Soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) levels in serum of normal volunteers and type 2 Diabetics

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ABSTRACT

Oxidative stress is increased in diabetes mellitus and is thought to be involved in the pathogenesis of the chronic complications of diabetes. Oxidized low density lipoprotein (LDL) causes endothelial activation, injury and dysfunction. Serum oxidized LDL receptors (sLOX-1) levels have been reported to be associated with atherosclerosis and diabetic vasculopathy.

Objectives: To compare soluble Lectin-like Oxidized Low Density Lipoprotein receptor-1 (sLOX-1) levels in serum of type 2 Diabetes mellitus patients with normal volunteers; and to study its association with other biochemical parameters.

Materials & Methods: Study participants included in this pilot study were males and females aged 25-50 years. Fasting blood specimens were collected from 21 normal volunteers and 51 type 2 diabetic patients visiting the outpatient department of the Gulf Medical College Hospital, Ajman, UAE. All diabetics included were on Metformin. None of them had renal, cardiovascular or liver disease. Routine biochemical investigations (Lipid profile, Fasting Plasma Glucose, HbA1C) were performed on Roche Cobas 6000 analyzer. Serum Total Antioxidant status was estimated using a kit from Sigma Aldrich, USA. sLOX-1 level in serum was estimated using Human LOX-1 ELISA Kit from Cell Biolabs, USA. Statistical analysis was done on IBM SPSS software version21.

Results: Serum sLOX-1 levels did not follow a "normal" statistical distribution in the population. No difference in the serum sLOX-1 levels was seen between the normal and diabetic participants. sLOX-1 levels did not correlate with age, BMI, fasting glucose, total-, LDL- or HDL-Cholesterol, triglyceride levels or total antioxidant status. However, within the diabetic population, sLOX-1 levels correlated weakly but significantly with the duration of diabetes.

Conclusion: Since sLOX-1 levels in serum showed high variability in the population and did not follow a normal distribution, further studies with larger sample sizes are needed. Correlation of sLOX-1 levels with duration of diabetes is interesting and needs to be investigated further.

Key words: Diabetes mellitus, oxidized LDL, sLOX-1, oxidative stress

INTRODUCTION

Diabetes mellitus is among the most common non-communicable diseases. International Diabetes Federation (2013) estimates indicate that 8.3% of adults which is 382 million people have diabetes worldwide. UAE is among the countries with very high (18.9%) prevalence of diabetes[1]. Diabetes mellitus raises the risk for atherosclerotic cardiovascular diseases which is the most important cause of death in diabetics. Metabolic abnormalities such as hyperglycemia, dyslipidemia with increased LDL and its modified forms, increased advanced glycation end products (AGES), increased free fatty acids and insulin resistance are seen in diabetes mellitus[2]. Oxidative stress is postulated to play a critical role in diabetes and its complications[3]. In diabetes there is high oxidative stress because of increase in the production of reactive oxygen species mainly due to the hyperglycemia and subsequent depletion of the antioxidants. High plasma glucose increases oxidative stress and facilitates LDL oxidation. Oxidized LDL (Ox-LDL) binds to several scavenger receptors expressed on the surface of the cells of the arterial wall and inflammatory circulating cells, leading to endothelial activation, injury and dysfunction. Majority of the effects of oxidized LDL are thought to occur through the expression and activation of a lectin-like receptor for oxidized LDL (LOX-1). LOX-1 is a class E scavenger receptor, expressed mostly on the cell surface of endothelial, macrophages and vascular smooth muscle cells. It was identified in bovine aortic endothelial cells by Sawamura et. al.[4] in 1997. LOX-1 is a 50-kDa membrane protein The OLR1 gene encoding the LOX-1 receptor is present on chromosome 12p13.2-p12.3. It consists of six exons and has four domains: a short N-terminal cytoplasmic, a transmembrane, a connecting neck, and a lectin-like domain at the C terminus which binds ox-LDL. The integrity of the lectin-like domain is important and is essential for its binding activity[5]. The expression of LOX-1 is increased by number of pro-inflammatory stimuli, such as tumor necrosis factor (TNF), C-reactive protein (CRP), interleukin-1 (IL-1), angiotensin II and endothelin-1[6]. Studies have shown that LOX-1 receptor protein is released as soluble forms (sLOX-1) after being proteolytically cleaved at its membrane proximal extracellular domain and this sLOX-1 can be measured in the serum. Soluble LOX-1 (sLOX-) levels have been implicated to be closely associated with the development of atherosclerosis and progression of diabetic vasculopathy which is an important underlying mechanism of diabetic complications[7]. Increased oxidative stress seen in diabetics is known to increase the oxidation of LDL. Ox-LDL is taken up by macrophages via scavenger receptors, such as SR-A1, SR-A2 and LOX-1[6]. LOX-1 is a type II membrane protein receptor for ox-LDL. Endothelial cells primarily express LOX-1 as receptor for ox-LDL and ox-LDL has been shown to up regulate expression of LOX-1[8]. Ox-LDL also promotes the growth and migration of smooth muscle cells, monocytes/macrophages and fibroblasts. Ox-LDL and LOX-1 are thought to play important roles in genesis and progression of atherosclerosis, cardiovascular disease, high blood pressure, myocardial infarction, congestive heart failure and thrombosis[5].

In coronary artery disease, sLOX-1 level has been reported to be an important biomarker for early detection of acute coronary syndrome[9]. It has also been proposed that LOX-1 may be an attractive therapeutic target for vascular disease[10]. Some of the drugs used in the treatment of cardiovascular disease as well as diabetes favorably reduce LOX-1 activation leading to the idea that LOX-1 may play a vital part in development and progression of diabetic nephropathy, neuropathy and cardiovascular diabetic complications[6].

The objectives of this study were to compare serum sLOX-1 levels in normal volunteers and patients with type 2 Diabetes Mellitus; and to study the association between serum sLOX-1 and other parameters including total anti-oxidant status, dyslipidemia, hypertension, obesity and glycemic status.

MATERIALS AND METHODS

Study design and participants: This was a pilot study. Participants were Type 2 diabetes mellitus patients (N=51) on oral antidiabetic drugs, attending the internal medicine out-patient department of GMC Hospital Ajman, and normal adults (N=21) who volunteered to participate from among the GMC Hospital staff. Both males and females aged 25-60 years were included in the study. Those on vitamins, antioxidant supplements, and diabetic patients with known renal, cardiovascular or liver disease were excluded. Approval was obtained from the Ethics and Research committees of Gulf Medical University before the start of the study. Any information revealing the identity of the patient was not recorded.

Methods: Patients were diagnosed as having Type 2 diabetes by the clinician using the American Diabetes Association (2013) criteria.[11] After obtaining informed consent, a questionnaire was completed for each participant comprising of items on socio-demographic characteristics such as age, ethnicity, height, weight, Body Mass Index, duration of diabetes, medication, blood pressure (systolic/diastolic) and details regarding smoking.

Laboratory investigations: Venous blood samples were collected after 8 hours of fasting from the participants and plasma glucose, HbA1c and serum lipids estimated in the GMC Hospital laboratory using established validated methods. An aliquot of the serum collected was stored at -80^{o} C for Total antioxidant status (TAS) and sLOX-1 estimations. Lipid profile was estimated by homogenous enzymatic colorimetry, Plasma glucose by the enzymatic spectrophotometric method using hexokinase and HbA1c by turbidimetric inhibition immunoassay (TINIA) on the Roche Cobas 6000 analyzer. Serum total antioxidant status was assayed in duplicate by the Metmyoglobin-ABTS method using a kit from Sigma Aldrich, USA using Trolox as the standard.[12] Serum sLOX-1 was assayed in duplicate using the Human LOX-1 ELISA Kit from Cellbio labs, USA.[13] The kit had a detection sensitivity limit of 40 pg/mL of human LOX-1. The intra-assay and inter-assay CV were less than 5% and 9% respectively.

Data Analysis: Statistical calculations were done using IBM SPSS Statistics 21.0. Pearsons and Spearmans Rho tests were used for correlation and Independent samples t-test and Mann Whitney U was used for testing significance between the groups. Kolmogorov-Smirnov test was used to test normality of sLOX-1 distribution. One-way analysis of variance (ANOVA), Least Significant difference tests (LSD) were used for comparison within the groups among the diabetics. P value < 0.05 was considered as statistically significant.

RESULTS

Descriptive statistics of the participants

Among the diabetic participants, 36 (71%) were of Asian origin, 11 (21%) were from the Middle-east and 4(8%) of African origin. 90% of diabetic participants were male and 10% were female. The mean age of the diabetic participants was 49 ±10 years. Most of the participants (18) belonged to the age group of 55-64 years and 15 participants belonged to age group 45-54 years. 38 (75%) of the diabetic participants had a family history of diabetes while 13 (26%) did not.

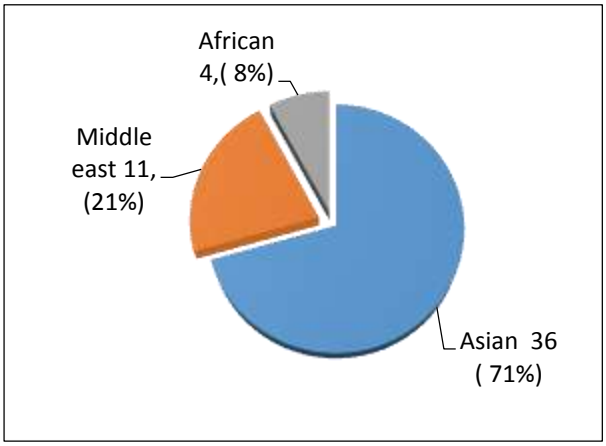

Figure 1A. Ethnicities of the diabetic participants

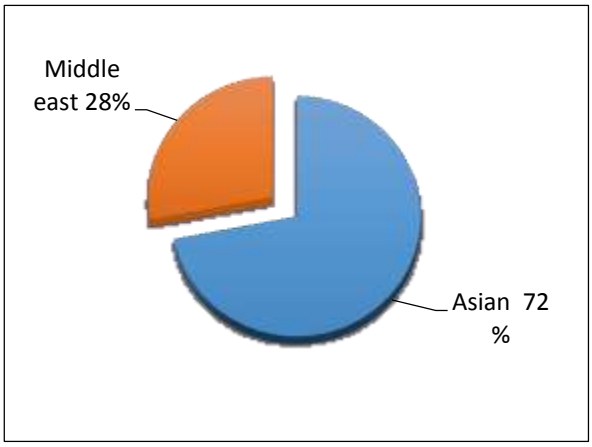

Figure 1B. Ethnicities of the normal volunteers

Among the normal volunteers 72% were Asian and 28% from the Middle-east. 8 (38%) of normal participants were male, 13 (62%) were female. Among the normal volunteers, 18 (86%) belonged to the age group 35- 44yrs.

Table 1. Comparison of parameters between normal and diabetic participants

Parameter	Mean ± SD	
	Normal (n=21)	Diabetic (n=51)
Age* (yrs.)	35.7±13.5	48.9±10.3
BMI* (kg/m^2)	25.70±4.73	27.36±5.52
FBS* (mg/dl)	94.62±6.17	148.45±45.79
HbA1c (%)	5.26±0.59	7.55±1.58
Serum total Cholesterol* (mg/dl)	177.31±21.27	157.44±43.48
Serum Triglyceride* (mg/dl)	90.48±34.20	140.71±73.04
LDL cholesterol* (mg/dl)	111.28±18.68	87.47±36.88
HDL cholesterol (mg/dl)	47.93±14.42	41.12±11.26
TAS mM	1.24±0.35	1.32±0.43

* statistically significant between two groups (P<0.05) as tested by Independent samples t-test

Serum sLOX-1 levels in diabetic and normal participants

Serum sLOX-1 levels were not found to follow a 'normal' distribution in either the diabetic or the normal volunteer group when tested by the Kolmogorov Smirnov test. Therefore nonparametric tests were used for statistical analysis of the data. Table 2 shows the range, mean, median and interquartile interval of serum sLOX-1 levels in diabetic participants and normal volunteers. They were compared using the Mann Whitney-U test. No statistically significant differences were seen in the mean or median serum sLOX-1 levels in the normal and the diabetic participants when tested by independent sample t-test.

Table 2. Serum sLOX-1 levels in Diabetic participants and normal volunteers

Group	No.	sLOX-1 levels (pg/mL)			
		Range	Mean	Median	Interquartile interval
Diabetic participants	51	397.89 - 2842.10	1209.4	1076.84	716.84-1582.10
Normal volunteers	21	310.52 - 3063.15	1325.9	1105.26	631.57-2121.05

Correlation of Serum sLOX-1 with various biochemical parameters

The correlation of the plasma sLOX-1 with other biochemical parameters was analyzed by Spearman's rank correlation test. There were no statistically significant or strong correlations between sLOX-1 levels and any of the biochemical parameters tested in the normal and diabetic population.

There was no statistically significant difference in the mean serum s-Lox-1 levels between the hypertensive and non-hypertensive, dyslipidemic and non-dyslipidemic, and the smokers and non-smokers when tested by the Independent sample t-test (Table 3).

Table 3. Serum sLOX-1 levels in diabetic participants with/without hypertension, dyslipidemia, smoking, and statin therapy.

Condition		n	Mean sLOX-1(pg/ml)
Hypertension	Yes	21	1306.46
	No	30	1141.47
Dyslipidemia	Yes	27	1333.99
	No	24	1069.25
Statin therapy	Yes	25	1325.92
	No	26	1125.51
Smoking	Smokers	15	1325.7

Non-smokers	36	1160.5

Serum sLOX-1 levels and duration of diabetes among the diabetic participants

Serum sLOX-1 levels did not correlate with smoking, dyslipidemia or hypertension. However sLOX-1 levels were found to correlate weakly but statistically significantly with the duration of diabetes (Table 4).

Table 4. Correlation of sLOX-1 levels with Duration of diabetes

	Correlation with serum slox-l levels
Duration of diabetes in months	0.445**

** Correlation is significant at the 0.01 level (2-tailed) as tested by the Spearman's correlation test

In order to study the association of the duration of diabetes with the sLOX-1 levels further, the diabetic participants were divided into three groups according to the duration of diabetes in months. Table 5 shows the various parameters in the three groups.

Using the multiple comparisons test of significance, mean sLOX-1 levels was found to be significantly different between the group-1 (0-24 months) and group-3 (>60 months) and between the group-2 (25-60 months and group-3 (>60 months). Results are shown in Table 6.

Table 5. Mean serum sLOX-1 levels and other biochemical parameters in diabetic participants distributed according to duration of diabetes

Parameters	Duration of diabetes		
	Group-1 0-24 months	Group-2 25-60 months	Group 3 >60 months
N (%)	18 (35.3%)	20 (39.2%)	13 (25.5%)
sLOX-1 (pg/mL)*	939	1192	1609
TAS (mM)	1.320	1.297	1.349
FPG (mg/dl	129	165	149
PPS (mg/dl)	204	258	255
HbA1c (%)	7.4	7.6	7.5
T. Cholesterol (mg/dl)	160.7	164.9	141.3
Triglyceride (mg/dl)	135.9	160.1	117.3
HDL (mg/dl)	42.5	40.5	39.9
LDL (mg/dl)	90.94	92.4	75.0

*. The mean difference was significant at the 0.05 level using Analysis of variance (ANOVA)

Table 6. Multiple comparisons test of mean sLOX-1 levels between the groups of diabetic participants.

Duration of diabetes	P value		
	Group-1 (0-24 months)	Group-2 (25-60 months)	Group-3 (>60 months)
0-24 months		0.157	0.001*
25-60 months	0.157		0.036*
>60 months	0.001*	0.036*	

* statistically significant between two groups (P<0.05)

DISCUSSION

Diabetic participants in this study had the mean age of 48.9±10.3 yrs. The mean age of the normal volunteers in this study was 35.7±13.5yrs. There was a significant age difference between the two groups. Normal volunteers were included based on history as well as biochemical parameters being within normal reference range, therefore most of them were younger. Most of the participants both diabetic and normal were of Asian ethnicity. This represented the diabetic participants visiting the clinician in the outpatient department of the GMC Hospital. While prevalence of diabetes mellitus is not known to be very different between men (13.6%) and women (11.2%),[14] most diabetic participants in this study were male (90%) again representing the diabetic population visiting the GMC hospital.

Most of the diabetic participants were overweight and had a BMI between 25 and 29 Kg/m2. Obesity is a known risk factor for diabetes and causes peripheral resistance to insulin-mediated glucose uptake[15]. Family history is known to be an important risk factor for type 2 diabetes mellitus. Thirty-nine percent of patients with type 2 diabetes have been reported to have at least one parent with the disease.[16] Family history of diabetes was positive in 74% of the diabetic participants. In this study, we found 29% of the diabetic participants to have hypertension and 52 % dyslipidemia. However since many of them were on statin drugs for treatment of hypercholesterolemia, the serum cholesterol, LDL and HDL were not found to be significantly different between the normal and the diabetic groups. Serum triglyceride was, however, found to be significantly different. In Type 2 diabetes dyslipidemia is an important and common risk factor for coronary heart disease (CHD) that is the leading cause of morbidity and mortality worldwide[15].

Oxidative stress has been reported to be increased in diabetics and it has been associated with the hyperglycemia. Antioxidants have been reported to be decreased in diabetic participants.[17] In this study, we did not find a significant difference in the total antioxidant status between the diabetics and the normal volunteers. This could also be due to the fact that many of the diabetic participants were also on statins for treatment of dyslipidemia and statins are known to decrease oxidative stress[18].

sLOX-1 levels in the normal and diabetic populations in this study was not found to be normally distributed. This is similar to reports in literature.[9] sLOX-1

levels in normal participants (median 1105 pg/mL) observed in this study is similar to that reported by Inoue et.al[19] where a median value of 1060 pg/ml was found in Japanese men and a median value of 797.8 pg/mL was found in women. The range of sLOX-1 levels observed by us is also similar to that reported by them.

A number of studies report increased LOX-1 expression in the presence of hyperglycemia in tissue culture and animal models.[20] However there are very few studies on sLOX-1 levels in type 2 diabetes. In a study by Tan et.al,[21] diabetic participants were found to have significantly higher (10%) sLOX-1 levels than non-diabetic controls. However in our study there was no significant difference between the diabetic and the normal participants. The diabetic group had a median sLOX-1 value of 1076 pg/ml and the normal had 1105pg/ml. This is similar to the observations of Kume et.al[9] on 176 patients undergoing coronary angiography, among which 35% were diabetic, where no significant difference in sLOX-1 levels was seen between diabetic and non-diabetic patients. Further, no difference was seen between groups with and without conventional risk factors such as hypertension and smoking which is similar to that reported by us in this study.

LOX-1 expression has been reported to be highly inhibited by treatment with cholesterol lowering drugs[22]. In this study there was no significant difference in sLOX-1 levels between those diabetic participants on statin therapy and those who were not taking statins. Tan et al[21] have reported that in participants with type 2 diabetes mellitus, with improvement in the glycemic control there is decrease in the circulating levels of sLOX-1. In this study, no correlation was observed between sLOX-1 levels and the HbA1c levels. The diabetic participants were on oral anti-diabetic drugs and though the fasting plasma glucose levels were higher, there was no significant difference in the HbA1c levels between the normal volunteers and diabetics suggesting that the hyperglycemia is well controlled. It is possible that there was no difference in sLOX-1 levels or total antioxidant status due to the tight control of the plasma glucose levels observed in these participants.

As diabetes progresses elevated free radical formation, elevated inflammatory cytokines and an overwhelming endogenous anti-inflammatory response, propagate the oxidative stress in diabetic participants[3]. In this study, sLOX-1 levels correlated weakly but significantly with the duration of diabetes. Further, when the diabetic participants were divided into groups based on duration of diabetes, significant difference was found in sLOX-1 levels between those who had diabetes of duration less than 5 years compared with those with more than 5 years. These observations are similar to that reported by Nakhjavani et al [23] who found Ox-LDL was significantly associated with diabetes duration ($r = 0.519$, $P = 0.001$) and serum ox-LDL level increases with the length of diabetes, even though the patients' LDL-cholesterol level is maintained at a desirable level.

CONCLUSION

This was a pilot study to compare serum sLOX-1 levels between normal and diabetic participants. No difference was observed in the serum sLOX-1 levels between normal and diabetic participants. There were no correlations between serum sLOX-1 levels and other parameters including age, BMI, fasting plasma glucose, HDL-, LDL- or total-Cholesterol, serum triglyceride and serum total antioxidant status, in the diabetic or the normal participants.

However in the diabetic population, sLOX-1 levels correlated weakly but very significantly with the duration of diabetes. Significant difference in sLOX-1 levels were found between participants who were diabetics for more than 5 years compared to those who had duration of diabetes less than five years.

Recommendations and limitations of the study

While the significant correlation of sLOX-1 levels with duration of diabetes is very interesting, more studies with larger number of diabetic participants are needed to draw firm conclusions since sLOX-1 in serum is not normally distributed and the range is very large.

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