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Oxidized low-density lipoprotein and intimal medial thickness in subjects with glucose intolerance—The Chennai Urban Rural Epidemiology Study-25☆

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Abstract

The aim of the present study was to assess the association of oxidized low-density lipoprotein (OX-LDL) with carotid intimal medial thickness (IMT) in different grades of glucose intolerance in Asian Indians. Three groups were recruited from the Chennai Urban Rural Epidemiology Study, a population-based study: group 1, normal glucose tolerance (NGT) (n = 175); group 2, impaired glucose tolerance (IGT) (n = 175); and group 3, type 2 diabetes mellitus (n = 175). Oxidized LDL (enzyme-linked immunosorbent assay) and carotid IMT (high-resolution B-mode ultrasonography) were assessed. Subjects with diabetes had higher IMT values (0.85 ± 0.30 mm) compared with those who have IGT (0.79 ± 0.16 mm, $P < .05$) and NGT (0.71 ± 0.12 mm, $P < .001$). Subjects with diabetes (40.1 ± 13.1 U/L) and IGT (34.3 ± 12.8 U/L) had significantly higher mean OX-LDL values compared with the NGT group (26.2 ± 16.6 U/L, $P < .001$). Oxidized LDL showed a correlation with IMT (total population: $r = 0.294$, $P < .001$; subjects with NGT: $r = 0.444$, $P < .001$; and subjects with IGT: $r = 0.481$, $P < .001$). In multiple linear regression analysis, OX-LDL showed a strong association with IMT ($\beta = .005$, $P < .001$), even after adjusting for age, sex ($\beta = .003$, $P < .001$), and glucose intolerance ($\beta = .002$, $P < .001$). In conclusion, OX-LDL levels increase with increasing glucose intolerance. Oxidized LDL is associated with carotid IMT and this is independent of age, sex, and glucose intolerance status.

1. Introduction

Several studies have established that there is an increased risk for coronary artery disease (CAD) not only in subjects with diabetes, but also in those with impaired glucose tolerance (IGT) [1-3]. It has also been shown that subclinical atherosclerosis as measured by intimal medial thickness (IMT) increases with increasing degrees of glucose intolerance [4,5]. Several mechanisms have been proposed to explain the accelerated atherosclerotic changes seen with glucose intolerance, and this includes inflammation [6,7], increased platelet activation [8,9], and oxidative stress [10]. After the initial studies by Goldstein et al [11] on modification of low-density lipoprotein (LDL) and its role in atherosclerosis, several studies have investigated the role of modified LDL as a biochemical risk factor for atherosclerosis [12-15]. Of the various modified forms of LDL, oxidized LDL (OX-LDL) has gained a lot of interest recently [13,14].

The oxidative conversion of LDL to OX-LDL is considered to be an important event in the biologic process that initiates and accelerates the development of the early atherosclerotic lesion [15-18]. Furthermore, circulating OX-LDL has also been proposed to give additive information to that provided by Global Risk Assessment Scoring [14] for assessing the risk for CAD.

Although several studies have reported on increased circulating levels of OX-LDL in relation to late end points of atherosclerotic process [12-14], very few have looked at the relation of OX-LDL with subclinical atherosclerotic markers such as IMT [7,19], and none in an Asian population. Asian Indians are known to have very high prevalence of diabetes [20,21] and premature CAD [1,22] compared with Europeans. In this study we report on the association of

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OX-LDL with IMT in Asian Indians with IGT and type 2 diabetic subjects, and compare them with subjects with normal glucose tolerance (NGT).

2. Research design and methods

The study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiologic study conducted on a representative population (aged ≥20 years) of Chennai (formerly Madras), the fourth largest city in India. The methodology of the study has been published elsewhere [23,24]. Briefly, in phase 1 of the urban component of CURES, 26 001 individuals were recruited based on a systematic random-sampling technique, which is described in our Web site www.drmohansdiabetes.com (under the link "Publications"). Fasting capillary blood glucose was determined using a One Touch Basic glucose meter (Life scan, Johnson & Johnson, Milpitas, CA) in all subjects. Subjects were classified as "known diabetic subjects" if they stated that they had diabetes and were on the treatment [25].

In phase 2 of CURES, all the known diabetic subjects (n = 1529) were invited to the center for detailed studies on vascular complications; 1382 responded (response rate, 90.3%). From the rest of the study subjects, 10% of newly diagnosed diabetic subjects (n = 320; response rate, 98.8%), 15% of subjects with impaired fasting glucose (n = 866; response rate, 99.1%), and 10% of subjects with normal fasting glucose (n = 1494; response rate, 97.0%) were recruited. Those who were confirmed with oral glucose tolerance test to have 2-hour plasma glucose value of 11.1 mmol/L or more (200 mg/dL) based on World Health Organization consulting group criteria [26] were labeled as "newly detected diabetic subjects," those with 2-hour postglucose value of ≥7.8 mmol/L (140 mg/dL) and <11.1 mmol/L (200 mg/dL) [26] as IGT, and those with 2-hour postglucose value of less than 7.8 mmol/L (140 mg/dL) as NGT.

Group 1 was composed of 175 control subjects with NGT. Group 2 included 175 subjects with IGT and group 3, 175 subjects with type 2 diabetes, all chosen randomly from those who satisfied the inclusion criteria. The inclusion criteria for all groups were not smoking; normal resting 12-lead electrocardiogram; absence of angina, myocardial infarction, or history of any known vascular, infectious, or inflammatory diseases; and not on statins or aspirin. The study had adequate power (90%) for obtaining a correlation coefficient of 0.3 between the study parameters, with an α error of .01 in each study group (NCSS and PASS, Number Cruncher Statistical System, Kaysville, UT). It also had a power of 87% to detect a difference of 5 U/L between groups for OX-LDL, with an SD of 15 and an α error of .05 (PS sample size calculations version 2.1.3.1).

2.1. Measurement of intima-media thickness

The method used for measurement of carotid IMT at our center has been described in earlier publications [27,28] but will be briefly outlined here. The intima plus medial thickness of the right common carotid artery was determined using a high-resolution B-mode ultrasonography system (Logic 400 GE, Milwaukee, WI) having an electrical linear transducer midfrequency of 7.5 MHz. The axial resolution of the system was 0.3 mm. The images were recorded as well as photographed. The scanning was done for an average of 20 minutes.

The IMT was measured as the distance from the leading edge of the first echogenic line to the second echogenic line during the diastolic phase of cardiac cycle. Six well-defined arterial wall segments were measured in the right carotid system: the near wall and far wall of the proximal 10 mm of the internal carotid artery, the carotid bifurcation beginning at the tip of the flow divider and extending 10 mm below this point, and the arterial segment extending 10 mm below the bifurcation in the common carotid artery. Essential in defining these segments is the identification of a reliable longitudinal marker, which is the carotid flow divider as performed in the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE) study [29].

Images were captured using a special grabber card, and the measurements were performed offline, manually. This method was standardized at our center and, for quality check, the videotapes were sent to Hamilton, Canada, the central laboratory for the SECURE and Global Registry of Acute Coronary Events (GRACE) studies. All scanning were conducted by a trained ultrasonologist who was unaware of the clinical status of the study subjects. The reproducibility of the IMT measurement was examined by conducting another scan 1 week later on 20 subjects by the same sonographer. The mean difference in IMT between the first and second measurements was 0.02 mm, the SD 0.06 mm, and the mean difference ranged between −0.09 and +0.09 mm.

2.2. Anthropometric measurements

Anthropometric measurements, including those of weight, height, and waist, were obtained using standardized techniques as detailed elsewhere [23]. Height was measured with a tape measure to the nearest centimeter. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Waist was measured using a nonstretchable fiber measuring tape. The body mass index was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 minutes apart, and the mean of the two was taken as the blood pressure.

2.3. Biochemical parameters

Fasting plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase-peroxidase-amidopyrine method), and

Table 1
Clinical characteristics of study subjects

Parameters	NGT (n = 175)	IGT (n = 175)	DM (n = 175)
Age (y)	45 ± 10	51 ± 10 ***	52 ± 9***
Male, n (%)	70 (33.2)	51 (31.5)	89 (39.7)
Waist circumference (cm)	85.7 ± 10.9	90.1 ± 11.9***	89.6 ± 10.2**
Systolic blood pressure (mm Hg)	119± 15	130 ± 21***	128 ± 18***
Diastolic blood pressure (mm Hg)	76 ± 10	79 ± 11*	76 ± 11
Fasting plasma glucose (mmol/L)	4.8 ± 0.4	5.3 ± 0.7	9.1 ± 3.8***,‡
HbA_{1c} (%)	5.7 ± 0.53	6.2 ± 0.69**	8.7 ± 2.2***,‡
Serum cholesterol (mmol/L)	4.91 ± 1.0	4.96 ± 0.9	5.2 ± 1.0**,†
Serum triglycerides (mmol/L)	1.41 ± 0.88	1.61 ± 0.89	1.84 ± 1.0***
LDL cholesterol (mmol/L)	3.0 ± 0.85	3.1 ± 0.83	3.2 ± 0.8
Carotid IMT (mm)	0.71 ± 0.12	0.79 ± 0.16***	0.85 ± 0.30***,†

* $P < .05$ compared to NGT.
** $P < .01$ compared to NGT.
*** $P < .001$ compared to NGT.
† $P < .05$ compared to IGT.
‡ $P < .001$ compared to IGT.

high-density lipoprotein cholesterol (direct method-polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). The intra- and interassay coefficient of variation for the biochemical assays ranged between 3.1% and 7.6%. Low-density lipoprotein cholesterol was calculated using the Friedewald formula [30]. Glycated hemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA). The intra- and interassay coefficient of variation of HbA_{1c} was less than 10%.

2.4. Oxidized LDL

Oxidized LDL was measured using commercially available sandwich enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden) in which 2 monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. During incubation, OX-LDL in the sample reacts with anti–OX-LDL antibodies bound to the microtitration well. After washing, which removes nonreactive plasma components, a peroxidase-conjugated antihuman apolipoprotein B antibody recognizes the OX-LDL bound to solid phase. After a second incubation and simple washing step that removes unbound enzyme-labeled antibody, the bound conjugate is detected by colorimetry with substrate. Absorbance was read at 450 nm. The intra- and interassay coefficient of variation for the assay ranged between 5.5% and 8.6%.

2.5. Statistical analysis

Student t test or 1-way analysis of variance (with Tukey honestly significant difference test) was used to compare groups for continuous variables, and χ^2 test or Fisher exact test as appropriate was used to compare proportions. Pearson correlation analysis was carried out to determine the relation of OX-LDL with other risk variables. Regression analysis was done to determine the association of OX-LDL with IMT. All analyses were done using Windows-based SPSS statistical package (Version 10.0, Chicago, IL), and P values of less than .05 were taken as significant.

3. Results

The clinical and biochemical profiles of the study subjects are shown in Table 1. Subjects with diabetes and IGT were older ($P < .001$) and had significantly higher waist circumference ($P < .001$) and systolic blood pressure ($P < .001$) compared with those who have NGT. Diabetic subjects had significantly higher serum cholesterol levels compared with those who have NGT ($P < .01$) and IGT ($P < .05$), and higher triglyceride levels compared with subjects with NGT ($P < .001$).

Subjects with diabetes had higher IMT values (0.85 ± 0.30 mm) compared with those who have IGT (0.79 ± 0.16 mm, $P < .05$) and subjects with NGT (0.71 ± 0.12 mm, $P < .001$); however, after adjustment for age, the difference reached statistical significance only with respect to subjects with NGT ($P < .001$). Subjects with diabetes (40.1 ± 13.1 U/L) and IGT (34.3 ± 12.8 U/L) had significantly higher mean OX-LDL values compared with the NGT group (26.2 ± 16.6 U/L) ($P < .001$). The P for trend was

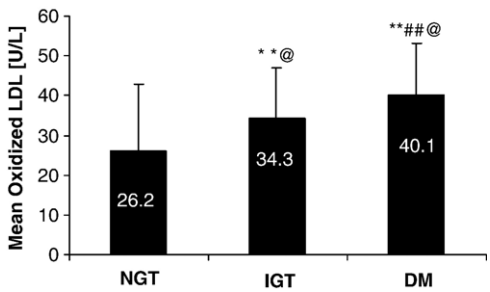

Fig. 1. Mean OX-LDL values in the study groups. Error bars indicate SD. $^{**}P < .001$ compared to NGT. $^{\#\#}P < .001$ compared to subjects with IGT. $^{@}P < .001$ compared to NGT after adjusting for age.

Table 2
Pearson correlation analysis of OX-LDL with other risk variables

Variables	OX-LDL	
	r	*P*
Age	0.297	<.001
Systolic blood pressure	0.106	.015
Diastolic blood pressure	0.053	.219
Waist circumference	0.080	.070
Fasting plasma glucose	0.218	<.001
2-h Postload plasma glucose[a]	0.324	<.001
HbA_{1c}	0.279	<.001
Serum cholesterol	0.139	<.001
Serum triglycerides	0.079	.067
HDL cholesterol	−0.004	.933
LDL cholesterol	0.122	.005

HDL indicates high-density lipoprotein cholesterol.
[a] Known diabetic subjects were excluded from the analysis.

statistically significant ($P < .001$), and adjustment for age did not alter the statistical significance (Fig. 1).

Table 2 presents the Pearson correlation analysis in the total population, which reveals that OX-LDL was significantly correlated with age ($P < .001$), systolic blood pressure ($P = .015$), fasting plasma glucose ($P < .001$), 2-hour postload plasma glucose ($P < .001$), HbA_{1c} ($P < .001$), serum cholesterol ($P < .001$), and LDL cholesterol ($P = .005$).

Fig. 2 presents the scatterplot of OX-LDL and IMT. Oxidized LDL had a strong correlation with IMT in the total population ($P < .001$). When categorized as NGT, IGT, and diabetes, the correlation remained significant only in subjects with NGT ($P < .001$) and IGT ($P < .001$).

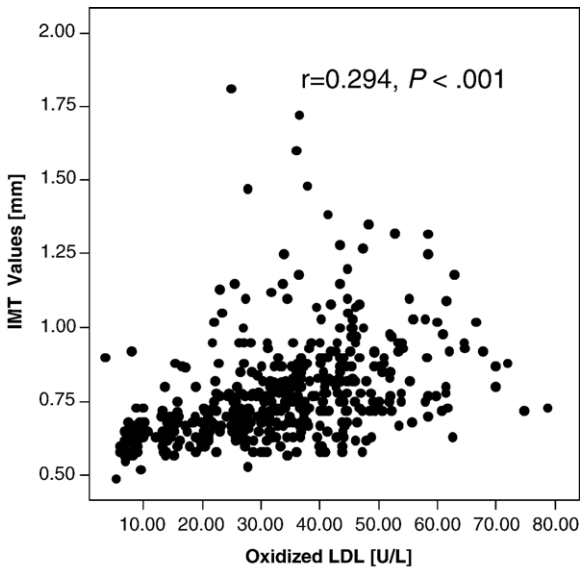

Fig. 2. Correlation of IMT with OX-LDL in the total study population.

Table 3
Multiple linear regression analysis

Parameters	β	*P*
OX-LDL	.005	<.001
OX-LDL	.003	<.001
Age	.009	<.001
Sex (male = 0, female = 1)	−.033	.077
OX-LDL	.002	<.001
Age	.008	<.001
Sex (male = 0, female = 1)	−.038	.077
Glucose intolerance (NGT = 0, IGT = 1, DM = 2)	.027	.021

IMT is the dependent variable. DM indicates diabetes mellitus.

In multiple linear regression analysis, OX-LDL showed a strong association with IMT ($\beta = .005$, $P < .001$), which remained significant even after adjusting for age, sex ($\beta = .003$, $P < .001$), and glucose intolerance ($\beta = .002$, $P < .001$) (Table 3).

When segregated based on glucose intolerance status, OX-LDL showed a strong association with IMT even after adjusting for age and sex in subjects with NGT ($\beta = .003$, $P < .001$) and IGT ($\beta = .004$, $P < .001$), but not in subjects with diabetes.

4. Discussion

There are 3 main findings in this study. Firstly, OX-LDL values increased with increasing severity of glucose intolerance, with diabetic subjects having the highest values followed by those with IGT and NGT. Secondly, OX-LDL showed an association with IMT, which was independent of age, sex, and glucose intolerance status. Thirdly, OX-LDL is associated with IMT in those with IGT and NGT. This study is of significance as this is the first report in Asian Indians on the association of OX-LDL and one of the few to demonstrate the association with IMT particularly in prediabetic subjects.

The role of OX-LDL in atherosclerosis is well recognized [15]. It has been hypothesized that LDL is oxidized in the intima of the arterial vessel wall. Oxidized LDL increases the adherence and penetration of monocytes, by stimulating monocyte chemoattractant protein-1 (MCP-1) and the inflammatory process, resulting in foam cell formation and thereby triggering the atherosclerotic lesion [31]. Oxidized LDL is found in monocyte-derived macrophages in atherosclerotic lesion, but not in normal arteries [32]. It has also been suggested that OX-LDL induces smooth muscle cell proliferation [33].

Earlier studies have documented that in diabetic subjects, hyperglycemia modifies LDL and makes it more vulnerable to oxidation [34,35]. This has now been extended to subjects with prediabetes [36]. In the present study, subjects with IGT had higher OX-LDL values compared with subjects with NGT, and this increase remained significant even after adjusting for age. This is consistent with the well-known phenomenon that even in the stages of prediabetes, the atherosclerotic changes are seen [5].

This study also supports an earlier prospective study in Sweden, which showed that baseline OX-LDL was associated with number of plaques in the carotid artery [37]. Furthermore, OX-LDL has been shown to be directly related to the severity of the disease, assessed by angiography [7]. In this study, OX-LDL was associated with IMT, and these associations remained significant even after adding age, sex, and glucose intolerance status into the regression model.

The results of the present study are also in accordance with that of Hulthe and Fagerberg [7] and Metso et al [19] who showed that OX-LDL showed a strong association with IMT. In the study by Hulthe and Fagerberg [7], OX-LDL showed a good correlation with cytokines, indicating its role in triggering the immune system. Furthermore, earlier studies provided evidence for the role of OX-LDL in triggering adhesion of monocytes favoring the progression of atherosclerotic process [15].

An interesting observation in the study is that the OX-LDL showed a good correlation with IMT in subjects with NGT and IGT, but not in subjects with diabetes. The probable explanation for this finding could be that modification of LDL occurs relatively early in the natural history of diabetes, that is, even before clinical diabetes sets in. It is also possible that back diffusion of OX-LDL from the intimal space to the circulating blood may be altered in subjects with diabetes, which results in nonsignificant association between the OX-LDL and IMT.

There are several limitations to this study. Firstly, the sample size is relatively small. However, the subjects were recruited from a population-based study and careful inclusion criteria were used. Secondly, being a cross-sectional study, a cause-and-effect relation of OX-LDL with atherosclerosis cannot be determined.

In conclusion, OX-LDL has a significant association with carotid IMT, and this is independent of age, sex, or glucose intolerance.

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