Oxidized Low-Density Lipoprotein (Ox-LDL) and Malondialdehyde (MDA) and Menopause: Loss of Protective Effect of Premenopuasl Status in Type 2 Diabetes Mellitus

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Research

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Abstract

Background: A network of factors suggested to influence oxidative modification of serum low-density lipoprotein (ox-LDL) concentrations and it plays critical role in diabetic copmlications.This study aimed to assess the joint effect of Type 2 diabetes mellitus (T2DM), gender and menopausal status on ox-LDL and Malondialdehyde (MDA) levels.

Material and Method: A total of 594 female and male participants were divided into six groups: 189 postmenopausal women with T2DM, 79 premenopausal women with T2DM, 54 premenopausal women without T2DM, 53 postmenopausal women without T2DM, 186 men with T2DM and 33 men without T2DM. Laboratory and anthropometric measurements, metabolic syndrome (MetS) and the 10-year risk score for atherosclerotic cardiovascular disease (ASCVD) were assessed.

Results: Ox-LDL in women with T2DM was three times higher than women without T2DM. However, ox-LDL in men with T2DM was 1.3 times higher than men without T2DM. In non-T2DM participants, postmenopausal women had higher 10-year ASCVD risk score, concentrations of ox-LDL and MDA and higher prevalence of MetS compared to premenopausal women. However, this difference disappeared in participants with T2DM. In The interaction analysis did not show an additional effect of menopausal status and T2DM for ox-LDL and MDA serum levels in female participants (respectively $p= 0.310$, $p= 0.922$). In multivariate analysis, TG was independently associated with ox-LDL concentrations (OR: 1.27, $p \leq 0.001$). In T-test analysis, participants with MetS, higher risk of ASCVD had higher levels of ox-LDL.

Conclusion: The magnitude of the increase in ox-LDL and MDA levels in women with T2DM was significantly higher than men with T2DM. The differences between pre- and postmenopausal women with and without T2DM in circulating ox-LDL and MDA mostly were dependent on T2DM, regardless of menopausal status. T2DM override the effect of menopausal status, gender and age on ox-LDL and MDA concentrations. Having MetS, increased TG levels and higher 10-year ASCVD risk score suggested to be associated with higher levels of ox-LDL.

Background

Oxidized low-density lipoprotein (ox-LDL) is produced from low-density lipoprotein cholesterol (LDL-C) under the oxidative condition and could lead to the sub-endothelial accumulation of foam cells. These foam cells take part in the evolvement of the fibro-fatty complex and atherosclerotic plaques [1]. Ox-LDL particles play an active role in developing endothelial dysfunction and atherosclerotic disease [2-6]. Holvet et al. indicated that the sensitivity of ox-LDL levels was higher than the Global Risk Assessment Score for Cardiovascular Risk prediction (GRAS) introduced for coronary artery disease (CAD) incidence [7]. Similar to ox-LDL, MDA is an end product of polyunsaturated fatty acid peroxidation and considered as an independent biochemical marker for atherosclerosis.[8].

The origin of ox-LDL is not entirely well-known. A network of risk factors suggested influencing ox-LDL concentrations, such as age, gender, T2DM, dyslipidemia, menopause, hypertension, and genetic predisposition. The role of T2DM on endothelial dysfunction has been well known [9-12]. Prolonged

exposure to hyperglycemia in T2DM could increase the imbalance of oxidative and anti-oxidative markers. Ox-LDL may take part in the progression of the macrovascular and microvascular diseases such as diabetic nephropathy and neuropathy [13, 14].

Men have higher levels of peroxide productions and oxidative stress biomarkers in the vascular cells compared to women [15]. Moreover, clinical and experimental data reported more significant antioxidant potential in pre-menopausal women compared to men, which made women less susceptible to oxidative stress [16]. Some studies showed a lower risk and incidence of disease related to oxidative stress such as cardiovascular disease (CVD) in pre-menopausal women compared to age-matched men, with the gradual elimination of this gender-linked preference after menopause [17, 18].

The aim of this study was first; to assess the joint effect of T2DM, gender, menopausal status on serum ox-LDL and MDA levels. Second, to examine serum levels of ox-LDL and MDA in pre- and post-menopausal women with and without T2DM compared to men with T2DM and non-diabetic male controls; third to evaluate independent risk factors, which predict serum level of ox-LDL.

Methods

Current study is a cross-sectional study conducted in the diabetes clinic of Vali-Asr Hospital affiliated with Tehran University of Medical Sciences (TUMS) in 2018. The study population consisted of 594 male and female participants selected from outpatients who attended our adult diabetes clinic and have similar environmental circumstances, lifestyle and diets. They divided into six groups of: 189 post-menopausal women with T2DM, 79 pre-menopausal women with T2DM, 54 pre-menopausal women without T2DM, 53 post-menopausal women without T2DM, 186 men with T2DM and 33 men without T2DM. The duration of T2DM ranged from 1 to 20 years. The participant's age was between 30-79 years. Diabetes was diagnosed according to the criteria of the American diabetes association (ADA) [19]. Menopause classification was based on women’s history. The 10-year ASCVD risk score was defined as the risk of developing the first ASCVD event over a 10-year period which was calculated using the simplified scoring developed by American College of Cardiology/American Heart Association eigher for our diabetic and non-diabeteic participants [20]. Patients divided into fourgroups of low risk (<5%), borderline risk (5%≤ and<7%), intermediate-risk (7%≤ and<19.5%), and high risk (≥19. 5%). Exclusion criteria were smoking, pregnancy, proteinuria, renal involvement (creatinine > 1.5 mg/dl or glomerular filtration rate (GFR) < 70 cc/min) and not having atherosclerotic cardiovascular disease (ASCVD) events at baseline. Also, women on hormone replacement therapy, ones at the age of menopause and women with history of surgical menopause were excluded. None of the patients with T2DM had any overt diabetic complications. Drug history, such as taking anti-hypertensive and lipid-lowering drugs, was not considered as a restriction for inclusion. The study complied with the principles of the Declaration of Helsinki [21]. The Local ethics review committee approved the study protocol. Written informed consent obtained from all patients.

Anthropometric measurements

Well-trained examiners conducted anthropometric measurements. We measured weight by a calibrated balance beam scale. Patients’ heights were measured in centimetrs with shoes off and weights were

measured in kilograms in indoor clothing. Body mass index (BMI) was calculated using the formula BMI= weight (kg)/ height^2 (m^2). After 15 minutes of rest in the sitting position, blood pressure (BP) was applied to the right arm measured with a digital sphygmomanometer.

Blood samples and laboratory evaluations

Morning, blood samples collected after almost 12 h fasting, centrifuged and were kept at -70°C until analysis. Fasting blood samples were obtained for measuring fasting blood sugar (FBS), total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and haemoglobin (HbA1c). Total cholesterol, HDL-C, and TG were measured by the Trinder method, immunoinhibitory method and enzymatic method respectively with the commercially available kit (Lipid, Pars Azmoon Co., Karaj, Iran). Also, LDL-C was calculated using the Friedewald formula. HbA1c was estimated by high-pressure liquid chromatography. Measurement of ox-LDL was performed using a commercially available sandwich ELISA method (Mercodia, Uppsala, Sweden) (Intra-assay CV= 4%, inter-assay CV= 7.3%).

Metabolic syndrome (MetS) was defined based on the modified National Cholesterol Education Program Adult Treatment Panel III definition (NCEP ATP III)[22]. Participants with three or more than the five following criteria were considered as having the MetS: high BP (≥130/≥85 mmHg) or known hypertensive patients, elevated FBS (≥110 mg/dl) or known diabetic patients, hypertriglyceridemia (≥150 mg/dl), low HDL-C (women <50 mg/dl and men<40 mg/dl), and high abdominal obesity (waist circumference >88 cm for women and >102 for men). Measurements for Serum creatinine (Cr) levels were done by kinetic colourimetric Jaffe with the sensitivity of 0.2 mg/dL (range0.2–15 mg/dL). The detection limit was 0.6 mU/l. Serum MDA was measured using a colourimetric method (Cayman, USA) with a dynamic assay range of 0-50 μM (Intra-assay CV= 5.5%, inter-assay CV= 5.9%).

Statistical analysis

The number of the patients(454) was estimated through the routine sample size calculation, (with alpha=0.05 and d=0.05, the prevalence of 62.22% for dyslipidemia in Iranian diabetic population-based on Janghorbani et al. study[23]. The statistical package SPSS 21 for windows (Chicago, Illinois, USA), was used for the analysis. Data were presented as mean ± standard deviation for continuous and number (%) for dichotomous variables. One-way analysis of variance (ANOVA), chi-square test, and T-test analysis were used for group comparisons, as appropriate. Pearson's correlation test was employed to study the correlation between ox-LDL, age, BMI, duration of T2DM, HbA1c, TG, LDL-C, HDL-C, MDA, MetS and 10-year risk score among our participants. The univariate and multivariate linear regression analysis was employed for data modelling. P-values <0.05 were considered statistically significant.

Results

T2DM and Gender

The study population consisted of 594 female and male participants. The baseline characteristics of the study population are presented in Table 1-4. Age, systolic blood pressure (SBP), TG, WC, HDL-C, FBS, HbA1c, the prevalence of MetS, and 10-year ASCVD risk score were significantly different between men and women with and without T2DM. Men and women with T2DM had 1.3 times and 3.0 times higher levels of ox-LDL compared to non-diabetic counterparts, respectively. Moreover, in patients with T2DM, women had about 1.2 times higher levels of ox-LDL in comparison with men (92.11±38.07 vs. 79.18±34.63) . However, in non-diabetic participants, men had about 1.8 times higher levels of ox-LDL in comparison with women (59.54±14.36 vs. 33.94±6.48). Similar to ox-LDL, men and women with T2DM had increased levels of MDA in comparison to non-diabetic control groups (p=0.018 and p= 0.001 respectively)(figure 1). In patients with T2DM, women had significantly higher levels of TG, HDL-C, the prevalence of MetS, and 10-year ASCVD risk score compared to men. However, in non-diabetic participants, men had significantly higher levels of TG, HDL-C, the prevalence of MetS and 10-year ASCVD risk score compared to women.

T2DM and Menopause

Comparing pre- and post-menopausal women with and without T2DM; age, SBP, WC, TG, HbA1c, ox-LDL, MDA levels, the prevalence of MetS, and 10-year ASCVD risk score were significantly different between the groups. Also, the duration of T2DM was significantly longer in post-menopausal women compared to pre-menopausal ones (median of 120.20 months (interquartile range [IQR], 72.00-156.00months) vs. median of 36.00 ([IQR], 2.40-96.00 months), p<0.001). Levels of ox-LDL, MDA, TG, 10-year ASCVD risk score, and the prevalence of MetS were more than two-fold higher in both pre-menopausal and post-menopausal patients with T2DM compared to non-diabetic women. Ox-LDL, MDA, 10-year ASCVD risk score, and the prevalence of MetS had no significant difference between pre- and post-menopausal women with T2DM. However, in non-diabetic participants, post-menopausal women had higher levels of MDA (1.95±.0.50 vs. 1.90±0.93 vs.) and ox-LDL (37.48±6.48 vs. 30.66±5.16) compared to pre-menopausal ones(figure 1). Moreover, interaction analysis did not show additional effect between menopausal status and T2DM for serum levels of ox-LDL and MDA (p = 0.310, p = 0.922 respectively) (figure2).

In both table1 and table3, BMI, diastolic blood pressure (DBP), LDL-C, total cholesterol, creatinine, family history of ASCVD, lipid lowering-drugs and glucose-lowering drugs were not significantly different between groups.

Associations

The ox-LDL levels were significantly correlated with TG (r = 0.68, p ≤ 0.001), HbA1c (r = 0.23, p ≤ 0.001), FBS (r =0.29, p ≤ 0.001), MetS (r=0.61, p ≤ 0.001) and 10-year ASCVD risk score (r=0.82, p<0.001). In T-test analysis, participants with MetS had higher levels of ox-LDL (p=0.003; not shown in tables).Aslo, ox-LDL was significantly different among the ASCVD risk groups (figure 3).In univariate linear regression age, HDL-C, SBP, TG, FBS, HbA1c, and 10-year ASCVD risk score were significantly associated with ox-LDL levels (p ≤0.001) (table 5). In multivariate analysis, TG remained the strongest factor which significantly associated with circulating ox-LDL concentrations between our groups (OR: 1.27, p ≤0.001) (table 5). In linear regression, ox-LDL and MDA were significantly different between participants with and without T2DM after adjustment for age (R=0.514, p ≤0.001).

Discussion

In the present study, T2DM overrided the effect of menopausal status, gender, and ageing on ox-LDL and MDA concentrations. In participants without T2DM, ox-LDL was 1.8 times higher in men compared to women; however, in participants without T2DM, ox-LDL was about 1.2 higher in women in comparison with men. The magnitude of the increase in ox-LDL and MDA levels in women with T2DM was significantly higher than men with T2DM. Diabetes could increase the concentration of lipid peroxidation products following the impairment of oxidation/ anti-oxidation balance induced by prolonged exposure to hyperglycemia [24]. Here we observed that diabetes could eliminate the protective effect of female sex on ox-LDL and MDA and reverse the difference between men and women.

Our results indicated that although ox-LDL and MDA were higher in post-menopausal women without T2DM, this difference was disappeared in patients with T2DM, either pre- or post-menopausal (figure1). Yichuan Wen et al. reported that ox-LDL mainly increased following menopause in non-diabetic women due to loss of anti-oxidative properties of estrogen after menopause [25] similar to MDA [26]. The interaction analysis showed that T2DM and menopausal status had no significant joint effects on ox-LDL and MDA levels.

The current study showed that ox-LDL levels had a powerful association with the 10-year ASCVD risk score. This result could support previous researches showing the association of cardiovascular events and ox-LDL concentration [27, 28]. In our study, men with T2DM had a lower 10-year ASCVD risk score compared to women; while among participants without T2DM, men had a higher 10-year ASCVD risk score compared to women. Some population-based studies have shown that diabetes imposes a greater risk of CVD in women than in men [29, 30]. It may suggest that diabetes could change the gender difference in ASCVD predisposition under the influence of ox-LDL levels. Moreover, our result indicated that the 10-year ASCVD risk score was higher in postmenopausal women without T2DM, while this difference was not present in patients with T2DM either pre- or post-menopausal. The same incidence of myocardial infarction in pre- and postmenopausal women with T2DM could support this issue [31]. It could be stated that diabetes could eliminate the difference between pre- and postmenopausal women in ASCVD events subsequently by changing in ox-LDL levels.

Our result indicated that ox-LDL levels were higher among participants with MetS compared to participants without MetS. In this line, Holvoet et al. mentioned that individuals with the MetS had two-fold more elevated levels of ox-LDL compared to those without MetS, independent of age, sex, ethnicity, LDL-C, and smoking status[32]. A population-based cohort study showed that higher concentrations of circulating ox-LDL are associated with the incidence of MetS as well as the accumulation of three of its component: hyperglycemia, abdominal obesity, and hyperglycemia [33]. In consistence with a meta-analysis report, in participants without T2DM, MetS was more prevalent among post-menopausal women compared to pre-menopausal[34] while participants with T2DM had the similar incidence of MetS. Based on our analysis, this difference was more attributed to BP among the components of MetS. It seems that T2DM made pre-menopausal women more susceptible to hypertension and the incidence of MetS as a result.

Our multivariate linear regression analysis showed that TG was an independent predictor for ox-LDL levels after adjusting for age, BMI, WC, duration of diabetes, SBP, HDL-C, LDL-C, FBS and HbA1c. We previously

mentioned the higher prevalence of hypertriglyceridemia in women with T2DM compared to men with T2DM [35]. Several studies have demonstrated the association between TG and oxidative markers [33, 36]. As plasma oxidized lipids have a relation with future subclinical and clinical atherosclerosis [14, 37]; this result could justify the long-standing association between hypertriglyceridemia and CVD [38, 39]. In this regard, the results of a decade follow-up of the Iranian population revealed that the increased risk for coronary heart disease (CHD) was attributed to TG [40]. A possible explanation is that higher TG levels in plasma could enhance the production of small, dense LDL particles, which are known to be more susceptible to oxidation [33].

Another important finding is that although the ox-LDL/LDL, as a lipid biomarker for estimation of oxidation[41], was significantly different among the participants, mean levels of total cholesterol and LDL-C did not differ by gender and T2DM status in this study. Infact, we found no significant correlation between ox-LDL and LDL-C. Moreover, ox-LDL and MDA were not significantly different between patients who were and were not treated by lipid-lowering drugs. This finding is aligned with our previous study suggesting that maintaining an optimized level of LDL-C, according to guidelines for the management of lipids in patients with T2DM does not sufficiently influence the ox-LDL levels [42].

The strength of this research was an adequate sample size to show the age-adjusted joint effect of diabetes, menopausal status, and gender and the independent association of TG on serum ox-LDL levels. The current research was a cross-section from a cohort study, so the main limitation was the lack of follow-up of the patients. Also, accurate analysis of lipid peroxidation like MDA and ox-LDL in serum is confounded by enzymatic and non-enzymatic lipid peroxidation that occurs during serum formation. So further studies should be conducted using plasma to handle procedures for prevention of lipid peroxidation.

In conclusion, T2DM overrides the effect of menopausal status, gender, and age on ox-LDL and MDA concentrations. Differences between women in circulating ox-LDL and MDA mostly dependent on T2DM, regardless of menopausal status and ageing. Besides, TG was independently associated with ox-LDL concentration. Also, we could show a positive correlation between 10-year ASCVD risk score and MetS with serum levels of ox-LDL.

Abbreviations

Oxidized low-density lipoprotein: ox-LDL; Type 2 diabetes mellitus: T2DM; Malondialdehyde: MDA; Metabolic syndrome: MetS; Atherosclerotic cardiovascular disease: ASCVD

Declarations

Ethics approval and consent to participate

All work was conducted in accordance with the Code of Ethics of the World Medical Association Helsinki Declaration. The privacy and rightsof all subjects were preserved

Consent for publication

Not Applicable.

Availability of data materials

The dataset supporting the conclusions of this article is included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RHA contributed to conception and design, development of methodology, analysis and interpretation of data, and drafting and re-vision of the manuscript, SR contributed to conception and design, develop-ment of methodology.AR contributed to development of methodology and analysis and interpretation of data. FH and HM contributed to drafting and revision of the manuscript. AE and MH contributed to acquisition of data, analysis and interpretation of data, drafting and revision of the manuscript, and study supervision. MN contributed to conception and design, developmentof methodology, analysis and interpretation of data, drafting and re-vision of the manuscript, and study supervision. Manouchehr Nakhjavani had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis.

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Tables

Table 1.Baseline characteristics of men and women with and without type 2 diabetes

	T2DM		Non-T2DM		
	women N=268	Men N=186	women N=107	Men N=33	p-value
Age (years)	54.51±9.06	56.47±10.67&	50.82±8.81	52.39±6.26	0.011
BMI (kg/m^2)	25.83±4.22	26.05±4.86	26.15±4.07	26.67±2.60	0.351
SBP (mmHg)	129.74±17.56	134.84±17.44^	126.65±15.88	120.94±10.47	0.008
DBP (mmHg)	83.89±13.37	86.65±21.10	80.05±8.82	85.45±7.47	0.326
WC(cm)	88.41±21.71	92.42±25.31&	82.97±22.43	87.01±19.13	0.046
Duration of diabetes (months)	96.21 (24.23,144.07)	108.02 (48.06,150.11)	-	-	0.275
Family history of ASCVD, n(%)	35(13.0)	23(12.3)	11(10.2)	4(12.1)	0.481
TG (mg/dl)	196.96±95.61&^*	164.63±77.86&^	80.63±23.08^	126.24±51.07	<0.001
LDL-C (mg/dl)	97.82±29.32	99.08±32.72	103.12±20.41	103.28±23.23	0.235
HDL-C (mg/dl)	42.62±13.51&^*	38.36±11.51&^	49.08±11.51^	48.41±15.36	<0.001
Total cholesterol (mg/dl)	203.04±52.80	193.42±48.36	209.45±32.06	195.75±37.84	0.659
FBS (mg/dl)	183.21±63.40&^	179.09±61.86&^	90.50±24.27	88.01±8.85	<0.001
Creatinine	0.94±0.31	0.97±0.22	0.90 ± 0.13	0.99±0.19	0.268
HbA1c (%)	8.25±1.75&^	8.23±1.94&^	4.90±0.33	5.15±0.30	<0.001
MDA(lU/l)	3.90±0.97&^*	3.13±1.32&^	1.96±0.58^	2.63±1.45	<0.001
Ox-LDL(mU/l)	92.11±38.07&^*	79.18±34.63&^	33.94±6.48^	59.54±14.36	<0.001
Ox-LDL/ LDL-C	1.04±0.96&^*	0.90±0.53&^	0.33±0.09^	0.60±0.19	<0.001
Glucose-lowering medication					0.198
- OAD, n(%)	123(45.8)	98(52.6)			
- Insulin, n(%)	102(38.0)	59(31.7)			
-Insulin+OAD, n(%)	39(14.5)	22(11.8)			
Lipid-lowering medication, n(%)	111(41.4)	101(54.3)	44(41.1)	15(45.4)	0.273

Data are presented as mean ± SD and percent (%), duration of diabetes presented as median (interquartile range[IQR])

& p<0.05 vs. female patients without T2DM

^p<0.05 vs. male patients without T2DM

* p<0.05 vs. male patients with T2DM

BMI=Body mass index; SBP=systolic blood pressure; DBP=Diastolic blood pressure; WC= waist circumference; TG= triglyceride; LDL-C=Low dense lipoprotein; HDL-C=high dense lipoprotein; MDA= Malondialdehyde; FBS=Fasting blood sugar; HbA1c= hemoglobin A1c; ox-LDL= oxidized Low dense lipoprotein; OAD=Oral Antidiabetic Drugs, n= number

Table 2. Ten-year ASCVD risk score and metabolic syndrome of men and women with and without T2DM

	T2DM		Non-T2DM		
	women N=268	Men N=186	women N=107	Men N=33	p-value
10-year ASCVD risk score(%)	10.36±6.88&^*	8.39±5.91&^	2.85±0.48^	4.24±1.24	<0.001
Risk groups, n(%)					0.034
- Low risk(<5%)	61(23.7)&^	34(18.2)&^	73(68.2)^	19(57.5)	
- Borderline risk (5%≤ and <7%)	25(9.3)	12(6.4)	10(9.3)	3(9.0)	
- Intermediate risk (7%≤ and <19.5%)	84(31.3)&^*	46(24.7)&^	14(13.0)	5(15.1)	
- High risk (≥19. 5%)	103(38.4)&^*	54(29.0)&^	15(14.0)^	7(21.2)	
Metabolic syndrome, n(%)	235(87.6)&^*	145(77.9)&^	42(39.2)^	8(24.2)	0.002
ASCVD=atherosclerosis cardiovascular disease; n=number					

Table3. Baseline characteristics of Premenopausal and Postmenopausal women with T2DM (268) and without T2DM (107)

	T2DM		Non-T2DM		
	Premenopausal women	Postmenopausal women	Premenopausal women	Postmenopausal women	p-value
	N=79	N=189	N=54	N=79	
Age (years)	42.59±5.28#	58.85±7.22*&	43.33±4.40#	56.43±5.81	<0.001
BMI (kg/m^2)	25.70±5.48	27.22±4.42	25.10±4.40	26.27±4.07	0.164
SBP (mmHg)	121.22±16.31	132.73±19.52*	130.32±17.82	127.14±11.88	0.028
DBP (mmHg)	86.01±20.60	83.07±10.37	80.07±8.78	82.02±8.81	0.141
WC(cm)	87.3±23.19	89.11±18.71&	81.87±20.31	85.17±19.72	0.032
Duration of T2DM (months)	36.00 (2.40, 96.00)	120.00 (72.00,156.00)*	-	-	<0.001
Duration of menopause (years)	-	8.70(3.70,12.70)	-	5.70(2.70,11.70)	0.321
Family history of ASCVD, n(%)	7(8.8)	19(10.0)	5(9.2)	5(9.4)	0.542
TG (mg/dl)	207.95±122.16&#	192.54±82.88&#	80.18±23.95	81.07±24.00	<0.001
LDL-C (mg/dl)	100.27±22.15	96.72±31.32	104.58±22.91	101.31±16.42	0.134
HDL-C (mg/dl)	42.59±16.60&	42.77±12.62&	48.72±11.54	47.10±9.52	0.049
Total cholesterol (mg/dl)	206.44±36.41	201.51±48.80	203.81±46.31	215.56±44.65	0.955
FBS (mg/dl)	191.53±71.81#&	179.65±60.40#&	87.58±8.7	94.10±35.38	<0.001
Creatinine	0.90 ± 0.18	0.96±0.33	0.88±0.11	0.92 ±0.15	0.231
HbA1c (%)	8. 21±2.33#&	8.26±1.75#&	4.83±0.39	4.99±0.26	<0.001
MDA(lU/l)	3.86±0.96&#	3.89±1.00&#	1.90±0.93#	1.95±.0.50	<0.001
Ox-LDL(mU/l)	91.38±34.73&#	92.08±39.40&#	30.66±5.16#	37.48±6.48	<0.001
Ox-LDL/LDL	0.89±0.42&#	1.01±0.57&#	0.30±0.07	0.37±0.09	<0.001

Glucose-lowering medication					0.367
- OAD, n(%)	38(48.1)	77(40.7)			
- Insulin, n(%)	17(21.5)	56(29.6)			
- Insulin +OAD, n(%)	10(12.6)	27(14.2)			
Lipid-lowering medication use, n(%)	24(30.3)	80(42.3)	18(33.3)	20(37.7)	0.256

Data are presented as mean ± SD and percent (%) duration of diabetes and menopause presented as median (interquartile range[IQR])

* p<0.05 vs. premenopausal patients with T2DM

# p<0.05 vs. postmenopausal patients without T2DM

& p<0.05 vs. premenopausal patients without T2DM

BMI=Body mass index; SBP=systolic blood pressure; DBP=Diastolic blood pressure; WC= waist circumference; T2DM=type 2 diabetes; TG= triglyceride; LDL=Low dense lipoprotein; HDL=high dense lipoprotein; MDA=Malondialdehyde; FBS=Fasting blood sugar; HbA1c= hemoglobin A1c; ox-LDL= oxidized Low dense lipoprotein, n=number

Table4. Ten-year ASCVD risk score and metabolic syndrome of Premenopausal and Postmenopausal women with T2DM (268) and without T2DM (107)

	T2DM		Non-T2DM		
	Premenopausal women N=79	Postmenopausal women N=189	Premenopausal women N=54	Postmenopausal women N=53	p-value
10-year ASCVD risk score(%)	9.36±4.51&#	10.61±4.22&#	3.20±0.03#	4.18±0.47	<0.001
Risk groups, n(%)					0.021
- Low risk(<5%)	16(21.5)&#	37(19.75)&#	30(55.5)#	22(41.5)	
- Borderline risk (5%≤ and <7%)	7(8.8)	15(7.9)	5(9.2)	4(7.5)	
- Intermediate risk (7%≤ and <19.5%)	22(27.8)&#	66(34.9)&#	6(11.1)#	11(20.7)	
- High risk (≥19. 5%)	30(37.9)&#	78(41.2)&#	7(12.9)#	13(24.5)	
Metabolic syndrome, n(%)	65(82.2)&#	170(89.9)&#	11(20.3)#	23(43.3)	0.001
ASCVD=atherosclerosis cardiovascular disease; n=number					

Table 5. correlation coefficients, univariate and multivariate linear regression analyses of the relationships between ox-LDL and biochemical and anthropometric parameters in 594 participants

Variables	Correlation		Univariate Regression		Multivariate Regression	
	r	ρ	OR	ρ	OR	ρ
age	0.09	0.055	1.38	<0.001	1.22	0.156
BMI	0.02	0.636	1.17	0.696	1.10	0.772
WC	0.04	0.341	1.09	0.404	1.01	0.541
Duration of diabetes	0.09	0.210	1.18	0.367	1.08	0.492
SBP	0.03	0.598	1.47	0.011	1.10	0.103
TG	0.68	<0.001	1.54	<0.001	1.27	<0.001
HDL-C	-0.08	0.131	0.40	<0.001	0.78	0.244
LDL-C	0.06	0.436	1.12	0.696	1.01	0.872
FBS	0.29	<0.001	1.33	0.018	1.10	0.162
HbA1c	0.23	<0.001	1.34	0.021	1.12	0.189
10-year ASCVD risk score(%)	0.82	<0.001	-		-	
Metabolic syndrome	0.61	<0.001	-		-	

BMI=Body mass index, WC= waist circumference, SBP= systolic blood pressure; HbA1C= hemoglobin A1C; LDL-C= low density lipoprotein cholesterol; HDL-C= high density lipoprotein; FBS= fasting blood sugar; TG= triglycerides, ASCVD= atherosclerosis cardiovascular disease

Figures

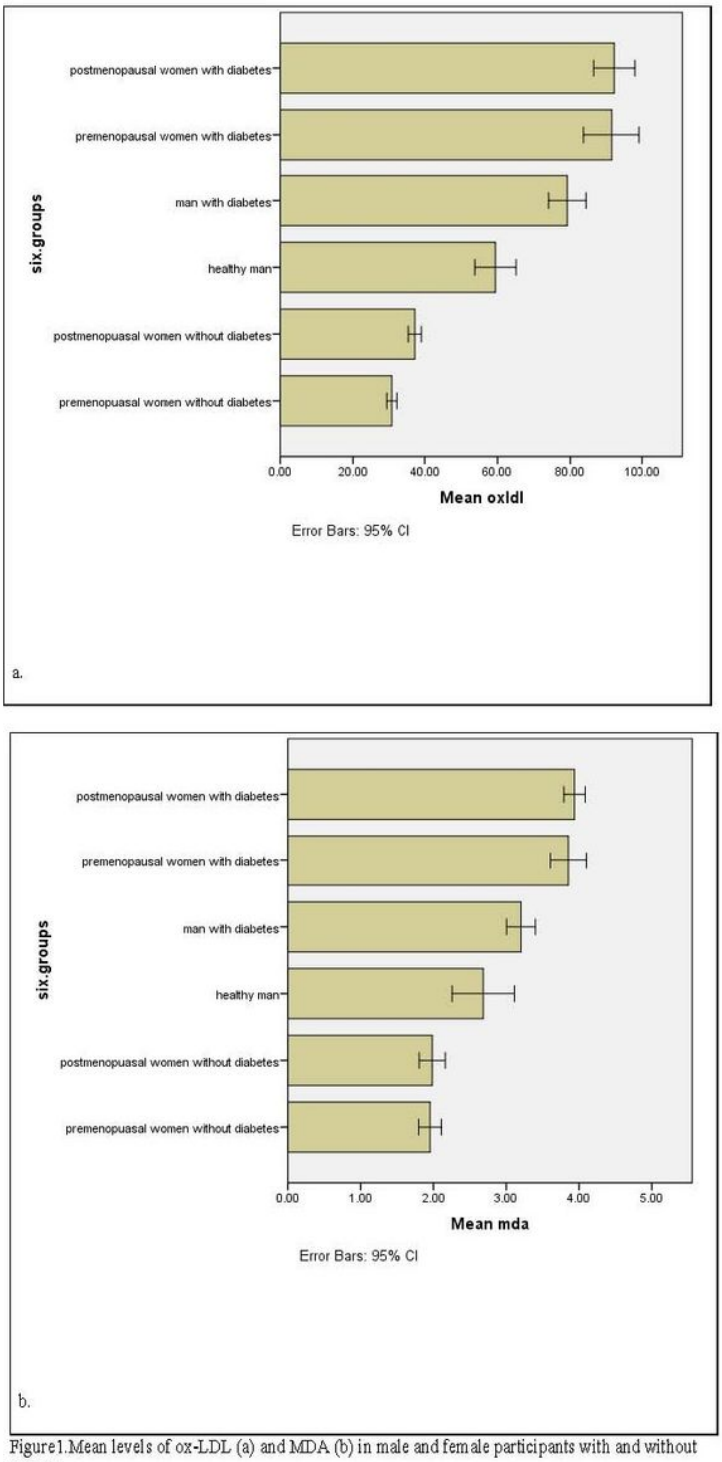

Figure1.Mean levels of ox-LDL (a) and MDA (b) in male and female participants with and without T2DM

Figure 1

Mean levels of ox-LDL (a) and MDA (b) in male and female participants with and without T2DM

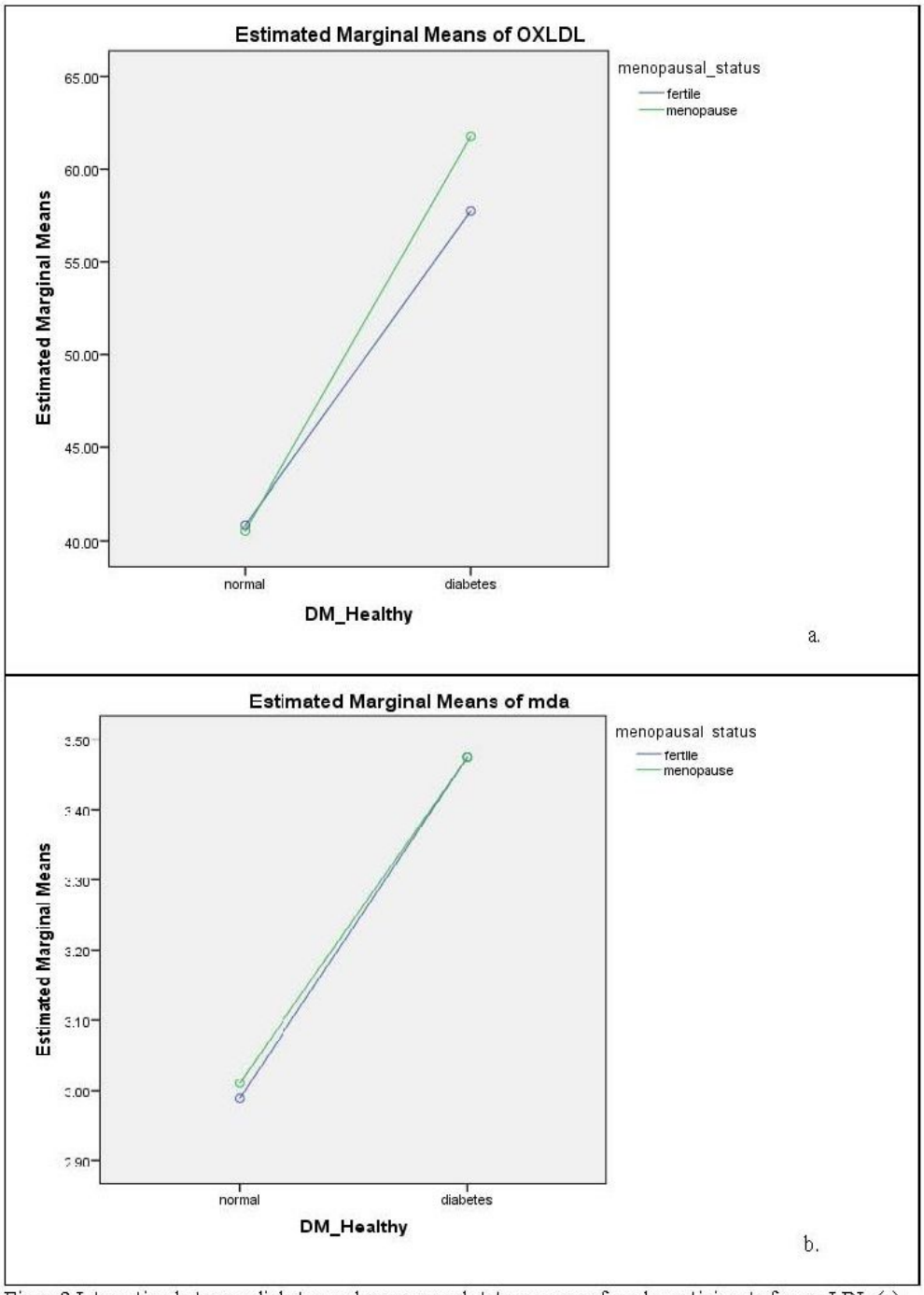

Figure2.Interaction between diabetes and menopausal statues among female participants for ox-LDL (a) and MDA (b) serum levels

Figure 2

Interaction between diabetes and menopausal statues among female participants for ox-LDL (a) and MDA (b) serum levels

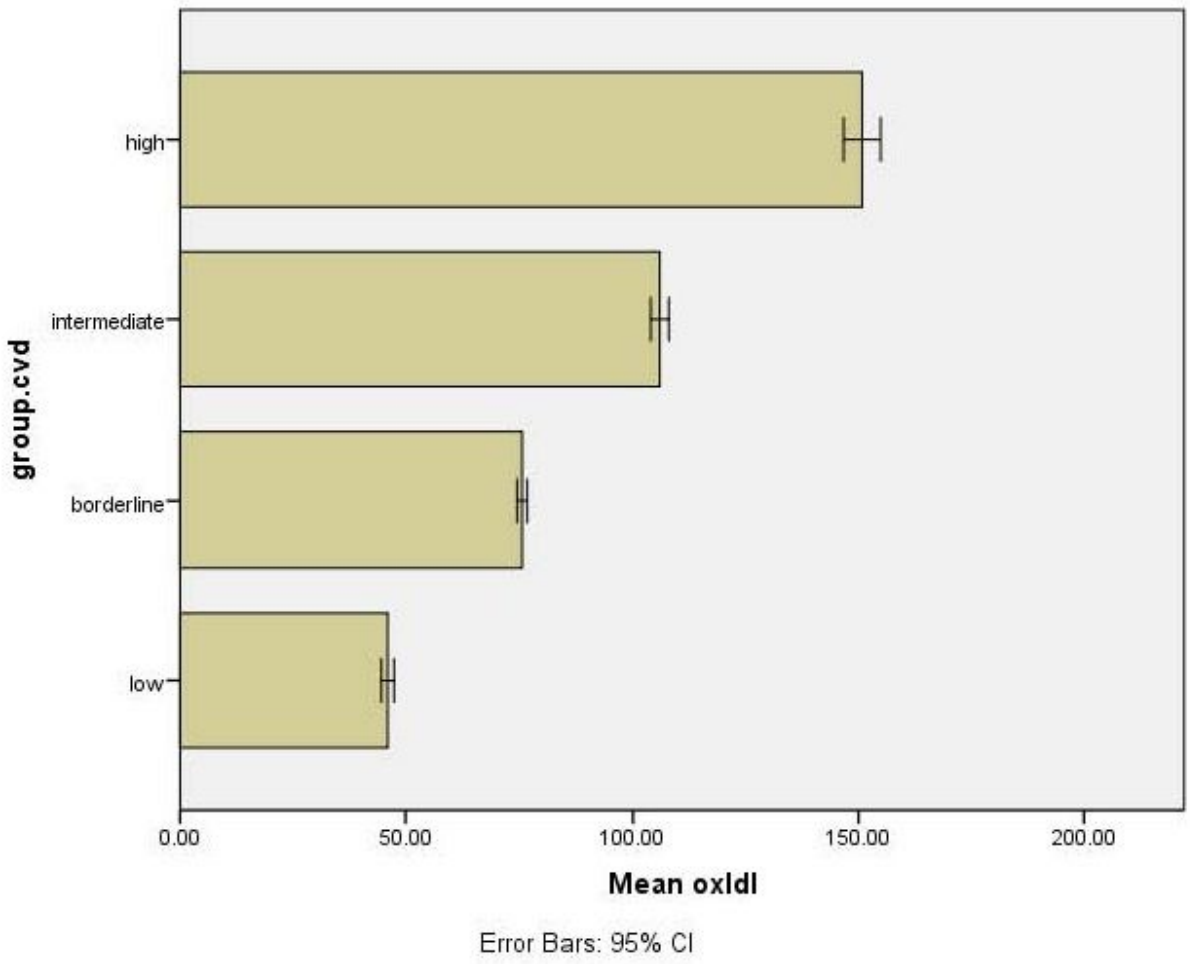

Figure3.Mean levels of ox-LDL in four risk groups of ASCVD

Figure 3

Mean levels of ox-LDL in four risk groups of ASCVD