

Plasma oxidized low-density lipoprotein is an independent risk factor in young patients with coronary artery disease

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Abstract. *Objectives:* Oxidized low-density lipoprotein (ox-LDL) is considered to be a key factor of initiating and accelerating atherosclerosis. The objective of this study was to investigate the role of ox-LDL in young patients with coronary artery disease (CAD).

Methods: 128 consecutive angiographically proven young CAD patients (aged ≤ 55 years) were enrolled, and 132 age-matched non-CAD individuals (coronary angiography normal or negative finding by coronary ultrafast CT) were set as control group. Conventional risk factors (hypertension, dyslipidemia, diabetes mellitus, obesity, smoking) were evaluated in the two groups. Ox-LDL was measured by competitive ELISA. Framingham risk score (FRS) and absolute 10-year CAD events risk were calculated for each individual.

Results: Male sex was more prevalent in group CAD than in control (87.5% vs. 62.1%; $P < 0.01$). There were significant differences in smoking history ($P < 0.01$) and triglyceride (TG) and ratio of apolipoprotein B/apolipoprotein A1 (ApoB/ApoA1) (both $P < 0.05$) but no remarkable difference in other conventional risk factors (all $P > 0.05$) between group CAD and control. Level of ox-LDL was significantly higher in group CAD than in control ($P < 0.01$). Multivariate logistic regression showed that male sex (OR, 4.54; 95%CI, 1.76–9.77), smoking quantity (OR, 2.78; 95%CI, 1.34–4.25), TG (OR, 1.42; 95%CI, 1.18–2.83), ApoB/ApoA1 (OR, 1.73; 95%CI, 1.32–4.23), and ox-LDL (OR, 2.15; 95%CI, 1.37–6.95) were independently correlated with CAD in young patients. Area under the curve (AUC) of receiver operating characteristic (ROC) curve of TG, ApoB/ApoA1, and ox-LDL was 0.831, 0.866, and 0.935, respectively ($P < 0.001$).

Conclusions: Ox-LDL is an important independent risk factor for CAD in young patients after adjusting other risk factors such as smoking, TG, and ApoB/ApoA1.

Keywords: Risk factors, young, coronary artery disease, oxidized low-density lipoprotein

1. Introduction

Coronary artery disease (CAD) is becoming the leading killer in developing countries, including China. It

usually involves the middle and older age. However, CAD has been reported in younger age more frequently in recent years [1]. Studies have shown that risk factors of young patients with CAD are different from those of older patients but controversies still remain. Lamm et al. [2] reported that conventional risk factors, including family history, plasma lipids, hypertension, diabetes mellitus (DM), and cigarette smoking were no different except low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)

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levels in young patients with CAD. While Fang et al. [1] found that smoking, CRP, ratio of apolipoprotein B/apolipoprotein A1 (ApoB/ApoA1) and triglyceride (TG) are independent risk factors for young patients with CAD. However, these studies focus only on conventional risks factors and did not explore nonconventional risk factors.

The Framingham risk score (FRS) is a well-known risk scoring system for predicting CAD [3,4]. However, it underestimates CAD risk in young individuals [5]. Conventional risk factors such as dyslipidemia, hypertension, DM, and smoking cannot account for all cases of young CAD. Thus there are other nonconventional risk factors that appear to be related to atherosclerosis in young patients. One plausible postsecretory modification of LDL-C that may render it more atherogenic is oxidation [6]. There are some reports showing that plasma oxidized LDL (ox-LDL) was elevated in CAD patients, suggesting that ox-LDL may be a marker for atherosclerosis [7,8]. We previously showed that ox-LDL is a better biomarker than total cholesterol (TC), TG, HDL-C, and LDL-C for discriminating between patients with CAD and healthy subjects [9]. However, there are no data about the relevance of ox-LDL in young individuals. The purpose of this study was to evaluate the prevalence of conventional risk factors in young patients with CAD in China and to assess the predictive value of ox-LDL as biomarker for CAD in these patients [10].

2. Methods

2.1. Subjects

In the present study, we defined young CAD patients as those aged ≤ 55 years, following the definition used in previous studies [11–13].

One hundred and twenty-eight young patients diagnosed as CAD by coronary angiography (CAG) were enrolled. Considering the excellent negative predictive value for CAD of ultrafast CT [14,15], 132 age-matched individuals with normal CAG or negative finding by coronary ultrafast CT were enrolled as control. The reason for coronary angiography or CT was owing to chest pain with ischemic findings such as ST deviation in electrocardiograph, regional dyskinesia in ultrasonic cardiography, positive treadmill test, and/or myocardial perfusion scintigraphy, or difficult for differential diagnosis. Patients with severe or uncontrolled

infectious diseases, malignancy, autoimmune disease, and psychiatric disorders were excluded.

Recruitment was from September 2008 to September 2010. Candidates should be willing to sign informed consent. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital at Shunde of Southern Medical University and the First Affiliated Hospital of Sun Yat-sen University.

2.2. Diagnosis of CAD

All CAD patients underwent selective CAG by means of Judkins technique using Allura Xper FD20 (Philips, Amsterdam, Netherlands). Coronary artery stenosis was analyzed by two independent interventional cardiologists who were blinded to the clinical characteristics of patients. CAD was diagnosed with the following standard: $\geq 50\%$ stenosis of the lumen diameter occurring in ≥ 1 major coronary artery including the left main coronary artery (LM), left anterior descending (LAD) branch, left circumflex (LCX) branch, right coronary artery (RCA), the larger diagonal (D) branch, and obtuse marginal (OM) branch.

2.3. Laboratory measurements

Venous blood samples were collected in EDTA vials (1 mg/dL) from all participants between 7 and 9 AM after 12-h fasting and centrifuged at 4500 rpm for 10 minutes. Sera were extracted from the samples and levels of TC, TG, LDL-C, HDL-C, ApoA1, ApoB, and glucose were determined with OLYMPUS AU2700 Automatic Biochemical Analyzer (Japan). The concentration of ox-LDL in plasma was measured by sandwich enzyme-linked immunosorbent assay (ELISA) procedure using the murine monoclonal antibody mAb-4E6 (Merckodia), which is directed against a conformational epitope in the ApoB-100 moiety of LDL that is generated as a consequence of substitution of lysine residues of ApoB-100 with aldehydes. The method was developed by Holvoet et al. [16] and described in detail in our previous report [9]. All samples were detected in triplicate. Coefficients of variation for intraassay and interassay in this study were 2.0% and 6.3%, respectively.

2.4. Determination of risk factors

Conventional risk factors of CAD included: (1) hypertension was defined according to Joint National

Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC) VII guideline [17]; (2) dyslipidemia was defined as history of receiving antidiyslipidemia agents or as satisfying criteria on admission such as TC \geq 240 mg/dL, LDL-C \geq 160 mg/dL, HDL-C $<$ 40 mg/dL, and/or TG \geq 150 mg/dL [18]; (3) DM was defined as history of treatment with either oral hypoglycemic medication or insulin and on the basis of American Diabetes Association criteria [19]; (4) body mass index (BMI) indicative of overweight (\geq 25.0) or obese (\geq 30.0) [20]; (5) As to smoking, the patients were classified as current smokers (if they reported to smoke regularly during the past 1 year preceding the enrollment) and nonsmokers (including former smokers if they had stopped smoking at least 1 year before the enrollment, and never smokers). For participants who reported past or current cigarette smoking, information on the number of cigarettes smoked was also collected and quantified as pack years from the number of packs per day multiplied by number of years smoked; one pack contains 20 cigarettes [21]; (6) alcohol abuse history was defined as alcohol consumption \geq 100 g per day, whereas occasional drinkers or those drinking less were disregarded [1]. (7) Family history was deemed positive if any of these criteria were present: the diagnosis of CAD in any first-degree male relative $<$ 55 years of age or in any first-degree female relative $<$ 65 years of age [22]. We also used the Framingham risk score system for the determination of absolute 10-year CAD events risk.

2.5. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science software release 13.0 (SPSS Inc, Chicago, IL). Continuous variables are presented as mean \pm standard deviation. Categorical variables are expressed as percentages. After testing for normality using the Kolmogorov–Smirnov test, continuous variables were compared by Student's *t*-test or otherwise the Mann Whitney *U*-test was implemented. Categorical variables were compared by chi-square test or Fisher's exact test as appropriate. Associations between variables were evaluated by Spearman rho and Pearson tests. Multicollinearity (strong correlations among independent variables) was examined by collinearity diagnostic statistics. Variance inflation factor values $>$ 2.5 or tolerance $<$ 0.4 may indicate concern for multicollinearity in logistic regression models [23]. To delineate predictive factors for coronary artery disease, multivariate logistic regression

analysis was performed and adjusted odds ratio (OR) as an estimate of relative risk and 95% confidence intervals (95%CI) were calculated. For logistic regression, smoking history was quantified as pack-years and dichotomized at the median value of all subjects, BMI at 25 kg/m² (the point of overweight), TC, LDL-C, HDL-C, and TG at 240, 160, 40, and 150 mg/dL, respectively (the points defined as dyslipidemia), ApoB/ApoA1 at the mean value of all subjects, and ox-LDL at the mean value of all subjects. Finally, discriminatory power of independent parameters was quantified in terms of area under receiver operating characteristics (ROC) curve. A *P* value $<$ 0.05 was accepted as significant.

3. Results

3.1. Clinical characteristics of patients

One hundred twenty-eight young patients diagnosed as CAD (112 men, 16 women) by CAG and 132 age-matched controls with CAG normal (*n* = 65; 40 men, 25 women) or negative finding by coronary ultrafast CT (*n* = 67; 42 men, 25 women) were enrolled; their demographics and clinical characteristics are shown in Table 1.

Compared with control, CAD group had a greater percent of male (*P* $<$ 0.01) and current smoking and dyslipidemia (both *P* $<$ 0.05), but the differences in percent of positive family history, DM, hypertension, overweight, and obesity were not significant (all *P* $>$ 0.05). Pack years of smoking (median for all subjects, 9.8) was more in CAD than control. There was no significant difference of use of statins, which can reduce the amount of ox-LDL, between the groups (*P* $>$ 0.05). FRS and estimated 10-year absolute CHD event risk were low in both groups and did not show statistical difference (Table 1). Average level of TG, ApoB/ApoA1 (mean value of all subjects, 0.97), and ox-LDL (mean value of all subjects, 1.90 mg/dl) was higher in CAD than control (*P* $<$ 0.05), but no remarkable difference was found in other lipid markers between the two groups (*P* $>$ 0.05) (Table 2).

3.2. Correlation of variables and risk factors in young patients with CAD

Correlations between variables are shown in Table 3. Level of ox-LDL was significantly correlated with current smoking, pack years of smoking, TG, and

Table 1
Demographic and clinical characteristics of patients in CAD/Control Group

	CAD group (n = 128)	Control group (n = 132)
Age (years)	47.2 ± 5.6	47.5 ± 5.7
Men [n(%)]	112 (87.5%)	82 (62.1%)**
Women [n(%)]	16 (12.5%)	50 (37.9%)**
Hypertension [n(%)]	20 (15.6%)	22 (16.7%)
Systolic blood pressure (mm Hg)	120.5 ± 8.4	121.6 ± 8.9
Diastolic blood pressure (mm Hg)	75.8 ± 5.3	76.2 ± 6.1
Dyslipidemia	46 (35.9%)	37 (28.0%)*
TC ≥ 240 mg/dL [n(%)]	24 (18.8%)	23 (17.4%)
HDL-C < 40 mg/dL [n(%)]	20 (15.6%)	22 (16.7%)
LDL-C ≥ 160 mg/dL [n(%)]	18 (14.1%)	18 (13.6%)
TG ≥ 150 mg/dL [n(%)]	40 (31.3%)	32 (24.2%)*
Diabetes mellitus [n(%)]	12 (9.4%)	13 (9.8%)
Fasting blood glucose (mg/dl)	91.4 ± 8.5	92.1 ± 8.8
Overweight [n(%)]	44 (34.4%)	47 (35.6%)
Obesity [n(%)]	2 (1.6%)	2 (1.5%)
Body mass index (kg/m ²)	25.5 ± 0.5	25.4 ± 0.6
Current smokers [n(%)]	80 (62.5%)	33 (25.0%)*
Pack-years of smoking	14.2 ± 6.4	5.2 ± 4.3**
Alcohol abuse [n(%)]	16 (12.5%)	19 (14.4%)
CAD family history [n(%)]	21 (16.4%)	20 (15.2%)
Statins therapy at baseline [n(%)]	6 (4.7%)	8 (6.1%)
Framingham risk score	5.2 ± 0.6	5.0 ± 0.5
10-year CAD event risk (%)	4.4 ± 0.3	4.1 ± 0.3

Abbreviations: CAD, coronary artery disease, TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol.
* $P < 0.05$; ** $P < 0.01$.

Table 2

Comparison of blood lipid profiles between group CAD and control

	CAD group (n = 128)	Control group (n = 132)
TC (mg/dl)	205.5 ± 31.4	194.6 ± 30.7
HDL-C (mg/dl)	40.1 ± 9.2	41.3 ± 9.8
LDL-C (mg/dl)	120.2 ± 21.7	117.3 ± 19.4
TG (mg/dl)	165.0 ± 29.6	145.6 ± 28.8*
ApoB/ApoA1	1.08 ± 0.15	0.85 ± 0.07*
ox-LDL (mg/dl)	2.95 ± 0.33	0.83 ± 0.15**

CAD, coronary artery disease, TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol; ox-LDL, oxidized low-density lipoprotein

* $P < 0.05$; ** $P < 0.01$.

ApoB/ApoA1 ($P < 0.01$), but all correlations (r) were < 0.5 .

Multivariate logistic regression analysis demonstrated that male sex, smoking, TG, ApoB/ApoA1, and ox-LDL were enrolled into the regression equation. Related data are shown in Table 4. Additionally, collinearity statistics were > 0.4 for tolerance and < 2.5 for variance inflation factor, suggesting that multicollinearity was not a concern among the independent variables.

The area under curves (AUC) of TG, ApoB/ApoA1, and ox-LDL for predicting premature CAD was 0.853, 0.866, and 0.935, respectively (Fig. 1), all were > 0.50 ($P < 0.001$).

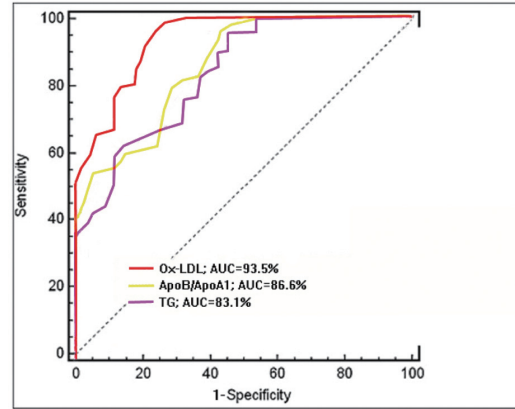


Fig. 1. Receiver-operating characteristic curves for predicting premature CAD. Ox-LDL = oxidized LDL; ApoB/ApoA1 = the ratio of ApoB to ApoA1; TG = triglyceride.

4. Discussion and conclusion

Many epidemiological, case-control, and prospective studies have shown that the most important risk factors for CAD include smoking, hypertension, dyslipidemia, DM, aging, and family history of premature atherosclerosis [6]. There has been an alarming increase in the prevalence of CAD in relatively young patients all over the world including China [1]; however,

Table 3
Correlation matrix of variables with corresponding *r*-values

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Current smoking	1.00								
(2) Pack-years of smoking	0.65**	1.00							
(3) Alcohol abuse	0.25	0.31	1.00						
(4) TC	0.09	0.11	0.17	1.00					
(5) HDL-C	-0.18	-0.16	-0.13	0.14	1.00				
(6) LDL-C	0.07	0.09	0.19	0.42*	-0.10	1.00			
(7) TG	0.22	0.15	0.36**	-0.05	-0.22	0.15	1.00		
(8) ApoB/ApoA1	0.11	0.21	0.18	-0.04	-0.32	0.16	0.31*	1.00	
(9) ox-LDL	0.26*	0.31*	0.17	0.36	-0.05	0.38	0.28*	0.33*	1.00

TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol; ox-LDL, oxidized low-density lipoprotein.

* $P < 0.05$ (2-tailed); ** $P < 0.01$ (2-tailed).

Table 4
Risk factors for CAD in young patients (multivariate logistic regression analysis)

Risk factors	OR	95% CI	<i>P</i> -value
Male gender (male vs female)	4.54	1.76 ~ 9.77	0.001
Pack-years of smoking (≥ 9.8 vs < 9.8)	2.78	1.34 ~ 4.25	0.005
TG (≥ 150 mg/dL vs < 150 mg/dL)	1.42	1.18 ~ 2.83	0.032
ApoB/ApoA1 (≥ 0.97 vs < 0.97)	1.73	1.32 ~ 4.23	0.025
ox-LDL (≥ 1.90 mg/dL vs < 1.90 mg/dL)	2.15	1.37 ~ 6.95	0.003

For logistic regression, smoking history was quantified as pack-years and dichotomized at the median value (9.8) of all subjects, BMI at 25 kg/m² (the point of overweight), TC, LDL-C, HDL-C, and TG at 240, 160, 40, and 150 mg/dL, respectively (the points defined as dyslipidemia), ApoB/ApoA1 at the mean value of all subjects (0.97), and ox-LDL at the mean value of all subjects.

TG, Triglyceride; ox-LDL, oxidized low-density lipoprotein; OR, odds ratio; 95% CI, 95% confidence interval.

conventional risk factors cannot account for all these patients. Thus there are other nonconventional risk factors that appear related to CAD in young patients.

For the first time, we report that ox-LDL is an independent risk factor of CAD in young Chinese patients after adjustment of other risk factors including ApoB/ApoA1 ratio. We found that male sex, smoking quantity, TG, ApoB/ApoA1, and ox-LDL are independent risk factors for young patients with CAD.

Ox-LDL is more atherogenic than native LDL; ox-LDL is taken up by scavenger receptor system ultimately leading to generation of foam cells and development of early lesions, and is a chemoattractant for monocytes and T lymphocytes, and also inhibits macrophage motility, thereby promoting retention of macrophages in the arterial wall [6]. Ox-LDL is also cytotoxic and impairs endothelial function as well as induces expression of genes such as interleukin-1 that can promote proliferation of smooth muscle cell and procoagulant state [6]. Furthermore, ox-LDL may promote platelet adhesion, trigger DNA strand to break, and promote apoptosis, all of which contribute to the development of atherosclerosis [24]. In this study, we found that increased LDL oxidation could explain pre-

mature atherosclerosis in the face of less conventional risk factors, and ox-LDL is an independent risk factor of CAD in young patients.

It has been suggested that ApoB is a strong predictor of CAD [25]. Because antibody 4E6 is specific for detection of oxidized ApoB-100 (the major component of ApoB), whether ox-LDL is independent of and adds prognostic information beyond ApoB itself is still controversial. Wu et al. [26] reported that circulating ox-LDL, measured with antibody 4E6, was not an independent predictor of CAD after adjustment of lipid markers and was less predictive of development of CAD than ApoB and TC/HDL-C ratio. However, Meisinger et al. [27] reported that ox-LDL was a predictor of CAD even after multivariable adjustment including TC/HDL-C ratio, although they did not include ApoB. It has been reported that Apo B/A1 ratio (reflecting the ratio of atherogenic/nonatherogenic apolipoproteins) is more accurate identification of cardiovascular risk and increased apolipoprotein B is mainly responsible for the higher apo B/A1 ratio [22]. In our study, we found that ox-LDL was related with ApoB/A1 ratio significantly but not strongly ($r = 0.33$; $P < 0.01$); after adjustment of lipid markers including ApoB, it

was still a risk factor of young patients with CAD. The possible interpretation for our inconsistent finding with Wu's study [26] may be different baseline characteristics of the individuals enrolled. In our study, we focused on relatively young patients aged < 55 years, most of whom were male with a lower percentage of hypertension and dyslipidemia, higher percentage of smoking; whereas in Wu's study, the patients were older, matched for both sexes, and with a higher percentage of hypertension and dyslipidemia but lower percentage of smoking. Increase of ox-LDL is related with smoking [28]; Sekher et al. [29] reported smoking has no effect on amino acid composition of ApoB-100 of LDL but directly influences the antioxidant status. We also found smoking is significantly correlated with levels of ox-LDL ($r = 0.26$; $P < 0.01$). The high prevalence of smoking in our patients may account for the importance of ox-LDL among these individuals besides ApoB.

When compared with TG and ApoB, ROC curve analysis confirmed superior performance of association between ox-LDL levels and CAD (Fig. 1). AUC of TG, ApoB/ApoA1, and ox-LDL for predicting young patients with CAD was 0.853, 0.866, and 0.935, respectively; all were > 0.50 ($P < 0.001$).

FRS is a well-known risk scoring system which incorporates age, TC, HDL-C, smoking status, and systolic blood pressure to calculate the score for CAD prediction [3,4]. In our study, although CAD patients were younger, differences in percentage of DM, hypertension, obesity, and level of LDL-C, HDL-C, and TG were not significant between CAD and control; therefore it was not surprising that FRS analysis had limited efficacy for CAD prediction in our study.

Some data had shown that circulating inflammatory markers such as C-reactive protein (CRP) are also associated with incidence of CAD in young patients [1, 30]. The absence of CRP measurement is a drawback of our study. However, many prospective studies evaluating predictors of CAD including CRP and ox-LDL suggest that there is no significant interactions between ox-LDL and CRP; adjustment for inflammatory markers in multivariate models did not alter the effect of ox-LDL for predicting CAD [26,27,31].

In conclusion, the present study suggests that smoking, hypertriglyceridemia, ApoB/ApoA1, and ox-LDL are independent risk factors of young patients with CAD. Increased LDL oxidation could explain premature atherosclerosis in the face of less conventional risk factors. These data may be useful in directing primary and secondary preventive measures.

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Conflict of interest

None to declare.

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