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Lipids (2012) 47:963–972
DOI 10.1007/s11745-012-3706-x

ORIGINAL ARTICLE

Serum Small Dense Low-density Lipoprotein Concentrations are Elevated in Patients with Significant Coronary Artery Stenosis and are Related to Features of the Metabolic Syndrome

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Received: 17 February 2012 / Accepted: 30 July 2012 / Published online: 18 August 2012
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Abstract Serum small dense low-density lipoprotein (sd-LDL) concentrations were measured in patients with angiographically defined coronary artery disease (CAD) and compared to concentrations in healthy subjects. Five hundred and seventy patients with stable CAD were divided into CAD− and CAD+ based on angiography. Patients in whom stenosis was <50 % in diameter were classified as having a 'normal' angiogram (CAD−), otherwise the patients were allocated to the CAD+ group. The CAD+ group was further subcategorized into single-, double- and triple-vessel disease (VD). Serum sd-LDL concentrations were significantly lower in controls compared with CAD+ and CAD− patients ($P < 0.001$). Moreover, CAD+ patients had higher concentrations of sd-LDL than CAD− patients ($P < 0.01$). sd-LDL levels were not significantly associated with severity of CAD defined by the number of stenosed coronary arteries ($P = 0.245$). All participants were also categorized into subgroups with or without metabolic syndrome. Subjects with metabolic syndrome had higher levels of sd-LDL than subjects without metabolic syndrome ($P < 0.01$). Multiple linear regressions showed that in CAD patients, triacylglycerol, total-cholesterol, body mass index, and waist circumferences were the most important determinants of serum sd-LDL concentrations. We found that sd-LDL levels were significantly higher in patients presenting with symptoms of CAD. Moreover, patients with significant stenosis of their coronary arteries (>50 % stenosis) had higher levels of sd-LDL compared to patients without significant lesions.

Keywords Small dense low-density lipoprotein · Coronary artery disease · Severity · Metabolic syndrome · Angiography

Abbreviations

AHA/NHLBI	American Heart Association/National Heart, Lung and Blood Institute
ANOVA	One-way analysis of variance
BMI	Body mass index
CVD	Cardiovascular disease
CAD	Coronary artery Disease
FBG	Fasting blood glucose
HDL-C	High density lipoprotein cholesterol
hs-CRP	High sensitive C-reactive protein
MS	Metabolic syndrome
Sd-LDL	Small-dense low-density lipoprotein
SPECT	Single photon emission computed tomography
SVD	Single vessel disease

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Introduction

A high serum low-density lipoprotein cholesterol (LDL-C) is a well established risk factor for cardiovascular disease (CVD) [1]. However, the qualitative features of LDL particles also appear to play an important role in the development of CVD as a novel risk factor, particularly the size spectrum of LDL-C particles and the predominance of small dense LDL (sd-LDL) particles. Several studies have investigated the possible role of sd-LDL in the pathogenesis of CVD and most have reported a positive association between the sd-LDL levels and the presence and severity of CVD [2–6].

It has been previously reported that the mean LDL particle size is smaller in those patients with proven coronary artery disease (CAD) based on angiography than for healthy controls; the authors observed that after multiple regression analysis the presence of high levels of sd-LDL was a significant and independent risk factor for CAD [2]. Moreover, it has been reported that men with an LDL particle size <25.6 nm had a significantly higher (2.2-fold increase) in the 5-year rate of ischemic heart disease compared with those men having an LDL particle size >25.6 nm [6] and hence it has been proposed that the presence of high concentrations of sd-LDL particles is a potent risk factor for CVD [3]. Previous studies have reported that there is a linear correlation between the sd-LDL concentrations and the risk of development of cardiovascular events [4, 5]. It has also been demonstrated that in healthy middle-age individuals the predominance of sd-LDL particles is associated with increased proinflammatory activation of peripheral mononuclear cells [7]. There is also evidence that sd-LDL is associated with the metabolic syndrome [8, 9]. In the present study, we aimed to evaluate the association between sd-LDL concentrations and severity of atherosclerosis defined by number of stenosed vessels in Persian population and also to assess the relationship between sd-LDL concentrations and several components of the metabolic syndrome.

Methods

Study Population

The study participants were selected from those subjects who underwent coronary angiography in the Quem Hospital, Mashhad in the north-eastern region of Iran. Angiography was principally indicated for stable angina, in patients who were positive for at least one objective test of myocardial ischemia including: exercise stress test, dobutamine stress echocardiography, and thallium SPECT (single photon emission computed tomography). The exclusion criteria for the study were as follows; oral contraceptives or hormone replacement therapy, pregnancy, prior history of coronary angioplasty or coronary artery bypass graft, having overt clinical features of infection or chronic inflammatory disease, and all subjects were negative for viral markers of hepatitis and anti-HIV antibody. Moreover, patients with myocardial infarction within the previous 3 months, renal, hepatic or malignant diseases were excluded. Finally, 570 patients fulfilled the inclusion criteria and blood samples were collected from all of them prior to the procedure. These patients included those with a primary diagnosis of CAD and included diabetic patients. Patients who were diagnosed with CAD were treated with a statin, and those who were diagnosed as diabetic were treated with anti-diabetic drugs. No subjects in the control group were treated with statins, whilst 22.6 and 32.4 % of patients in CAD− and CAD+ group were previously treated with statins respectively.

Coronary angiograms were performed using routine procedures using a femoral approach on patients who were fasted prior to the procedure. Analysis of the angiograms was performed offline by a specialist cardiologist. The presence of one or more stenoses ≥50 % in diameter of at least one major coronary artery (left main, right coronary artery, left anterior descending, circumflex) was considered evidence of significant CAD [10]. Patients in whom stenosis was <50 % in diameter were categorized as having a 'normal' angiogram (CAD−). The CAD+ patients were sub-classified according to the number of significantly affected stenotic vessels into: single-vessel ($n = 114$), double-vessel ($n = 123$), and triple-vessel ($n = 174$) disease groups. One-hundred and nine healthy volunteers were also recruited as a normal control group. The control group comprised subjects who had never experienced any symptom nor had any signs of CVD. These subjects had no other apparent major disease. Information on smoking, drug use and family history of CAD was obtained via a questionnaire. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS) and written informed consent was obtained from each participant.

Definition of Metabolic Syndrome

The American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) guideline was used to categorize subjects into metabolic syndrome-positive and metabolic syndrome-negative subgroups [11]. Metabolic syndrome was defined as the co-occurrence of at least three of the following five metabolic abnormalities: (1) elevated serum fasting glucose (≥100 mg/dl) or use of medication for hyperglycemia; (2) elevated serum triacylglycerols (≥150 mg/dl); (3) reduced serum high density lipoprotein-

cholesterol (HDL-C) (<40 mg/dl in males and <50 mg/dl in females); (4) elevated systolic (≥130 mmHg) or diastolic (≥85 mmHg) blood pressure or use of medication for hypertension; and (5) elevated waist circumference (≥102 cm in males and ≥88 cm in females).

Anthropometric and Other Measurements

Anthropometric parameters including weight, height, and body mass index (BMI) were measured in all subjects. Weight was measured with the subjects dressed in light clothing after an overnight fasting using a standard scale. BMI was calculated as weight (in kg) divided by height squared (in square meters). Blood pressure was measured twice while the patients were seated and rested, using a standard mercury sphygmomanometer. The systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1), and the diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflation of the cuff.

Blood Sampling and Biochemical Analysis

Blood samples were taken from each patient for analysis prior to the procedure. Following venipuncture, blood samples were collected into Vacutainer® tubes and centrifuged at 15,000*g* for 15 min at 4 °C. After separation, aliquots of serum were frozen at −80 °C until analysis. A full fasted lipid profile comprising total-cholesterol, triacylglycerols, HDL-C and LDL-C were determined for each subject. Serum lipid and fasting blood glucose (FBG) concentrations were measured enzymatically with the use of commercial kits using a BT-3000 autoanalyzer (Biotechnica, Rome, Italy). High sensitive C-reactive protein (hs-CRP) was measured by a PEG-enhanced immunoturbidimetry method with an Alcyon® analyzer (ABBOTT, Chicago, IL, USA).

Determination of Serum sd-LDL Levels

Serum sd-LDL concentrations were determined using a method previously described in detail by Hirano et al. [12]. Briefly, a precipitation reagent (150 U/ml heparin-sodium salt and 90 mmol/l $MgCl_2$) was added to 0.5 ml of serum sample, mixed and incubated for 10 min at 37 °C. The samples were placed in an ice bath and allowed to stand for 15 min, and centrifuged at 15,000 rpm for 15 min at 4 °C. An aliquot of the clear supernatant was removed for LDL-cholesterol and apo B analyses. The LDL-C in the heparin-Mg^{2+} supernatant (containing HDL and sd-LDL) was directly and selectively determined by a homogenous method. The concentration of the sd LDL-apoB in the heparin-Mg^{2+} supernatant was measured by an immunoturbidometric assay (Apo-B, Biosystems). In this method, as has been previously described, the cholesterol and apo-B values obtained by the precipitation method were similar to those obtained in the lipoprotein separated by ultracentrifugation, and the authors have found there was an excellent correlation between the two methods for sd LDL-cholesterol and apoB [12]. The coefficients of variation of inter- and intra-assay for the precipitation method were 1.3–1.6 and 1.7–3.7 %, respectively.

Statistical Analysis

All statistical analyses were performed using the SPSS for Windows™, version 16 software package (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± SD (for parameters with a normal distribution) or median and interquartile range (for non-normally distributed data). Data that were normally distributed were analyzed using Student's *t* test (for 2 groups) or one-way analysis of variance (ANOVA) (for >2 groups). Data found to be non-normally distributed were analyzed using the non-parametric Mann–Whitney test (for 2 groups) or Kruskal–Wallis (for >2 groups). Categorical data were compared using the Chi-square test. A two-sided *P* value <0.05 was considered statistically significant. A Bonferroni correction was applied in the comparison of sd-LDL values between control, CAD− and CAD+ groups. Bivariate correlations between different parameters and sd-LDL levels were performed using Spearman's rank correlation and Kendall's test. Stepwise multiple linear regression analysis was used to determine which of the conventional risk factors could influence sd-LDL values. The predictor variables classified as dichotomous (1 = yes/0 = no) including diabetes mellitus, hyperlipidemia, hypertension, sex and smoking were entered into the initial model. Height, weight, FBG, waist circumference, hip circumference, HDL-C, LDL-C, systolic and diastolic blood pressure, triacylglycerol and number of narrowed vessels were entered as continuous variables in the same model. The effect of statin therapy was also analyzed as a covariate variable.

Results

Demographic Characteristics

Clinical and biochemical characteristics of all 3 groups (CAD+, CAD−, and control) are summarized in Table 1. LDL-C values were found to be significantly higher in controls than for patients. This may be attributed to the fact that a proportion of the CAD patients were on treatment with a statin, whilst none of controls were on statin treatment. With regard to the subgroups of CAD+ patients with

Table 1 Demographic and clinical characteristics of CAD positive and negative and control participants

	CAD-positive	CAD-negative	Controls	*P* value
Number	411	159	109	
Gender (M/F)	260/151	48/111	49/60	<0.001
DM (%)	40.3	27.8	0.9	<0.001
Smoking (%)	46	26.6	3.7	<0.001
HTN (%)	69.1	72.8	4.2	<0.001
HLP (%)	40.8	34.8	0	<0.001
Statin (%)	32.4	22.6	0	<0.001
Age (year)	58.31 ± 10.72	52.71 ± 11.53	56.52 ± 6.70	<0.001
Height (cm)	161.10 ± 10.32	157.80 ± 9.76	162.88 ± 7.26	<0.001
Weight (kg)	70.54 ± 14.51	68.45 ± 13.46	71.95 ± 10.76	0.262
BMI (kg/m^2)	27.40 ± 8.02	27.62 ± 5.74	27.14 ± 3.95	0.799
WC/HC ratio	0.95 ± 0.09	0.92 ± 0.09	0.94 ± 0.07	<0.01
WC (cm)	91.63 ± 13.42	89.93 ± 14.33	97.95 ± 10.62	<0.05
HC (cm)	95.75 ± 12.08	97.43 ± 12.77	103.53 ± 7.54	<0.001
FBG (mg/dl)	126.92 ± 63.13	110.69 ± 45.89	80.53 ± 10.74	<0.001
TC (mg/dl)	176.29 ± 49.62	179.51 ± 48.04	185.68 ± 39.75	0.054
LDL-C (mg/dl)	102.91 ± 37.21	102.03 ± 40.01	120.79 ± 29.21	<0.001
HDL-C (mg/dl)	43.16 ± 13.03	44.01 ± 11.96	48.63 ± 39.47	0.133
TAG (mg/dl)	131.00 (94.00–188.00)	121.50 (86.00–179.00)	109.00 (93.00–139.00)	0.003
hs-CRP (mg/dl)	3.25 (1.33–7.72)	1.89 (1.00–6.28)	2.20 (1.40–3.01)	<0.001
SBP (mmHg)	142.47 ± 30.51	142.84 ± 26.47	121.84 ± 12.38	<0.01
DBP (mmHg)	78.29 ± 13.68	76.38 ± 14.46	76.58 ± 9.13	0.371
Sd-LDL (mg/dL)*	34.42 (19.68–53.37)	30.21 (16.52–46.52)	16.52 (11–27.5)	<0.001

Values are presented as means ± SD or median (interquartile range). Comparisons between controls, CAD+ and CAD− patients were performed using ANOVA or Kruskal–Wallis and Chi-square test

CAD coronary artery disease, *sd-LDL* small dense low density lipoprotein, *DM* diabetes mellitus, *HTN* hypertension, *HLP* hyperlipidemia, *WC* waist circumference, *HC*; hip circumference, *FBG* fasting blood glucose, *BMI* body mass index, *TC* total-cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TAG* triacylglycerol, *hs-CRP* high-sensitive C-reactive protein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

* Means before covariate analysis

different number of stenosed vessels [single-vessel disease (SVD), double-vessel disease (2VD), and triple-vessel disease (3VD)], no significant differences in demographic parameters were observed between different subgroups ($P > 0.05$, Table 2) except age. All subjects were divided into those with or without metabolic syndrome, based on AHA/NHLBI criteria. These data have been summarized in Table 3.

Sd-LDL Values Among Different Groups

Median sd-LDL values in the control group were [16.52 (11–27.5) (mg/dl)], being significantly lower than CAD− [30.21 (16.52–46.52) (mg/dl)] and CAD+ patients [34.42 (19.68–53.37) (mg/dl)] (comprising SVD, 2VD and 3VD) ($P < 0.001$). The results remained significant after Bonferroni correction. Moreover, CAD− patients had statistically lower median sd-LDL values in comparison with patients with SVD, 2VD and 3VD ($P < 0.01$). In the CAD+ group, median sd-LDL values were [36.26 (20.60–54.42) (mg/dl)], [29.16 (18.63–49.16) (mg/dl)] and [35.73 (19.55–57.05) (mg/dl)] in SVD, 2VD and 3VD patients respectively. Median sd-LDL levels were not significantly different among SVD, 2VD and 3VD patients ($P = 0.245$). Gender and smoking status did not alter the sd-LDL values significantly between controls, CAD+ and CAD− patients ($P > 0.05$).

The subjects were also divided into those with or without co-existing metabolic syndrome. As would be expected, the sd-LDL levels were significantly higher in patients with metabolic syndrome [33.9 (19.16–52.31) (mg/dl)] in comparison with patients without metabolic syndrome [27.6 (16.52–45.34) (mg/dl)] ($P = 0.006$). Sd-LDL levels where evaluated based on different components of the metabolic syndrome. In those subjects with several components of the metabolic syndrome such as increased waist circumference,

Table 2 Demographic and clinical characteristics of CAD positive participants

	1VD	2VD	3VD	*P* value
Number	114	123	174	
Gender (M/F)	66/48	83/40	111/63	0.305
DM (%)	38.4	42.9	39.8	0.855
Smoking (%)	50.0	45.8	43.5	0.664
HTN (%)	65.2	70.6	70.6	0.840
HLP (%)	33.9	44.5	42.7	0.391
Statin (%)	28.9	27.6	37.9	0.384
Age (years)	55.24 ± 10.78	57.67 ± 10.29	60.79 ± 10.44	<0.001
Height (cm)	162.18 ± 9.85	160.94 ± 12.12	160.51 ± 9.18	0.344
Weight (kg)	71.31 ± 14.74	71.58 ± 16.49	69.31 ± 12.77	0.786
BMI (kg/m^2)	27.14 ± 5.19	28.38 ± 12.69	26.89 ± 4.51	0.981
WC/HC ratio	0.95 ± 0.07	0.95 ± 0.12	0.96 ± 0.07	0.301
WC (cm)	92.26 ± 13.14	91.09 ± 15.46	91.58 ± 12.07	0.963
HC (cm)	97.14 ± 12.59	95.49 ± 12.93	94.99 ± 11.08	0.494
FBG (mg/dl)	122.23 ± 51.82	129.29 ± 69.54	128.20 ± 65.09	0.821
TC (mg/dl)	173.26 ± 44.48	169.86 ± 42.89	182.94 ± 56.43	0.214
LDL-C (mg/dl)	95.93 ± 35.75	104.17 ± 35.29	106.54 ± 39.11	0.118
HDL-C (mg/dl)	44.12 ± 12.91	42.75 ± 11.08	42.83 ± 14.44	0.652
TAG (mg/dl)	135.00 (91.75–193.00)	130.00 (92.00–183.00)	135.00 (96.25–189.75)	0.929
hs-CRP (mg/dl)	2.41 (1.14–5.69)	4.02 (1.22–15.08)	3.32 (1.55–7.67)	0.167
SBP (mmHg)	138.65 ± 32.52	143.85 ± 29.62	144.01 ± 29.72	0.290
DBP (mmHg)	78.22 ± 12.67	78.42 ± 14.39	78.24 ± 13.88	0.984
Sd-LDL (mg/dL)	36.26 (20.60–54.42)	29.16 (18.63–49.16)	35.73 (19.55–57.05)	0.245

Values are presented as means ± SD or median (interquartile range). Comparisons between 1VD, 2VD and 3VD patients were performed using ANOVA or Kruskal–Wallis and Chi-square test

CAD coronary artery disease, *sd-LDL* small dense low density lipoprotein, *DM* diabetes mellitus, *HTN* hypertension, *HLP* hyperlipidemia, *WC* waist circumference, *HC* hip circumference, *FBG* fasting blood glucose, *BMI* body mass index, *TC* total-cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TAG* triacylglycerol, *hs-CRP* high-sensitive C-reactive protein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *VD* vessel disease

reduced HDL-C, and hypertension had significantly higher levels of sd-LDL in comparison with those subjects without above components (Table 4). This was not however the case for hyperglycemia and hypertriacylglycerolemia. When the females and males with and without metabolic syndrome were analyzed separately, it was found that females ($P = 0.006$) but not in males ($P = 0.108$), sd-LDL levels were significantly different between patients with and without metabolic syndrome.

Covariate Analysis

The effects of statin therapy were analyzed as a covariate variable. We found that in the present study, statin therapy had a covariate effect in 3 groups of CAD+, CAD− and control subjects, whilst we did not observe this effect in the CAD+ subgroups. After covariate analysis, we found similar results, as CAD+ and CAD− patients had significantly higher levels of sd-LDL than controls ($P < 0.001$). Moreover, a significant difference was observed between patients with and without metabolic syndrome ($P < 0.05$). Covariate analysis also confirmed our previous results indicating that only in females ($P < 0.01$) but not in males ($P = 0.137$), sd-LDL levels were significantly different between patients with and without metabolic syndrome.

Correlation Between sd-LDL Values and CAD Risk Factors

Among the risk factors for CAD, in the CAD+ group waist and hip circumferences, waist/hip ratio, FBG, LDL-C, HDL-C, triacylglycerol (positively) and systolic blood pressure (inversely) were related to sd-LDL levels, however in CAD− patients sd-LDL levels were not associated with any of the classical CAD risk factors except waist circumference and triacylglycerol. In controls, FBG and triacylglycerol (positively) and LDL-C and hs-CRP (inversely) was associated with sd-LDL levels. No association was

Table 3 Demographic and clinical characteristics of patients with and without metabolic syndrome

	Total ($n = 587$)	MS+ ($n = 215$)	MS− ($n = 372$)	P value
Sd-LDL (mg/dL)*	29.7 (17–47.6)	33.9 (19.16–52.31)	27.6 (16.52–45.34)	0.006
Gender (F) (%)	47.4 %	61.4 %	34.9 %	<0.001
Age (years)	56.73 ± 10.60	57.50 ± 10.05	55.95 ± 11.36	0.091
Height (cm)	160.30 ± 10.15	158.24 ± 11.42	161.91 ± 9.23	<0.01
Weight (cm)	70.05 ± 14.09	74.06 ± 16.14	67.90 ± 12.40	<0.01
BMI (kg/m^2)	27.45 ± 7.31	29.96 ± 10.18	25.95 ± 4.71	<0.001
WC/HC ratio	0.94 ± 0.09	0.96 ± 0.07	0.93 ± 0.10	<0.001
WC (cm)	91.48 ± 13.63	96.63 ± 11.77	87.79 ± 13.55	<0.001
HC (cm)	96.58 ± 12.20	99.83 ± 10.24	94.30 ± 12.79	<0.001
FBG (mg/dl)	115.54 ± 56.58	135.71 ± 69.23	103.60 ± 41.47	<0.001
TC (mg/dl)	178.48 ± 47.91	177.00 ± 48.34	177.08 ± 44.99	0.937
LDL-C (mg/dl)	105.98 ± 37.12	102.64 ± 38.77	105.51 ± 36.35	0.191
HDL-C (mg/dl)	44.35 ± 20.56	37.89 ± 9.18	49.16 ± 25.44	<0.001
TAG (mg/dl)	124 (93.00–175.00)	166.5 (127.75–210.25)	105 (84.00–145.00)	<0.001
hs-CRP (mg/dl)	2.54 (1.26–6.20)	2.66 (1.26–6.22)	2.75 (1.26–6.88)	0.801
SBP (mmHg)	141.87 ± 29.26	155.67 ± 27.89	132.48 ± 26.42	<0.001
DBP (mmHg)	77.72 ± 13.77	81.99 ± 14.47	74.94 ± 12.55	<0.001
1VD (%)	16.8	29.4	27.6	0.895
2VD (%)	18.1	28.0	30.6	0.696
3VD (%)	25.6	42.7	41.8	0.914

Values are presented as means ± SD or median (interquartile range). Comparisons between patients with and without metabolic syndrome were performed using Student's t test or Mann–Whitney and Chi-square tests

Sd-LDL Small dense low density lipoprotein, *MS* metabolic syndrome, *WC* waist circumference, *HC* hip circumference, *FBG* fasting blood glucose, *BMI* body mass index, *TC* total-cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TAG* triacylglycerol, *hs-CRP* high-sensitive C-reactive protein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *VD* vessel disease

* Means before covariate analysis

Table 4 Sd-LDL values in subgroups with and without metabolic syndrome components

	Statin	MS− ($n = 372$)	MS+ ($n = 215$)	P value
Hypertriacylglycerolemia	No	23.37 (12.84–38.63)	32.31 (19.68–50.34)	0.129
	Yes	20.21 (8.5–38.23)	42.58 (19.68–61.13)	
Reduced HDL-C	No	23.37 (15.74–39.16)	32.84 (19.68–50.21)	<0.001
	Yes	19.68 (10.21–27.05)	36.79 (17.31–52.58)	
Elevated WC	No	31.79 (19.16–52.84)	33.89 (19.68–50.73)	0.005
	Yes	29.16 (17.71–47.71)	39.95 (22.58–56.79)	
Hypertension	No	43.10 (25.74–56.52)	33.37 (16.5–52.31)	0.002
	Yes	45.47 (24.95–58.63)	42.58 (27.97–60.73)	
Hyperglycemia	No	24.95 (11–44.95)	32.05 (17.01–48.89)	0.583
	Yes	28.10 (18.24–62.58)	44.68 (22.71–61.13)	

Group comparisons were performed using Kruskal–Wallis test with Bonferroni correction. As statin therapy was as a covariate variable, patients who were treated with stains were analyzed separately

Sd-LDL small dense low density lipoprotein, *MS* metabolic syndrome *HDL-C* high density lipoprotein-C, *WC* Waist circumference

found for traditional CAD risk factors such as diabetes mellitus, smoking, hypertension and hyperlipidemia with sd-LDL in 3 groups (Data have not been shown).

When the association between different biochemical and CAD risk factors with sd-LDL levels were assessed among the 3 subgroups of CAD patients (SVD, 2VD and 3VD), we found that LDL-C and triacylglycerol (positively) and smoking (inversely) were associated with serum sd-LDL concentrations in patients with SVD. In the 2VD subgroup, only triacylglycerol was associated with the sd-LDL

concentrations; whilst in the 3VD subgroup, waist and hip circumference, waist/hip ratio, FBG, LDL-C, HDL-C, triacylglycerol (positively) and systolic blood pressure (inversely) were associated with the sd-LDL concentrations (Data not shown).

Association Between sd-LDL Values and CAD Risk Factors

Stepwise multiple linear regressions in CAD patients yielded the following equation for the prediction of serum sd-LDL values [sd-LDL values = 0.115 (triacylglycerol) + 0.154 (total-cholesterol) − 0.891 (BMI) + 0.306 (waist circumferences)] (Table 5). Moreover, in patients with metabolic syndrome, the regression model yielded the following equation for the prediction of serum sd-LDL values in patients with metabolic syndrome [sd-LDL values = 0.149 (triacylglycerol) + 0.173 (total-cholesterol)] (Table 6). In patients without metabolic syndrome, the following equation for the prediction of serum sd-LDL values was found [sd-LDL values = 0.148 (total-cholesterol) + 9.07 (gender) + 0.061 (triacylglycerol) + 0.107

Table 5 Stepwise multiple linear regressions of factors affecting sd-LDL levels in CAD patients

Independent variable	Before covariate analysis			After covariate analysis		
	β	% Variation	*P* values	β	% Variation	*P* values
Triacylglycerol	0.115	16	0.0001	0.115	16.1	<0.001
Total-cholesterol	0.154	5.6	0.0001	0.16	6.1	<0.001
Body mass index	−0.891	1.2	0.002	−0.904	1.2	0.002
Waist circumferences	0.306	1.3	0.024	0.319	1.3	0.019

Diabetes mellitus, hyperlipidemia, hypertension, sex and smoking were entered into the initial model. Height, weight, fasting blood glucose, waist and hip circumference, high and low density lipoprotein-cholesterol, systolic and diastolic blood pressure blood pressure, triacylglycerol and number of narrowed vessels were entered as continuous variables in the same model. Regression analyses were performed before entering the effect of statin consumption as covariate variable

Sd-LDL small dense low density lipoprotein

Table 6 Stepwise multiple linear regressions of factors affecting sd-LDL levels in subjects with metabolic syndrome

Independent variable	Before covariate analysis			After covariate analysis		
	β	% Variation	*P* values	β	% Variation	*P* values
Triacylglycerol	0.149	19.6	0.0001	0.146	19.5	<0.001
Total-cholesterol	0.173	5.9	0.002	0.177	6.2	0.002

Diabetes mellitus, hyperlipidemia, hypertension, sex and smoking were entered into the initial model. Height, weight, fasting blood glucose, waist and hip circumference, high and low density lipoprotein-cholesterol, systolic and diastolic blood pressure blood pressure, triacylglycerol and number of narrowed vessels were entered as continuous variables in the same model. Regression analyses were performed before entering the effect of statin consumption as covariate variable

Sd-LDL small dense low-density lipoprotein

Table 7 Stepwise multiple linear regressions of factors affecting sd-LDL levels in subjects without metabolic syndrome

Independent variable	Before covariate analysis			After covariate analysis		
	β	% Variation	*P* values	β	% Variation	*P* values
Total-cholesterol	0.148	10.7	0.0001	0.146	10.5	<0.001
Sex	9.07	4.2	0.006	9.223	4.4	0.006
Triacylglycerol	0.061	2.5	0.015	0.062	2.7	0.015
Systolic blood pressure	0.107	1.9	0.043	–	–	–

Diabetes mellitus, hyperlipidemia, hypertension, sex and smoking were entered into the initial model. Height, weight, fasting blood glucose, waist and hip circumference, high and low density lipoprotein-cholesterol, systolic and diastolic blood pressure blood pressure, triacylglycerol and number of narrowed vessels were entered as continuous variables in the same model. Regression analyses were performed before entering the effect of statin consumption as covariate variable

Sd-LDL small dense low density lipoprotein

(systolic blood pressure)] (Table 7). After regression analysis and entering statin consumption as a covariate variable, we found similar results in all three regression analysis, but only in patients without metabolic syndrome systolic blood pressure lost its significance.

Discussion

To our knowledge, the predominance of the sd-LDL phenotype has not previously been studied in Persian CAD patients. We have however recently found higher levels of sd-LDL in SVD versus 3VD patients among 204 patients presenting with acute coronary syndrome [13]. Thus, we aimed to evaluate the possible relationship between the severity of CAD and sd-LDL concentrations within a larger sample size in which CAD was defined angiographically. In the present study, we found higher levels of sd-LDL concentrations in CAD+ patients compared with CAD− and healthy controls. Moreover, the sd-LDL values were significantly higher in CAD− patients, who had no significant stenosis in their coronary arteries based on angiography results, than healthy controls.

There have been previous studies that have reported a positive association between the sd-LDL levels, LDL particle size and the presence and severity of CVD [2–6]. It has also been reported that the mean LDL size was smaller in those patients with proven CAD based on angiography results than the healthy controls; Moreover, after multiple regression analysis, the presence of sd-LDL was found to be an important and independent risk factor for CAD development apart from the traditional cardiovascular risk factors [2]. Furthermore, it has been reported that men with an LDL particle size <25.6 nm had a significant 2.2-fold increase in the 5-year rate of ischemic heart disease compared with those men having an LDL particle size >25.6 nm [6] and it has been proposed that sd-LDL particles and high concentrations of sd-LDL are both important risk factors for CVD [3]. Previous reports indicate that there is a linear correlation between the sd-LDL concentrations and the risk of development of cardiovascular events [4, 5].

It has been proposed that sd-LDL may be involved in the pathogenesis of CVD via several mechanisms. Sd-LDL is one of the most atherogenic lipoprotein classes, and in comparison with larger-sized lipoproteins, sd-LDL particles have (1) a lower affinity for the LDL-receptors [14], (2) higher susceptibility to oxidative modification and (3) lesser antioxidants concentrations [15], (4) longer retention time in the circulation [16] and (5) enter the arterial wall more easily [17].

Although our method is less precise than the recently developed quantitative angiographic techniques such as those using the Gensini coronary atherosclerosis scores and angiography itself may not accurately measure the CAD severity, but based on the number of stenosed vessels, we did not find any association between sd-LDL levels and severity of CAD; LDL- and total-cholesterol levels were comparable between patients with SVD, 2VD and 3VD. Some studies have reported results consistent with ours. Koba et al. [18] found that LDL size is not related to the severity and extent of coronary lesions determined by Gensini score. In other study by Kwon et al. [2], it was found that patients with more extensive CAD had smaller LDL particles. In other study, both sd-LDL mass concentrations and also sd-LDL particle size were measured simultaneously in patients with CAD and their association with severity of disease (based on Gensini score) was determined. The authors found that high sd-LDL concentrations were closely related to the CAD severity independently of classical coronary risk factors, while LDL particle size was not related. Thus, they suggested that the progression of CAD is closely linked to the amount of sd-LDL but not with the LDL particle size [3].

There is also strong evidence suggesting that sd-LDL is independently associated with the metabolic syndrome; Haffner et al. [19] have reported a decreased LDL size in subjects with multiple metabolic disorders. Moreover, several studies have suggested sd-LDL as a valuable marker for diagnosis and severity of the metabolic syndrome [8, 9]. It has been previously reported that increased plasma triacylglycerol and decreased HDL-C concentrations are usually accompanied by the presence of sd-LDL particles comprising together the atherogenic lipoprotein phenotype [20, 21]. Furthermore, in regard to important role of triacylglycerol in the composition and metabolic fate of lipoproteins, it has been reported that 80 % of patients with serum triacylglycerols above 1.5 mmol/L are characterized by the presence of sd-LDL subfraction pattern [22]. In the present study, as would be expected the subjects with metabolic syndrome had higher levels of sd-LDL values compared with subjects without metabolic syndrome. Moreover, when sd-LDL levels were analyzed with respect to metabolic syndrome components separately and statin therapy analyzed as a covariate variable, we found that all of metabolic syndrome components except hypertriacylglycerolemia and hyperglycemia appear to affect sd-LDL levels significantly. In a recent cross-sectional study by Kathiresan et al. [23], the number of sd-LDL particles was found to be greater in patients with metabolic syndrome and to increase with the number of components of metabolic syndrome.

In addition, multiple linear regressions showed that triacylglycerol, total-cholesterol, BMI, and waist-circumferences were the most important determinants of sd-LDL levels in CAD patients. Increased plasma triacylglycerol

plays a critical role in preponderance of sd-LDL in CAD patients, as our results and other studies support that LDL particle size is associated with serum triacylglycerol level [24]. Hirano et al. [12] have reported that sd-LDL is positively associated with LDL-C and triacylglycerol and inversely with HDL-C. When the females and males with and without metabolic syndrome were analyzed separately, it was found that only in females but not in males, sd-LDL levels were significantly different. As Coresh et al. [25], have previously reported, this finding can be explained by the fact that female subjects had significantly higher levels of HDL-C and lower levels of triacylglycerol in comparison with the male subjects.

In the present study, the regression model showed that sd-LDL levels were negatively associated with BMI in the CAD patients. While it would be expected that patients with a high BMI, would have high levels of sd-LDL, the regression model did not show this in our sample population. This may be explained by this notion that more than 30 % of the subjects in CAD group were treated with statins. Moreover, it has been previously revealed that statin therapy does not decrease the proportion of sd-LDL among total LDL particles, but in fact increases it, while predictably reducing total LDL-C, absolute amounts of sd-LDL, and absolute amounts of large, buoyant LDL [26]. It has been proposed that statins are able to up-regulate the activity of LDL receptor and decreases large, buoyant LDL more than sd-LDL, as statins increase LDL receptor activity; large, buoyant LDL is a better ligand for the LDL receptor than sd-LDL [27]. Hence, it may be concluded that those patients who have higher BMI, are more prone to development of CAD and these patients usually receive statins lifelong. Thus, it could be possible those patients, who have higher BMI and are treated with statins, would have lower sd-LDL values.

In conclusion, we found that sd-LDL levels are significantly higher in patients presenting with symptoms of CAD. Moreover, patients with evident stenosis in their coronary arteries had also higher levels of sd-LDL levels compared with those patients without overt lesions. Due to our results, sd-LDL concentrations are not associated with severity of CAD defined by number of stenosed coronary arteries. In addition to assessing the impact of lipid-lowering agents on sd-LDL size further studies are needed to assess the effect of lipid lowering drugs in reduction of sd-LDL concentrations.

Limitations

This study has a number of limitations; first, it was not possible to control for the dosage, or type of statin in our subgroups of our patients. It is possible that patients with more severe disease were on higher doses of statin, or more potent statins, and this will cause increasing effects on sd LDL. We did not examine the size distribution of sd-LDL. It is not clear whether the LDL size profile or sd-LDL concentration is the better risk marker. There was a potential problem of selection bias in our study, as patients included were those with extant CAD rather than an unselected metabolic syndrome population. As we looked at a rather selected group of patients with metabolic syndrome, that may not be representative of all patients with metabolic syndrome. We hope future studies provide more information about this that which one (size or concentrations of sd-LDL) would be more useful in clinical practice.

Acknowledgments The Mashhad University of Medical Science has provided the financial supports for this study. We are particularly grateful to the patients and their family members who volunteered to participate in this study. We are particularly grateful to Ms. Zahra Gharaie Sheyda for data analysis. The results presented in this work have been taken from Shima Yazdandoust's thesis in Mashhad University of Medical Science.

Conflict of interest None.

References

1. Ross R (1999) Atherosclerosis–an inflammatory disease. N Engl J Med 340:115–126
2. Kwon SW, Yoon SJ, Kang TS, Kwon HM, Kim JH, Rhee J et al (2006) Significance of small dense low-density lipoprotein as a risk factor for coronary artery disease and acute coronary syndrome. Yonsei Med J 47:405–414
3. Koba S, Hirano T, Ito Y, Tsunoda F, Yokota Y, Ban Y et al (2006) Significance of small dense low-density lipoprotein-cholesterol concentrations in relation to the severity of coronary heart diseases. Atherosclerosis 189:206–214
4. Gardner CD, Fortmann SP, Krauss RM (1996) Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 276:875–881
5. St-Pierre AC, Cantin B, Dagenais GR, Mauriege P, Bernard PM, Despres JP et al (2005) Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol 25:553–559
6. Lamarche B, St-Pierre AC, Ruel IL, Cantin B, Dagenais GR, Despres JP (2001) A prospective, population-based study of low density lipoprotein particle size as a risk factor for ischemic heart disease in men. Can J Cardiol 17:859–865
7. Norata GD, Raselli S, Grigore L, Garlaschelli K, Vianello D, Bertocco S et al (2009) Small dense LDL and VLDL predict common carotid artery IMT and elicit an inflammatory response in peripheral blood mononuclear and endothelial cells. Atherosclerosis 206:556–562
8. Krayenbuehl PA, Wiesli P, Schmid C, Lehmann R, Spinas GA, Berneis K (2008) Insulin sensitivity in type 2 diabetes is closely associated with LDL particle size. Swiss Med Wkly 138:275–280
9. Gazi I, Tsimihodimos V, Filippatos T, Bairaktari E, Tselepis AD, Elisaf M (2006) Concentration and relative distribution of low-density lipoprotein subfractions in patients with metabolic syndrome defined according to the National Cholesterol Education Program criteria. Metabolism 55:885–891

10. Marroquin OC, Kip KE, Kelley DE, Johnson BD, Shaw LJ, Bairey Merz CN et al (2004) Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women: a report from the Women's Ischemia Syndrome Evaluation. Circulation 109:714–721
11. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C (2004) Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 109:433–438
12. Hirano T, Ito Y, Saegusa H, Yoshino G (2003) A novel and simple method for quantification of small, dense LDL. J Lipid Res 44:2193–2201
13. Emadzadeh MR, Alavi MS, Soukhtanloo M, Mohammadpour T, Rahsepar AA, Tavallaie S et al (2012) Changes in small dense low-density lipoprotein levels following acute coronary syndrome. Angiology, s1–7. doi:10.1177/0003319712441855
14. Chen GC, Liu W, Duchateau P, Allaart J, Hamilton RL, Mendel CM et al (1994) Conformational differences in human apolipoprotein B-100 among subspecies of low density lipoproteins (LDL). Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from hypertriglyceridemic subjects. J Biol Chem 269:29121–29128
15. Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ, Krauss RM (2001) Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. Am J Med 110:103–110
16. Packard CJ, Demant T, Stewart JP, Bedford D, Caslake MJ, Schwertfeger G et al (2000) Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. J Lipid Res 41:305–318
17. Bjornheden T, Babyi A, Bondjers G, Wiklund O (1996) Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. Atherosclerosis 123:43–56
18. Koba S, Hirano T, Kondo T, Shibata M, Suzuki H, Murakami M et al (2002) Significance of small dense low-density lipoproteins and other risk factors in patients with various types of coronary heart disease. Am Heart J 144:1026–1035
19. Haffner SM, Mykkanen L, Robbins D, Valdez R, Miettinen H, Howard BV et al (1995) A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non-diabetic subjects. Diabetologia 38:1328–1336
20. Rizzo M, Berneis K (2006) Low-density lipoprotein size and cardiovascular risk assessment. QJM 99:1–14
21. Austin MA, King MC, Vranizan KM, Krauss RM (1990) Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 82:495–506
22. Demacker PN, Veerkamp MJ, Bredie SJ, Marcovina SM, de Graff J, Stalenhoef AF (2000) Comparison of the measurement of lipids and lipoproteins versus assay of apolipoprotein B for estimation of coronary heart disease risk: a study in familial combined hyperlipidemia. Atherosclerosis 153:483–490
23. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW et al (2006) Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation 113:20–29
24. Tsunoda F, Koba S, Hirano T, Ban Y, Iso Y, Suzuki H et al (2004) Association between small dense low-density lipoprotein and postprandial accumulation of triglyceride-rich remnant-like particles in normotriglyceridemic patients with myocardial infarction. Circ J 68:1165–1172
25. Coresh J, Kwiterovich PO Jr, Smith HH, Bachorik PS (1993) Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. J Lipid Res 34:1687–1697
26. Choi CU, Seo HS, Lee EM, Shin SY, Choi UJ, Na JO et al (2010) Statins do not decrease small, dense low-density lipoprotein. Tex Heart Inst J 37:421–428
27. Nigon F, Lesnik P, Rouis M, Chapman MJ (1991) Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. J Lipid Res 32:1741–1753

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