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Original Article

Beyond LDL-c: The importance of serum oxidized LDL in predicting risk for type 2 diabetes in the middle-aged Asian Indians

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ABSTRACT

Aims: Oxidized low-density lipoprotein (OxLDL) as the residual lipid plays a crucial role in cardiovascular complications and type 2 diabetes. This study aimed to evaluate the relationship of OxLDL with the conventional risk markers and to find the association of OxLDL with the risk of development of type 2 diabetes in middle-aged (30–50 years) Asian Indians.

Materials and methods: A total of 78 type 2 diabetes patients and 78 age-matched controls were recruited. The serum OxLDL concentration was assessed by enzyme-linked immunosorbent assay (ELISA). Other anthropometric and biochemical measures were also carried out. Multiple logistic regression was used to determine the association of OxLDL and OxLDL to non-oxidized lipoproteins with the occurrence of type 2 diabetes.

Results: OxLDL was significantly higher in type 2 diabetes cases than controls ($p < 0.001$) even though there was no significant difference in LDL cholesterol (LDL-c) between type 2 diabetes patients and controls. OxLDL correlated significantly with fasting plasma glucose (FPG) and insulin resistance (HOMA-IR). OxLDL did not show any significant correlation with LDL-c. Multiple logistic regression showed a significant association of OxLDL, OxLDL/LDL-c and OxLDL/HDL-c with type 2 diabetes ($p < 0.001$). LDL-c showed no association with type 2 diabetes. ROC-AUC curve analyses showed OxLDL/HDL-c to have highest discriminatory power for type 2 diabetes (AUC: 0.710 with 95% CI: 0.629–0.791, $p < 0.001$).

Conclusion: Our findings highlight the possibly more attention has to be given to OxLDL for managing lipids and diabetes progression as well as reducing cardiac risk in middle-aged type 2 diabetes patients.

1. Introduction

India has become an epitome of non-communicable diseases such as type 2 diabetes mellitus and cardiovascular diseases (CVDs). According to the International Diabetes Federation (IDF) (2017) estimates, India is home for about 72.9 million diabetic individuals with an average prevalence rate of 10.4% [1]. The high prevalence of diabetes also incurred an economic burden of the country with the total annual expenditure for the diabetes care would be approximately 180,000 million rupees [2]. The genetic predisposition coupled with rapid urbanization, unhealthy dietary habits, and sedentary lifestyles, have altogether increased the risk of developing of type 2 diabetes and associated complications at much younger age among Asian Indians than the western population [3]. The development of type 2 diabetes at a relatively young age not only inclines an individual to the risk of early development of chronic vascular complications but causes morbidity and mortality in the most productive years of life.

According to the Indian Council of Medical Research–India DIABetes (ICMR-INDIADB) population-based cross-sectional study (2011), the majority of the Indians had onset of diabetes below 50 years of their age [4]. Another study in an urban population of India also showed the high prevalence of diabetes below 44 years [5]. A recent rural community based Indian study reported about 16.22% and 24.32% prevalence of type 2 diabetes in the age group 30–40 years and 41–50 years respectively [6].

Hyperglycemia, dyslipidemia, and oxidation of lipoproteins are considered as the major risk factors for CVDs in type 2 diabetes patients, affecting about 10%–73% diabetic population [7]. CVDs are the most reported co-morbidities responsible for about 60–80%

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deaths in diabetic individuals worldwide [8]. It is widely accepted that low-density lipoprotein (LDL) particles are highly sensitive to oxidative damages and oxidative modification of native LDL particles makes them highly pathogenic, immunogenic and, atherogenic [9–11]. The oxidation of LDL is also considered as an initiating event in various pathological conditions including CVDs and upregulation of oxidized low-density lipoprotein (OxLDL) is a hallmark feature of atherosclerotic development [12]. OxLDL attracts monocytes, induces inflammatory reactions in the arterial wall, thereby, playing an important role in endothelial dysfunction, atherosclerotic plaque formation, progression, destabilization, and premature cardiovascular ageing [13,14]. Increased lipid peroxidation results in high malondialdehyde (MDA) level which deposits in atherosclerotic plaques [15].

OxLDL and oxidation ratio of LDL-c (OxLDL to LDL-c) are often considered as the better predictors of coronary artery disease (CAD), myocardial infarction than conventional lipid measures [16,17]. The association between high levels of OxLDL and dyslipidemia has been reported in the elderly (60–75 years) of prediabetic and type 2 diabetes population [18]. In another study, Gradinaru and colleagues reported about the increased levels of OxLDL, AGEs, advanced oxidation protein products (AOPP), NOx (sum of nitrites and nitrates) and reduced levels of adiponectin in the serum samples of elderly subjects (64–76 years) with metabolic syndrome [19].

Since the prevalence of type 2 diabetes is now rapidly growing in the 30's and 40's, it is a matter of concern to identify reliable and sensitive biomarkers for early prediction of type 2 diabetes and assessing cardiovascular risk. The primary focus of the physicians is directed towards lowering the conventional lipid parameters for minimizing the risk of type 2 diabetes and associated cardiac complications. OxLDL as the residual lipid has not been regarded as the traditional risk factor for type 2 diabetes. There are several reports of OxLDL correlating with various pathological conditions. Hence, OxLDL needs more attention, and comprehensive studies are required to explore the association of OxLDL with the risk of type 2 diabetes and associated cardiovascular complications. Thus, the present study aimed to evaluate the relationship of OxLDL with the conventional risk markers and to find the association of OxLDL with the risk of development of type 2 diabetes in middle-aged (30–50 years) Asian Indians.

2. Materials and methods

2.1. Study population

The study was conducted at Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Pune, India. This study enrolled participants (n = 156), both men and women aged 30–50 years of Pune city, India. Type 2 diabetes patients (n = 78) were recruited from diabetic clinics of Pune. Inclusion criteria for type 2 diabetes patients were in accordance with the American Diabetes Association (ADA) guidelines 2016 [20]. The age-matched "controls" (n = 78) included apparently healthy individuals without diabetes, not having a major medical illness, not on current medications and had a subjective perception of good health. Individuals suffering from common flu, fever, any chronic diseases like cancer, diabetic microvascular and macrovascular complications and neuro-degenerative diseases were excluded from the study. Pregnant and lactating women were not included in the study. The study protocol received approval of Independent Ethics Committee (IEC) of Symbiosis International (Deemed University) and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The written informed consent was obtained from all individual participants included in the study.

2.2. Questionnaire and anthropometric measures

The demographic data (age and gender), family history of diabetes and hypertension in first degree relatives, and lifestyle factors (dietary patterns, physical exercise, smoking, and alcohol consumption) were collected from each participant by using standard questionnaire. Type 2 diabetes patients were asked about their current treatment modalities. The systolic and diastolic blood pressures (SBP and DBP) were measured by Digital sphygmomanometer (Omron Corporation, Japan) in sitting position after the participant had been resting for at least 5 min. Two readings were taken after 5 min interval and their mean was considered as the blood pressure.

The physical examinations included height and weight measurements of participants in barefoot in the standing position nearest to 0.1 cm and 0.05 kg respectively using the standard technique. The body mass index (BMI) was calculated as the weight (kg) divided by height in meter square (m^2). The waist circumference (WC) was measured midway between the lower rib margin and the iliac crest by a plastic, flexible and inelastic measuring tape. The hip circumference (HC) was measured by the same measuring tape around the widest portion of the buttocks.

2.3. Biochemical methods

The venous blood sample was collected after an overnight fasting (10–12 h) under all aseptic conditions by the doctor or trained nurse. The blood samples were centrifuged at 3000 rpm for 15 min for plasma and serum separation and were stored at - 80 °C in separate aliquots. The laboratory investigations include fasting plasma glucose (FPG) (glucose oxidase-peroxidase method, Span-diagnostics), fasting plasma insulin or FPI (sandwich enzyme-linked immunosorbent assay or ELISA, (Invitrogen, ThermoFisher Scientific), with the analytical sensitivity of this kit was 0.17 μIU/ml of human insulin) and glycated hemoglobin (HbA_{1C}), which was estimated from whole blood by immunoturbidimetric method (Randox Laboratories). The insulin resistance (HOMA-IR) was calculated by Homeostatic model assessment (HOMA) methods [21] using the formula: HOMA-IR = FPG (mmol/l) x FPI (μIU/ml)/22.5.

The serum lipid profile (total cholesterol (TC), high-density lipoprotein (HDL-c) and triglycerides (TG)) was determined by the enzymatic colorimetric assay using Span Diagnostics kit. Low-density lipoprotein (LDL-c) was calculated by the Friedewald's formula [22].

2.4. Quantitative determinations of plasma malondialdehyde (MDA) and serum oxidized LDL (OxLDL)

The lipid peroxidation in the plasma was measured by thiobarbituric acid reacting substance method (TBARS) as described by Placer et al. [23]. Absorbance of the analyte was measured by UV–Vis spectrophotometer (UV-Evol 201, Thermoscientific) at 548 nm against a non-incubated blank. The concentration of plasma MDA was calculated by the formula = Absorbance x 4.6 (μmol/l).

The serum OxLDL was estimated by Sandwich ELISA based assay (USCN, Cloud-one, USA). The assay was conducted according to the manufacturer's protocol. The absorbance was read on ELISA plate reader (Biotek, Elx- 800) at 450 nm immediately after adding the stopping solution. The standard curve was plotted by drawing the best fit curve through the points. The concentration of OxLDL (pg/

ml) of each unknown sample was read from the standard curve and finally expressed in mg/l. This kit could measure the minimal detectable dose of OxLDL that was typically less than 26.9 pg/ml. The inter-assay and intra-assay coefficient of variations were <12% and <10% respectively.

2.5. Statistical analysis

The statistical analysis was done by using Statistical Package for the Social Science (SPSS) version 16.0. IL, USA. The assumptions of normal distribution of data and homogeneity in variances were determined by Shapiro Wilk test of normality and Levene's test of equality of variances. Data were represented in median and interquartile range (IQR) (Q1-Q3). Independent *t*-test assessed differences between groups for continuous and normally distributed variables and Mann–Whitney *U* test for not normally distributed variables. The categorical variables were expressed in frequency (%) and compared using the Pearson's chi-square test. The Spearman's correlation was performed to analyze the relationship between different continuous variables. The multiple logistic regression analysis (unadjusted and adjusted for gender, lifestyle factors) was performed to predict the odds ratio for type 2 diabetes with different risk factors. The statistical significance was set at $p < 0.05$. The areas under the receiver operating characteristic curves (ROC-AUC) of the adiposity indicators were measured to compare the discriminatory abilities of LDL-c and OxLDL to predict the type 2 diabetes risk correctly. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Baseline characteristics of the study participants

The study included 156 participants of age group 30–50 years, were divided into two major groups controls (n = 78) and type 2 diabetes patients (n = 78). The baseline characteristics of all study participants were presented in Table 1. There was no significant difference in age between controls and type 2 diabetes patients. BMI, WC, SBP and DBP were significantly higher in type 2 diabetes patients. All glycemic indices (FPG, FPI, HOMA-IR, and HbA_{1C}) showed significant differences between type 2 diabetes patients and controls. The lipid profile parameters, except TC and LDL-c, were significantly different between groups. TG/HDL-c was found to be significantly higher in type 2 diabetes patients than controls. MDA, OxLDL, OxLDL/LDL-c, OxLDL/HDL-c were significantly increased in type 2 diabetes patients. Among type 2 diabetes patients, 67.9% and 6.4% reported the family history of type 2 diabetes, and both type 2 diabetes and hypertension respectively. About 93.6%, 2.6%, and 3.8% of type 2 diabetes patients were on oral hypoglycemic agents (OHA), insulin, and both OHA and insulin respectively. 41% and 59% of type 2 diabetes patients were on statins and not on statins respectively. There were no significant differences in OxLDL-c level in type 2 diabetes patients with the difference in duration of disease (<5 years and ≥5 years) and use of statins.

3.2. Interactions of oxidized LDL with conventional risk markers of type 2 diabetes

The Spearman correlations of OxLDL with metabolic risk factors separately in type 2 diabetes patients and controls were shown in Table 2. OxLDL significantly and positively correlated with FPG and HOMA-IR but, LDL-c did not correlate significantly with these glycemic indices in type 2 diabetes patients (Fig. 1). OxLDL/LDL-c correlated well with FPG ($r = 0.250$; $p = 0.027$) and HOMA-IR ($r = 0.271$; $p = 0.016$) in type 2 diabetes patients. Likewise, OxLDL/HDL-c correlated significantly with FPG ($r = 0.303$; $p = 0.007$) and HOMA-IR ($r = 0.350$; $p = 0.002$). In the lipid parameters, OxLDL significantly correlated with TC, TG, TC/HDL-c, and TG/HDL-c in type 2 diabetes patients, whereas these correlations were non-significant in the controls. OxLDL did not show any significant association with LDL-c in both type 2 diabetes patients and controls. MDA, OxLDL/LDL-c, and OxLDL/HDL-c found to correlate significantly with OxLDL among type 2 diabetes patients and controls. MDA correlated significantly ($r = 0.328$; $p < 0.01$) with both OxLDL/LDL-c and OxLDL/HDL-c in type 2 diabetes patients.

3.3. Logistic regression analysis of metabolic risk factors using type 2 diabetes as the dependent variable

The multiple logistic regression analysis was next done to determine the association of various metabolic risk factors with type 2 diabetes (Table 3). Unadjusted model of logistic regression identified a significant association of different metabolic indicators except for TC, LDL-c and TC/HDL-c, with type 2 diabetes. In model 1, after adjusting for gender, the odds ratio of OxLDL/LDL-c increased to 5.779 (CI: 2.092–15.959; $p = 0.001$). In the model 2 after adjusting for various lifestyle factors such as diet, physical exercise, smoking, and alcohol consumption, OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c showed higher odds ratio and significant association with type 2 diabetes. OxLDL/LDL-c showed much higher odds ratio (OR: 6.620, CI: (2.248–19.493), $p = 0.001$) than lipid parameters for predicting the risk of type 2 diabetes (model 2). On the contrary, LDL-c did not show any significant association with type 2 diabetes in both model 1 and model 2.

3.4. ROC-curve analysis

The ROC-curve analysis (Fig. 2) was carried out to check the discriminatory power of LDL-c, OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c to predict the risk of type 2 diabetes correctly. The area under ROC (AUC-ROC) curve for LDL-c was 0.473 with 95% CI: 0.382–0.565, $p = 0.566$, standard error (S.E) = 0.047. The area under ROC (AUC-ROC) curve for OxLDL-c was 0.694 with 95% CI: 0.612–0.776, $p < 0.001$, S.E = 0.042. OxLDL/LDL-c showed AUC of 0.679 with 95% CI: 0.596–0.762, $p < 0.001$, S.E = 0.042. OxLDL/HDL-c showed highest AUC of 0.710 with 95% CI: 0.629–0.791, $p < 0.001$, S.E = 0.041.

4. Discussion

The present study showed significant correlations of OxLDL and the conventional risk markers (FPG, HOMA-IR, TC, TG, and MDA) of type 2 diabetes. The significant association of OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c with the risk of type 2 diabetes were observed in middle-aged Indian population. The oxidatively modified LDL-c was earlier reported to be involved in the CVDs e.g., atherosclerosis and deleterious role of increased in the pathogenesis of type 2 diabetes [13,24]. The chronic hyperglycemia triggers the production of excess free radicals which attack the lipids and causes peroxidation of lipid molecules in a chain reaction fashion [14].

In our study, type 2 diabetes patients of age group 30–50 years were mostly overweight and obese. A marked increase was found in FPG, HbA_{1C}, FPI, and HOMA-IR in type 2 diabetes patients as compared to the controls. OxLDL showed significant correlations with glycemic indices such as FPG and HOMA-IR as compared to its non-oxidized lipid counterpart that is LDL-c in type 2 diabetes patients, suggesting that a substantial relationship of oxidized modification of LDL-c with hyperglycemia and insulin resistance [25]. Harmon and colleagues [26] demonstrated that OxLDL/LDL-c

Table 1
Baseline characteristics of study participants.

Variables	Controls (n = 78)	Type 2 diabetes (n = 78)	p value
Clinical characteristics			
Age (years)	40 (34–45)	41 (35–46)	0.278
Gender (M/F), n (%)	M – 36 (46.2%) F-42 (53.8%)	M – 50 (64.1%) F-28 (35.9%)	0.024$
Body weight (kg)	65 (56.7–73.2)	69.2 (63.98–75)	0.006
Height (cm)	161 (153–168)	162.78 (154–169)	0.643#
BMI (kg/m^2)	25.22 (22.65–27.77)	27.1 (24.66–28.86)	0.004
WC (cm)	91 (85–95.13)	94 (87.75–100)	0.009
HC (cm)	98 (93–103)	101 (96–104.63)	0.024
WHR	0.93 (0.99–0.97)	0.94 (0.89–0.97)	0.596
SBP (mmHg)	116 (110–127.5)	125 (120–130)	<0.001
DBP (mmHg)	78 (72.38–81)	80 (80–83.25)	0.001
Duration of diabetes (years)	–	4 (2–8)	–
Metabolic and biochemical characteristics			
FPG (mmol/l)	4.58 (4.25–4.98)	7.38 (6.12–9.3)	<0.001
FPI (μIU/ml)	15.38 (9.62–22.78)	19.59 (13.17–34.59)	0.006
HOMA-IR	3.06 (1.95–4.63)	6.76 (4.27–11.78)	<0.001
HbA_{1C} (%)	4.9 (4.7–5.2)	7.5 (6.8–8.7)	<0.001
TC (mmol/l)	4.08 (3.31–4.71)	3.81 (3.4–4.71)	0.692#
TG (mmol/l)	1.13 (0.93–1.52)	1.37 (0.97–1.98)	0.005
HDL-c (mmol/l)	0.99 (0.89–1.19)	0.95 (0.83–1.104)	0.029
LDL-c (mmol/l)	2.39 (1.76–3.06)	2.21 (1.66–2.74)	0.614
TC/HDL-c	3.85 (3.17–4.69)	3.94 (3.36–4.99)	0.099
TG/HDL-c	1.13 (0.81–1.53)	1.41 (0.99–2.37)	0.002
MDA (μmol/l)	3.31 (2.35–7.04)	7.96 (6.18–10.08)	<0.001
OxLDL (mg/l)	0.57 (0.3–1.1)	1.03 (0.64–1.73)	<0.001
OxLDL/LDL-c (mg/mmol)	0.28 (0.14–0.28)	0.48 (0.29–0.84)	<0.001
OxLDL/HDL-c (mg/mmol)	0.59 (0.33–1.04)	1.08 (0.65–1.88)	<0.001

Data expressed in median and inter-quartile range (IQR); p value from Mann-Whitney test; $ p value from Chi-square test; #p value from Independent *t*-test; M: male, F: female; BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WHR: Waist to hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HOMA-IR: Homeostatic model assessment-insulin resistance; HbA1c: Glycated hemoglobin; TC: Total cholesterol; TG: Triglycerides; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; MDA: Malondialdehyde; OxLDL: Oxidized LDL.

Table 2
Correlation analyses of OxLDL with conventional risk markers of type 2 diabetes.

Variables	OxLDL	
	Controls	Type 2 diabetes
FPG	0.045 (0.696)	0.290 (0.010)
FPI	0.188 (0.099)	0.209 (0.067)
HOMA-IR	0.205 (0.072)	0.325 (0.004)
HbA_{1C}	0.093 (0.420)	0.090 (0.436)
TC	0.107 (0.350)	0.245 (0.031)
TG	−0.008 (0.942)	0.260 (0.021)
HDL-c	−0.043 (0.709)	−0.077 (0.504)
LDL-c	0.113 (0.323)	0.171 (0.134)
TC/HDL-c	0.061 (0.595)	0.278 (0.014)
TG/HDL-c	0.039 (0.731)	0.254 (0.025)
MDA	0.265 (0.019)	0.319 (0.004)
OxLDL/LDL-c	0.879 (<0.001)	0.836 (<0.001)
OxLDL/HDL-c	0.975 (<0.001)	0.964 (<0.001)

FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HOMA-IR: Homeostatic model assessment-insulin resistance; HbA1c: Glycated hemoglobin; TC: Total cholesterol; TG: Triglycerides; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; MDA: Malondialdehyde; OxLDL: Oxidized LDL.

and OxLDL/HDL-c ratios significantly correlated with HbA_{1C} and suggested to be more informative than OxLDL alone. We also obtained significantly higher correlation coefficients of OxLDL/HLD-c with FPG and HOMA-IR than OxLDL.

In serum lipid profile, there was no significant difference in TC and LDL-c levels between type 2 diabetes patients and controls which was in accordance with the previous study [27]. The use of statins by patients could be a possible reason. The significant increase in TG and considerable decrease in HDL-c levels observed in type 2 diabetes patients indicated the presence of dyslipidemia [27]. The high levels of TG and low levels of HDL-c increased the oxidation of LDL-c [28]. The level of oxidation of LDL-c was reported to be more dependent on glycemic control than the presence of diabetic complications [28].

In lipid ratios, we observed TG/HDL-c ratio was significantly higher in type 2 diabetes patients than controls. The higher TG/HDL-c ratio indicates higher risk of developing CVDs, insulin resistance in type 2 diabetes patients [29,30]. Dobiasova and Frohlich [31] revealed a strong association of that log (TG/HDL-c) with the diameter of LDL-c particles and suggested it to be a surrogate measure of atherogenic index. Armato et al. [32] reported the strong correlation of TG/HDL-c with blood pressure, insulin resistance and higher cardiometabolic risk in a population with higher susceptibility of type 2 diabetes. A significant positive correlation of OxLDL with lipid parameters such as TC, TG, and TC/HDL-c in type 2 diabetes patients suggested the relationship of dyslipidemia and oxidative stress, which was inconsistent with previous report [33].

Our findings alongside with the previous results showed that OxLDL correlated well with the surrogate atherogenic lipid TG/HDL-c in type 2 diabetes patients, emphasizing its role as a predictor of atherosclerosis [17]. A negative but non-significant correlation was observed between OxLDL and HDL-c in type 2 diabetes patients and controls similar to the previous evidence [27]. HDL-c is responsible for the reverse transportation of cholesterol from the periphery to the liver and contains anti-oxidative enzymes which prevent the formation of oxidized phospholipids [27,34]. HDL-c is further known to reverse the oxidation of LDL-c by removing oxidized phospholipids responsible for atherogenesis, thus acting as anti-atherogenic [34]. Although not estimated in this study, small dense LDL-c particles, abnormal HDL-c levels, and low antioxidants levels were reported to be strongly associated with type 2 diabetes and metabolic syndrome [35].

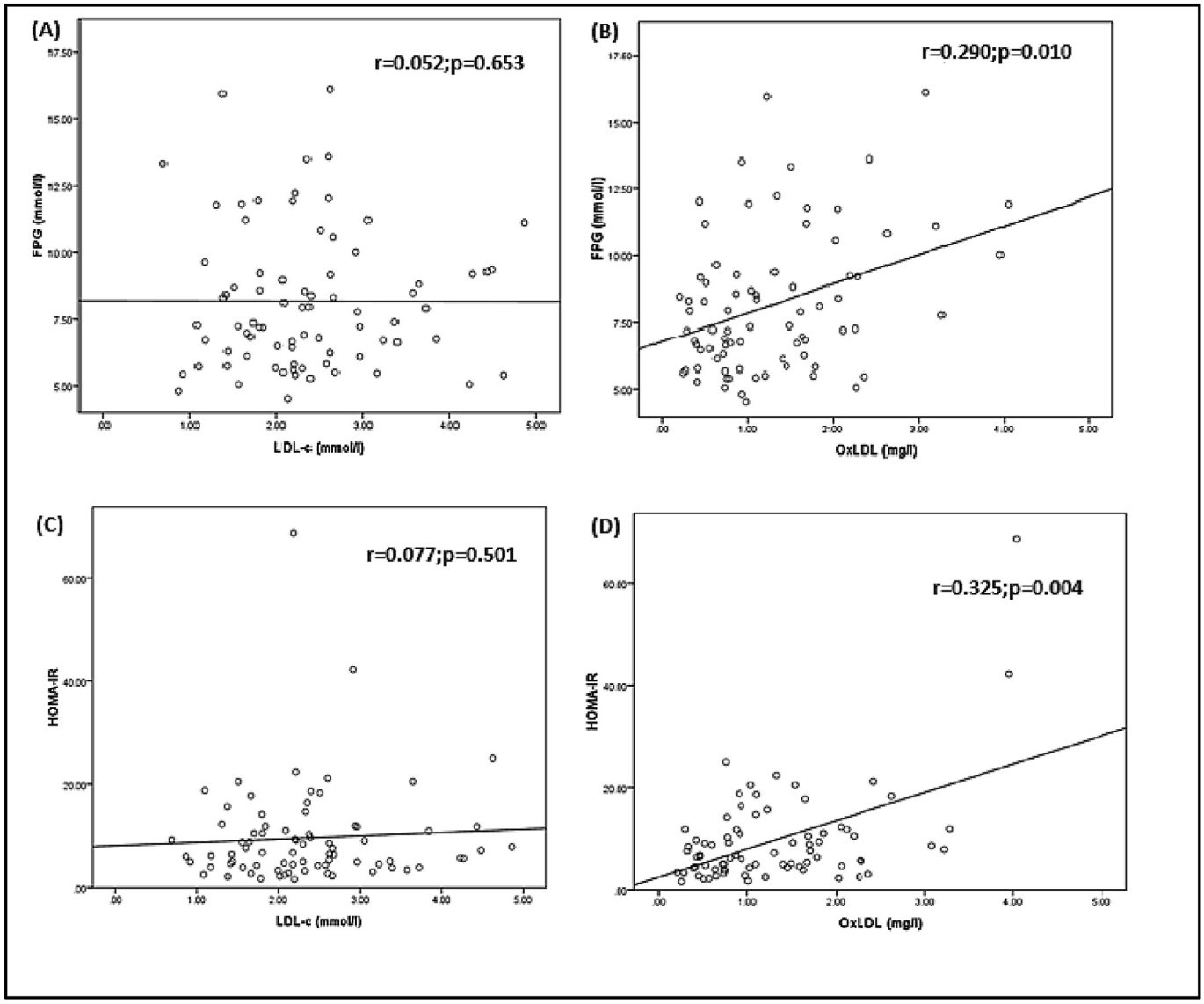

Fig. 1. Correlations(r) between (A) LDL-c and FPG, (B) OxLDL and FPG, (C) LDL-c and HOMA-IR, and (D) OxLDL and HOMA-IR in type 2 diabetes patients (n = 78); LDL-c: Low density lipoprotein cholesterol; OxLDL: Oxidized LDL; FPG: Fasting plasma glucose; HOMA-IR: Homeostatic model assessment-insulin resistance.

MDA, OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c levels were significantly higher type 2 diabetes patients than controls similar to previous studies [27,36,37]. Both MDA and OxLDL correlated significantly with OxLDL/LDL-c and OxLDL/HDL-c in type 2 diabetes patients. MDA is a recognized marker of end products of lipid peroxidation and is reported to modify apolipoprotein B. Apolipoprotein B is the primary apolipoprotein of LDL-c involved in atherosclerosis and CVDs, which also increases the susceptibility of LDL to oxidation and production of OxLDL [13,15]. Motamed and colleagues also determined a positive correlation of OxLDL/HDL-c with MDA and revealed OxLDL/HDL-c and OxLDL/LDL-c as potential biomarkers of oxidative stress in patients with type 2 diabetes patients [37]. On the contrary, Pawlak et al. reported the OxLDL/LDL-c ratio to be the markers of lipoprotein abnormalities rather than oxidative stress in dialyzed patients [38].

In our study, we did not find any significant association of LDL-c with the risk of type 2 diabetes, which was in agreement with the findings of Spessatto et al. in non-diabetic population [39]. The previous studies suggested that a change in OxLDL level may not be dependent on LDL-c level [40,41]. Interestingly, Nakhjavani and colleagues showed maintaining an optimized level of LDL-c did not affect the OxLDL level [41]. An increase in LDL-c oxidation may be associated with impaired glycemic status even with decreased or stable levels of LDL-c [40]. The sensitivity of LDL-c oxidation was found to be increased in diabetic state and along with the progression of diabetes [41]. Although advanced glycation end products do not interfere directly with the oxidation of LDL-c, they probably have a role in inducing the production of OxLDL from macrophages [42]. Interestingly, it was also reported that the level of OxLDL reduced by statins in patients with coronary artery diseases [43]. The ROC curve analyses also suggested the higher discriminatory power of OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c as compared to LDL-c in predicting the risk of type 2 diabetes.

Our study has several strengths which include the same strict methodology followed for both type 2 diabetes patients and controls. We computed the odds ratio of a large panel of conventional diabetes risk markers for predicting the risk of development of type 2 diabetes. To best of our knowledge, the present study is the first to

Table 3
Logistic regression of metabolic risk factors in type 2 diabetes.

Variables	Unadjusted		Model 1		Model 2	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
FPG	42.871 (9.350–196.574)	<0.001	42.643 (9.241–196.784)	<0.001	50.954 (9.856–263.433)	<0.001
FPI	1.027 (1.005–1.050)	0.017	1.027 (1.004–1.050)	0.022	1.036 (1.010–1.063)	0.006
HOMA-IR	1.336 (1.184–1.507)	<0.001	1.329 (1.178–1.500)	<0.001	1.431 (1.246–1.643)	<0.001
HbA_{1C}[a]	1.729 (1.326–2.256)	<0.001	1.774 (1.313–2.396)	<0.001	2.431 (1.231–4.801)	0.010
TC	1.071 (0.766–1.497)	0.690	0.996 (0.705–1.408)	0.983	1.056 (0.742–1.501)	0.763
TG	2.687 (1.469–4.913)	0.001	2.464 (1.336–4.545)	0.004	3.176 (1.609–6.268)	0.001
HDL-c	0.164 (0.031–0.860)	0.033	0.181 (0.034–0.972)	0.046	0.120 (0.019–0.740)	0.022
LDL-c	0.983 (0.690–1.401)	0.925	0.925 (0.643–1.331)	0.676	0.966 (0.666–1.402)	0.857
TC/HDL-c	1.309 (0.981–1.747)	0.067	1.235 (0.918–1.661)	0.163	1.338 (0.983–1.821)	0.064
TG/HDL-c	2.436 (1.477–4.018)	<0.001	2.207 (1.375–3.806)	0.001	2.887 (1.636–5.094)	<0.001
MDA	1.533 (1.336–1.760)	<0.001	1.522 (1.325–1.748)	<0.001	1.619 (1.383–1.894)	<0.001
OxLDL	2.499 (1.527–4.092)	<0.001	2.436 (1.473–4.027)	0.001	2.618 (1.557–4.403)	<0.001
OxLDL/LDL-c	5.726 (2.115–15.499)	<0.001	5.779 (2.092–15.959)	0.001	6.620 (2.248–19.493)	0.001
OxLDL/HDL-c	2.527 (1.566–4.077)	<0.001	2.480 (1.524–4.034)	<0.001	2.835 (1.679–4.789)	<0.001

[a] HbA_{1C} expressed in mmol/mol (IFCC unit); Model 1: adjusted for gender; model 2: adjusted for life style factors (diet, exercise, smoking, and alcohol consumption); OR: Odds ratio; CI: confidence interval; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HOMA-IR: Homeostatic model assessment-insulin resistance; HbA_{1c}: Glycated hemoglobin; TC: Total cholesterol; TG: Triglycerides; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; MDA: Malondialdehyde; OxLDL: Oxidized LDL.

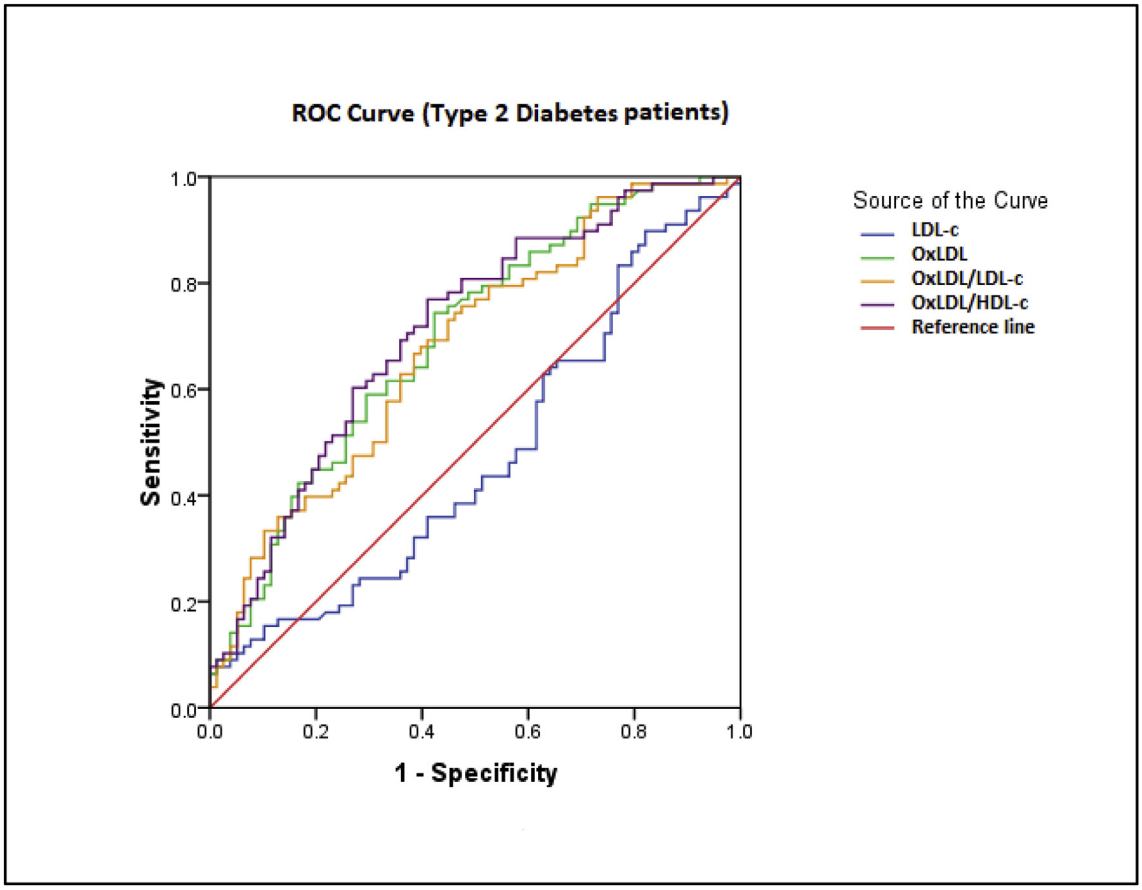

Fig. 2. Receiver operating characteristic (ROC) curves for LDL-c, OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c in type 2 diabetes patients; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; OxLDL: Oxidized LDL.

show the association of OxLDL, OxLDL/LDL-c, and OxLDL/HDL-c with type 2 diabetes in the middle-aged population of Asian Indians. However, one limitation of the study was that most of our controls were also overweight and obese.

In conclusion, this study showed that the serum OxLDL was significantly associated with type 2 diabetes, even though LDL-c was not a significant predictor of type 2 diabetes in the middle-aged Asian Indians. Both, OxLDL/LDL-c and OxLDL/HDL-c may be considered as the oxidative stress markers as well as markers of dyslipidemia. Our findings highlight the possibly more attention has to be given to OxLDL as the residual lipid marker along with the conventional lipid parameters for assessing cardiovascular risk and management of lipids in type 2 diabetes patients. Since the middle-aged population of India are more susceptible for early

development of type 2 diabetes and associated cardiac complications, it would thus be wise to reduce OxLDL level for controlling for diabetes progression as well as cardiac risk in this age group.

Conflicts of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dsx.2018.08.036.

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