella R. Inflammatory cytokine concentration are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* 2002; 106: 2067-2072.
29. Morohoshi M, Fujisawa K, Uchimura I, Numano F. Glucose-dependent interleukin-6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996; 45:954-959.