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Research Article

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Gender-Related Differences of Oxidized Low-Density Lipoprotein Levels in Adults with and without Type 2 Diabetes

Reyhane Hizomi Arani[1], Soghra Rabizadeh[1], Armin Rajab[1], Firouzeh Heidari[1], Alireza Esteghamati[1], Hossein Mirmiranpour[1] and Manouchehr Nakhjavani[1]

[1]Endocrinology and Metabolism Research Center (EMRC), Vali-Asr Hospital, Tehran University of Medical Sciences, Tehran, Iran.

***Corresponding author:**
Manouchehr Nakhjavani, M.D. Professor of Endocrinology and Internal Medicine, Endocrinology and Metabolism Research Center (EMRC), Vali-Asr Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

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Abstract

Objective

We aimed to evaluate potential gender differences in serum levels of oxidized low-density lipoprotein (ox-LDL) in patients with type 2 diabetes (T2D) and controls. We looked at the correlation between ox-LDL levels and 10-year cardiovascular disease (CVD) risk using Framingham risk score.

Materials and Methods

A total of 454 (268 women) patients with T2D and 140 (107 women) participants without T2D were recruited for this study. Ox-LDL, lipid profile, glycemic indices, anthropometric measurements, and 10-year CVD risk score were compared among the subgroups.

Results

In participants without T2D, women had lower levels of ox-LDL in comparison with men (33.94±6.48 U/L vs. 59.54±14.36 U/L; adjusted odds ratio (OR) of 0.968 (95% confidence interval (CI), 0.947-0.990). However, in participants with T2D, ox-LDL levels were higher in women compared to men (92.11±38.07 U/L vs. 79.18±34.63 U/L; adjusted OR of 1.008 (95%CI, 1.001-1.014). In addition, in female participants without T2D, ox-LDL levels in post-menopausal women were higher than the pre-menopausal ones (38.48±6.48 U/L vs. 29.66±5.16 U/L; adjusted OR of 1.036 (95%CI, 1.005-1.068). In contrast, this difference disappeared in female participants with T2D (92.08±39.40 U/L vs. 91.38±34.73 U/L; adjusted OR of 0.999 (95%CI, 0.994-1.024). Moreover, after controlling for potential confounders, the 10-year CVD risk score had a significant association with ox-LDL levels in men without T2D (R=0.899, p ≤0.001), women without T2D (R=0.318, p=0.012), men with T2D (R= 0.446, p=0.003) and women with T2D (R= 0.298, p=0.001).

Conclusions

Participants with T2D had higher levels of ox-LDL compared to the controls. Moreover, among participants with T2D, the levels of ox-LDL increased more adversely in women than men. T2D may override the effect of gender and menopausal status on ox-LDL.

Keywords: Type 2 Diabetes; Oxidized low-density lipoprotein; Oxidative stress; 10-year cardiovascular disease risk prediction; Framingham risk score.

Introduction

Oxidative modification of low-density lipoprotein (LDL) was introduced as a hallmark of atherosclerosis development [1, 2]. Holvet et al. showed that oxidized low-density lipoprotein (ox-LDL) levels was more sensitive than the Global Risk Assessment Score for Cardiovascular Risk prediction (GRAS) [3]. Moreover, the health, aging, and body composition (Health ABC) cohort identified ox-LDL as a potential factor for increased risk of myocardial infarction (MI) [4]. The role of type 2 diabetes (T2D) on increasing oxidative stress markers and endothelial dysfunction has been well known [5-7]. Njajou et al. showed a positive and strong association between ox-LDL and measures of insulin resistance, independent of visceral fat or body mass index (BMI) [8]. As diabetes progresses, inflammatory cytokines, free radical formation, and lipid peroxidation increase overwhelmingly [9].

Men have more oxidative stress biomarkers and peroxide production in the vascular cells compared to the same age women [10]. Moreover, experimental and clinical data indicated that women seem to have more significant antioxidant potential and consequently less susceptible to oxidative stress [11]. Furthermore, there are differences in the activities or expression of antioxidant enzymes between men and women [10]. More visceral fat accumulation in men could be associated with dyslipidemia and increased small dense LDL particles that are more susceptible to oxidation [8, 12]. Studies concerning the effect of gender on levels of ox-LDL are controversial and inconclusive. Hermsdorff et al. showed that men have significantly higher levels of ox-LDL compared to women [13]. However, Harmon et al. and Barbosa et al. showed no gender difference among healthy subjects [14, 15]. Data from the Health ABC cohort also revealed that white men and women had similar ox-LDL levels, whereas black women had significantly higher levels of ox-LDL compared to black men [8].

To the best of our knowledge, although the previous studies focused on the effect of gender on serum ox-LDL levels, there is no literature available studying the impact of gender on ox-LDL in patients with T2D. Most of the studies on this issue are mainly from developed countries. Here, we aimed to assess ox-LDL levels among women and men with and without T2D and evaluate potential gender differences in serum ox-LDL levels.

Materials and methods

Study design and population study

A cross-sectional study was conducted in Vali-Asr hospital affiliated with Tehran University of Medical Sciences (TUMS) in 2021. We randomly selected a total of 493 participants from the outpatients who visited our adult diabetes clinic and 169 healthy controls. We chose healthy controls from the patients who visited our endocrinology clinic for the reason except diabetes. All the participants were living in the capital city of Tehran with similar environmental circumstances and lifestyles. Past medical, drug, and family history, as well as physical examination were recorded. Exclusion criteria were age<20 and >80 years old, duration of diabetes>20, alcohol consumption, pregnancy, having malignancy, renal disease (creatinine > 1.5 mg/dL or glomerular filtration rate < 70 cc/min, proteinuria, and prevalent cardiovascular disease (CVD) at baseline. Additionally, women treated with hormone replacement therapy or consuming contraceptive medications and women with a history of surgical menopause were excluded. Also, we excluded subjects with missing data on relevant covariates and ox-LDL measurement. So, the remaining 454 participants with T2D and 140 controls were eligible for analysis.

Ethical considerations

This research complied with the principles of the declaration of Helsinki [16]. Ethics Committee of Tehran University of Medical Sciences approved this study (Code: 982826). Written informed consent was obtained from the patients for publication of this study. A copy of the written consent is available for review by the Series Editor of this journal.

Clinical and laboratory measurements

Anthropometric measurements were conducted by well-trained examiners. Weight was measured by a calibrated balance beam scale in kilograms wearing indoor clothing. We measured patients' height in centimeters with shoes off. We calculated BMI using the formula BMI= weight (kg)/ height² (m²). Blood pressure (BP) was applied to the right arm with a digital sphygmomanometer, after 15 minutes of rest in the sitting position.

Morning blood samples were collected after almost 12 hours of fasting. The samples were centrifuged, and the extracted serums were kept at -70°C until analysis. We measured total cholesterol (TC), triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glycated hemoglobin (HbA1c), fasting blood sugar (FBS), and serum creatinine. We estimated HbA1c by high-pressure liquid chromatography. Serum creatinine levels were measured using kinetic colorimetric Jaffe with the sensitivity of 0.2 mg/dL (ranged from 0.2 to 15 mg/dL).

HDL-C, TC, and triglyceride were measured using the immunoinhibitory method, Trinder method, and enzymatic method, respectively; using the available kit (Lipid, Pars Azmoon Co., Karaj, Iran). Also, if triglyceride <400 mg/dL, the Friedewald formula was applied for calculating LDL-C and if triglyceride ≥400 mg/dL, LDL-C was measured by direct assay. Ox-LDL was measured by the sandwich enzyme-linked immunoabsorbant assay (ELISA) method as previously described (Mercodia, Uppsala, Sweden). Briefly, we added diluted samples, standards, controls containing ox-LDL into the wells of microplates coated by high affinity monoclonal antibodies [17]. During the first incubation (2 hours at 37°C), antibodies immobilized on the microtiter wells and capture the antigen in the serum samples. Then we washed the samples to remove unbound components and added a peroxidase conjugated antibody to the microtiter wells. After the second incubation (2 hours at 37°C), we added an acidic stop solution to finish the reaction. There was a direct proportion between intensity of the yellow color and ox-LDL level in the samples. We generated a curve of absorbance unit vs. concentration applying the values

received from standards. Here, the ox-LDL in the samples was determined directly and we could read them by ELISA reader at the wavelength of 450 nm. The inter-assay and intra- assay coefficient of variation of the ox-LDL was 7.3% and 4%, respectively. The measurement range was 9 -140 U/L, and the detection limit was 1.0 mU/L.

Definition of terms

Diabetes was diagnosed as having FBS ≥7 mmol/L, or HbA1c≥6.5, or taking any glucose-lowering drugs based on the criteria of the American diabetes association (ADA) [18]. Menopause classification was according to women's medical history (menopause: a history of at least 12 months without menstrual bleeding). The 10-year CVD risk score is a sex specific risk prediction model which was developed by the Framingham Heart Study by following formula:

Risk factors = (ln (Age) * 2.32888) + (ln (TC) * 1.20904) - (ln (HDL-C) * 0.70833) + (ln (Systolic blood pressure) * Hypertension medication factor) + Smoking + Diabetes - 26.1931 Risk = 100 * (1 – (0.95012^ (e^ (Risk factors))) The 10-year CVD risk score used to estimate the risk of first CVD event development over the future 10 years in each person and to identify high risk subjects. Validity and reliability of it have been reported in an Iranian population previously [19, 20].

Statistical analysis

Routine sample size calculation (with alpha=0.05 and power=0.8) was employed for estimating the proper sample size. The Kolmogorov–Smirnov test, histogram, and P-P plot were run to test the normality of the study population. We presented the data of continuous variables as mean ± standard deviation and dichotomous variables as frequency (%). T-test and Chi-square test analyses were used for group comparisons, as appropriate. Mann-Whitney U test was performed to assess the differences of non-parametric variables (duration of diabetes). Using multinomial logistic regression, we compared the ox-LDL levels between women and men (reference group) in diabetic and non-diabetic groups, separately, by reporting odds ratios (OR) [95% confidence interval, 95%CI] in three levels of adjustment: 1) crude; 2) age and BMI adjusted; 3) adjusted for age, BMI, triglyceride, HDL-C, LDL-C, TC, smoking status, FBS, and family history of CVD. Moreover, we compared the ox-LDL levels between pre-and post-menopausal women (pre-menopausal as reference).

Pearson's correlation coefficients test was employed to evaluate the relationship between ox-LDL and 10-year CVD risk score. Multivariable-adjusted partial correlation coefficients (PCC) and scatter plot were used to reflect mentioned relationship after controlling for BMI, WC, HbA1C, LDL-C, triglyceride, family history of CVD, and T2D duration (among participants with T2D). We employed the statistical package SPSS 21 for windows (Chicago, Illinois, USA) for the analysis and considered p <0.05 as statistically significant.

Result

Baseline characteristics of 594 subjects, including 186 men with T2D, 33 men without T2D, 265 women with T2D, and 107 women without T2D were presented in Table 1. Among non-T2D participants, men had significantly higher levels of triglyceride, ox-LDL, ox-LDL/LDL-C, and 10-year CVD risk scores and lower HDL-C compared to women (p <0.01). However, in T2D group, men had significantly lower levels of triglyceride, ox-LDL, ox-LDL/LDL-C, and 10-year CVD risk score and higher HDL-C compared to women (p <0.01). Mean levels of ox-LDL and ox-LDL/LDL-C across the subgroups were illustrated in Figure 1 using bar chart. Men and women without T2D had ox-LDL levels of 59.54 ± 14.36 U/L and 33.94 ± 6.48 U/L. Also, men and women with T2D had ox-LDL levels of 79.18±34.63 U/L and 92.11±38.07 U/L. In addition, post-and pre-menopausal women without T2D had ox-LDL levels of 38.48±6.48 U/L and. 29.66±5.16 U/L, whereas post-and pre-menopausal women with T2D had ox-LDL levels of 92.08±39.40 U/L and 91.38±34.73 U/L.

Table 1. Baseline characteristics of participants with and without type 2 diabetes (T2D).

Number of participants Continuous variables	T2D			Non-T2D		
	Women	Men	p	Women	Men	p
	268	186		107	33	
Age (year)	54.5 (9.0)	56.4 (10.6)	0.26	50.8 (8.8)	52.3 (6.2)	0.18
BMI (kg/m²)	25.8 (4.2)	26.0 (4.8)	0.41	26.1 (4.0)	26.6 (2.6)	0.35
WC (cm)	88.4 (21.7)	92.4 (25.3)	0.17	82.9 (22.4)	87.0 (19.1)	0.20
SBP (mmHg)	129.7 (17.5)	134.8 (17.4)	0.29	126.6 (15.8)	120.9 (10.4)	0.30
DBP (mmHg)	83.8 (13.3)	86.6 (21.1)	0.09	80.0 (8.8)	85.4 (7.4)	0.12
FBS (mmol/L)	10.1 (3.5)	9.8 (3.4)	0.48	5.0 (1.3)	4.8 (0.5)	0.55
HbA1c (%)	8.2 (1.7)	8.2 (1.9)	0.67	4.9 (0.3)	5.1 (0.3)	0.54
Creatinine (mg/dL)	0.9 (0.3)	0.9 (0.2)	0.89	0.9 (0.1)	0.9 (0.1)	0.91

Triglyceride (mg/dL)	196.9 (95.6)	164.6 (77.8)	<0.001	80.6 (23.0)	126.2 (51.0)	<0.001
LDL-C (mg/dL)	97.8 (29.3)	99.0 (32.7)	0.44	103.1 (20.4)	103.2 (23.2)	0.85
HDL-C (mg/dL)	37.3 (10.5)	43.6 (11.5)	0.03	49.0 (11.5)	44.1 (12.3)	0.04
TC (mg/dL)	203.0 (52.8)	193.4 (48.3)	0.52	209.4 (32.0)	195.7 (37.8)	0.65
10-year ASCVD risk score (%)	10.8 (6.8)	8.3 (2.1)	0.02	2.8 (0.4)	4.2 (1.2)	<0.01
Duration of diabetes (year)	8.0(2.0-12.0)	8.0(4.0-12.5)	0.25	-	-	-
Categorical variables						
Family history of CVD, n (%)	35(13.0)	23(12.3)	0.61	11(10.2)	4(12.1)	0.48
Glucose lowering drug, n (%)			0.21			
Oral agent	123(45.8)	98(52.6)	-	-	-	-
Insulin	102(38.0)	59(31.7)	-	-	-	-
Insulin + Oral agent	39(14.5)	22(11.8)	-	-	-	-

Data are presented as mean (SD) for continuous variables/ frequency (%) for categorical variables. Duration of diabetes presented as median (interquartile range).

BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBS: Fasting blood sugar; HbA1c: Hemoglobin A1c; LDL-C: Low-density lipoprotein; HDL-C: High-density lipoprotein; TC: total cholesterol; Ox-LDL: Oxidized low-density lipoprotein; ASCVD: Atherosclerosis cardiovascular disease; CVD: Cardiovascular disease; n: Number.

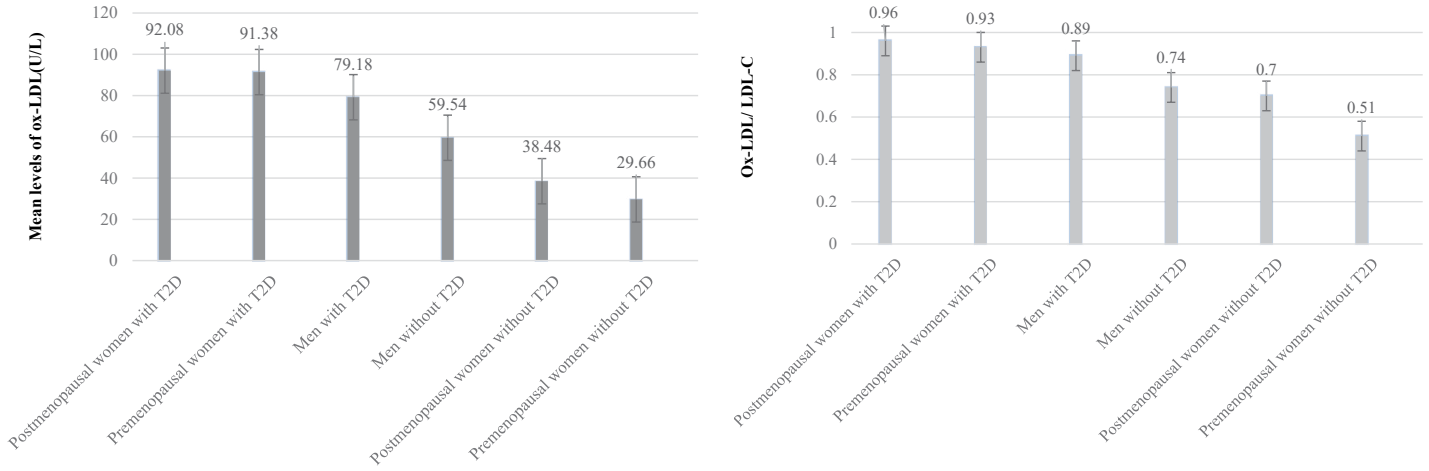


Figure1. Mean levels of ox-LDL and ox-LDL/ LDL-C among men and women participants with and without type 2 diabetes

In Table 2, after adjustment for age, BMI, triglyceride, HDL-C, LDL-C, TC, smoking status, FBS, and family history of CVD, we observed that in participants without T2D, women had lower levels of ox-LDL than men with the adjusted OR of 0.968 (95% CI, 0.947-0.990). However, in participants with T2D, ox-LDL levels were higher in women than men with the adjusted OR of 1.008 (95%CI, 1.001-1.014). Moreover, in female participants without T2D, ox-LDL levels in post-menopausal women were higher than the pre-menopausal ones with the adjusted OR of 1.02 (95%CI, 1.00-1.08). In contrast, this difference disappeared in female participants with T2D with the adjusted OR of 0.999 (95%CI, 0.994-1.024).

Table 2. Crude and adjusted odds ratios of ox-LDL level among participants with and without type 2 diabetes (T2D).

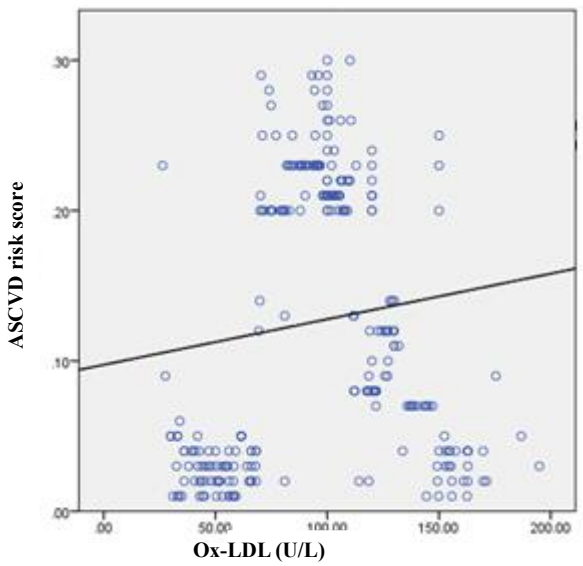
Variables	Crude odds ratio (95%CI)	Age and BMI adjusted odds ratio (95%CI)	Full-adjusted odds ratio (95%CI)†
T2D			
•	Women	1.012 (1.007-1.017)	1.012 (1.005-1.019)
•	Men	Reference	Reference
Non-T2D			
•	Women	0.976(0.962-0.990)	0.973(0.959-0.988)
•	Men	Reference	Reference
T2D women			
•	Premenopausal	Reference	Reference
•	Postmenopausal	1.006 (0.999-1.014)	0.999 (0.998-1.021)
Non-T2D women			
•	Premenopausal	Reference	Reference
•	Postmenopausal	1.019 (1.005-1.032)	1.026 (1.007-1.045)

In table 3, Pearson's correlation coefficients indicated that ox-LDL and the 10-year CVD risk score had a significantly positive association in four groups. PCC analyses were performed to quantify their association independent of other potential confounding variables which remained significant among men without T2D (R=0.899, p ≤0.001), women without T2D (R=0.318, p=0.012), men with T2D (R= 0.446, p=0.003) and women with T2D (R= 0.298, p=0.001) (Figure2).

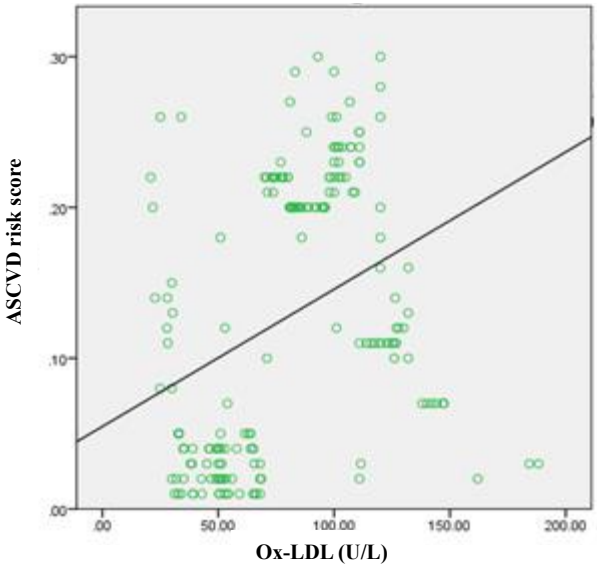
Table 3. Pearson's and Partial Correlation coefficients analyses of the relationships between 10-year CVD risk score and ox-LDL level among participants with and without type 2 diabetes (T2D).

	Pearson's Correlations Coefficient		Partial Correlations Coefficient†	
	R	p	R	p
Women withT2D	0.122	0.043	0.298	0.001
Men with T2D	0.355	<0.001	0.446	0.003
Men without T2D	0.812	<0.001	0.899	<0.001
Total	0.315	<0.001	0.398	<0.001

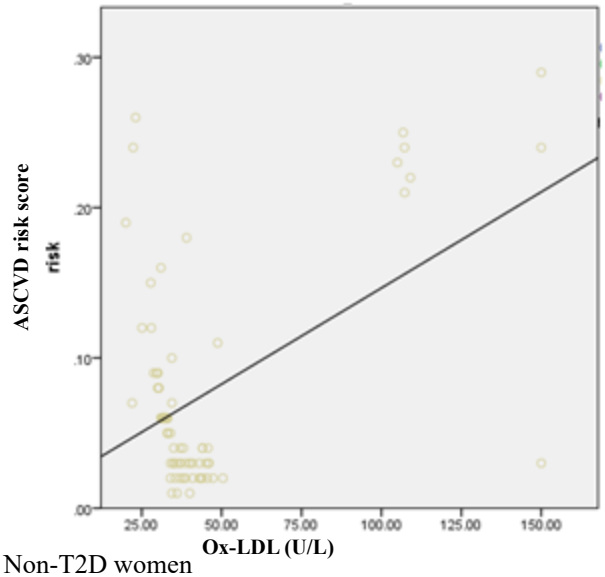
†Partial correlation coefficient from multiple linear regression model controlling for body mass index, waist circumference, hemoglobin A1c, low density lipoprotein cholesterol, triglycerides, family history of cardiovascular disease, and T2D duration (only among T2D participants).
CVD: Cardiovascular disease



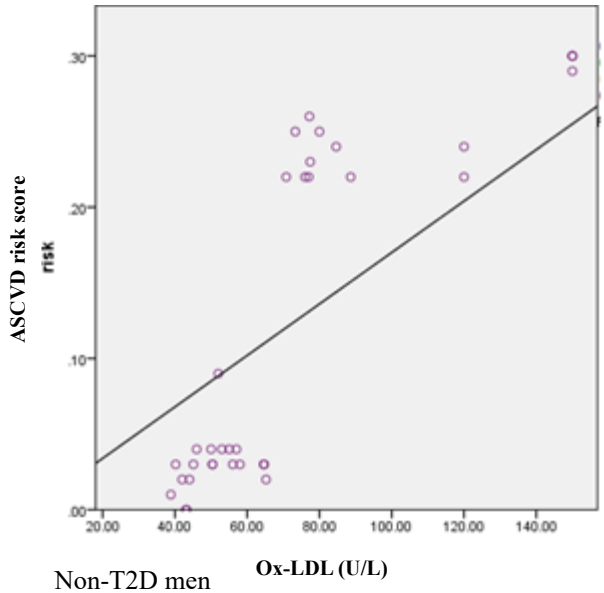
T2D women



T2D men



Non-T2D women



Non-T2D men

Figure2. Scatter plot demonstrating the significant correlation between ox-LDL and 10-year ASCVD risk score

Discussion

In the present study, we investigated the serum levels of ox-LDL across gender and diabetic status. Among non-T2D participants, male participants had higher ox-LDL levels. However, in participants with T2D, ox-LDL levels increased more adversely in women compared to men. In this sense, we might speculate that women with T2D could have more unfavorable modifications in oxidative stress markers and inflammation. The reason for this "female disadvantage" in diabetes status remains unclear. It may be due to sex hormones, and sex chromosome genes asset, as well as disparity across genders in the treatment of cardiovascular risk factors among individuals with diabetes [21].

Our subgroup analyses showed that post-menopausal women without T2D had higher levels of ox-LDL than pre-menopausal ones. It has been speculated that women in the menopause transition may have changes in hormones and metabolic profile that associated with change in body fat distribution specially increase in central adiposity leading to unfavorable modifications in markers of oxidative stress [13]. However, pre-and post-menopausal women with T2D had the same levels of ox-LDL. A study suggested women with diabetes lose the advantages associated with sex-hormones during premenopausal period in the clearance of dietary lipids in non-diabetic counterparts which may elevate the atherogenic lipoprotein profile. This could also contribute to the loss of protection against CVD events in women with diabetes [22]. Moreover, we previously mentioned that dyslipidemia in pre-menopausal women with T2D was similar to post-menopausal ones, and they have the same incidence of MI [23].

In the current study, the mean levels of LDL-C and TC did not differ considering gender and T2D status. Maybe because most of our participants were undertreated with statins. However, the levels of ox-LDL and ox-LDL/LDL-C were significantly different among subgroups. We previously introduced ox-LDL/LDL-C as a lipid biomarker for the estimation of oxidation [24]. This finding is in line with our previous research stated that keeping an optimized level of LDL-C, based on guidelines for the management of lipids in diabetes, does not sufficiently effect on the ox-LDL levels [9]. We guessed that despite controlling LDL-C and TC, the oxidation process and its adverse effects remain persistent.

In routine practice, traditional cardiovascular risk factors are applied for predicting CVD risk. However, the impact of more recent cardiovascular risk factors including endothelial dysfunction markers is not clear [25]. The current study showed that levels of circulating ox-LDL were independently associated with a 10-year CVD risk prediction using Framingham scoring before CVD events. We speculated that circulating ox-LDL is a helpful marker for identifying persons at higher risk for CVD besides the known factors including sex, age, total and HDL cholesterol, hypertension, diabetes mellitus, and smoking. Previous researches elaborate on the value of ox-LDL as a biomarker of CVD [26]. In this line, a systematic review indicates that increased levels of ox-LDL are significantly associated with clinical ASCVD events with an effect size of 1.79 (95% CI: 1.56-2.05). As LDL particles are oxidized within the sub-intimal of arteries, they deposited as atherogenic plaques in the arterial wall [1]. Moreover, subgroups analyses indicated a more pronounced association of ox-LDL and predicted 10-year CVD risk among male participants after adjustment for potential confounding variables. Thus, the effects of endothelial dysfunction biomarkers on ASCVD risk may differ among genders [25, 27]. Suzuki et al. also mentioned superior performance (sensitivity, specificity) of ox-LDL as a diagnostic marker of coronary artery disease as compared against other lipid markers (TC, triglyceride, HDL-C, LDL-C, and TC / HDL-C ratio) with optimal performance in younger men [28]. Understanding gender differences and the impact of diabetes on clinical presentation and pathogenetic mechanisms on ASCVD is essential, since women and men may experience diabetes-related diseases differently.

This research was a cross-sectional study, so the primary limitation was the lack of follow-up of the patients. Moreover, the data on components related to sex hormones were not available to assess their actual effect on ox-LDL levels. Furthermore, accurate analysis of lipid peroxidation is confounded by enzymatic and non-enzymatic lipid peroxidation during serum formation. Hence, more studies should be done using plasma to apply procedures to prevent lipid peroxidation.

Conclusions

Participants with T2D had higher ox-LDL levels compared to the non-T2D subjects. Moreover, among participants with T2D, the levels of ox-LDL increased more adversely in women than men. Also, in female participants without T2D, ox-LDL levels in post-menopausal women were higher than the pre-menopausal ones, whereas in women with T2D, ox-LDL was elevated to the same degree in pre-and post-menopausal ones.T2D may override the effect of gender and menopausal status on ox-LDL levels. Furthermore, in our data set, predicted 10-year CVD risk and serum levels of ox-LDL were significantly correlated independent of glycemic, lipid, and anthropometric indices. Current data suggested that ox-LDL could be applied to identify the patients with high risk of CVD among subjects with and without T2D to employ proper prevention management [29]. More studies are needed to confirm prognostic value of ox-LDL.

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