Original Article

Small,dense LDL and High-Sensitivity C-Reactive Protein (hs-CRP) in Metabolic Syndrome with Type 2 Diabetes Mellitus

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Aim: To clarify the clinical significance of small,dense LDL (sLDL) in the metabolic syndrome associated with type 2 diabetes.

Methods: One hundred and ten healthy non-diabetic and non-metabolic syndrome subjects (58 male/52 female), 77 non-metabolic diabetic subjects (62/15), 58 non-diabetic metabolic subjects (25/33), and 46 metabolic diabetic subjects (29/17) were enrolled in this study.

Results: The subjects with metabolic syndrome (both with and without type 2 diabetes) had significantly higher fasting blood glucose, total-cholesterol (C), LDL-C, triglyceride, sLDL-C and hs-CRP levels than non-metabolic and non-diabetic subjects. HDL-C levels were significantly decreased in the former compared to the latter. Among the metabolic syndrome subjects, those with type 2 diabetes had significantly higher fasting blood glucose, systolic blood pressure and hs-CRP values than those without diabetes. sLDL-C, LDL-C and hs-CRP were the highest and HDL-C was lowest in the metabolic syndrome with diabetes group. A multiple regression analysis revealed the most significant determinant of sLDL-C to be LDL-C, followed by HDL-C, total-C, metabolic syndrome, type 2 diabetes mellitus, and triglyceride.

Conclusion: Metabolic syndrome is a significant determinant of the plasma sLDL-C level. Hs-CRP was the highest in the metabolic syndrome patients with type 2 diabetes. Therefore, type 2 diabetes may further increase the risk of coronary artery disease in the metabolic syndrome subjects through cardiovascular inflammation.

Introduction

A definition and diagnostic criteria for metabolic syndrome in Japan were established in April 2005[1]. It is now well-known that the presence of metabolic syndrome is associated with an increased frequency of coronary artery disease (CAD) events, cardiovascular mortality, or a reduced survival rate[2-4]. Metabolic syndrome

is also reported to be a predictor of subclinical atherosclerosis. The purpose of diagnosis and intervention for metabolic syndrome is not only the early detection of subclinical atherosclerosis but also the prevention of type 2 diabetes mellitus. Patients with diabetes mellitus are at increased risk of developing CAD[5, 6]. Therefore, if patients with metabolic syndrome develop type 2 diabetes, their risk for CAD will increase further.

Small,dense LDL has been demonstrated to be an additional risk factor for the development of CAD in Western countries as well as in Japan[7, 8]. The size of LDL is usually measured by gradient gel electrophoresis using a polyacrylamide gel[7]. This procedure takes time and money, Hirano *et al*. have developed a simple and rapid method of measuring the concentration

of small, dense LDL-cholesterol using heparin-magnesium precipitation and a direct assay[8].

Recent research into the inflammatory nature of atherosclerosis suggests that inflammatory-response proteins may serve as potential predictors of clinical events. One of these proteins, high-sensitivity C-reactive protein (hs-CRP), has been the focus of much attention. Epidemiological data have shown an independent association between elevated hs-CRP levels and coronary risk[9-11]. Recently, several investigators have found that human atherosclerotic lesions, coronary artery smooth muscle cells, aortic endothelial cells and adipocytes all express CRP[12-14]. Therefore, the persistent production of a small amount of CRP by atherosclerotic lesions and coronary artery smooth muscle cells may lead to a slight but chronic elevation in CRP levels. Thus, the hs-CRP assay is useful in predicting CAD risk[15].

The present study was conducted to explore whether the risk of CAD is greater among patients with metabolic syndrome who develop type 2 diabetes than among those with metabolic syndrome or type 2 diabetes alone by measuring plasma small, dense LDL-cholesterol and hs-CRP concentrations.

Subjects and Methods

A total of 291 subjects including 174 males and 117 females, 30 to 80 years old, were recruited from among individuals visiting the Division of Diabetes, Metabolism and Endocrinology, Omori Hospital Medical Center or participating in a local health check-up at several private companies. All study protocols and procedures were approved by the Ethics Committee of Toho University Medical Center, Omori Hospital. The study objectives and intended measures were explained to all subjects individually. Written informed consent was obtained from all participants. Subjects who had hepatic, renal or thyroid disease were excluded by routine serum biochemical analysis. Fibrate and statin users were also excluded. Participants were divided into 4 groups, ie: healthy non-diabetic and non-metabolic syndrome group (58 male/52 female), non-metabolic diabetic group (62/15), non-diabetic metabolic group (25/33), and metabolic diabetic group (29/17) were enrolled in this study.

Metabolic syndrome was diagnosed according to Japanese Guidelines[1].

Normotensive subjects receiving drugs for hypertension were considered to have hypertension. After informed consent was obtained, waist circumference and blood pressure (supine) were measured. Blood sampling was done after an overnight fast. Blood glucose, hemoglobin (Hb) A1c and plasma lipids were measured using standard laboratory methods. Small,dense LDL-cholesterol and hs-CRP concentrations were measured according to Hirano et al.[8], and Ledue et al.[16], respectively.

Statistical Analysis

All values are expressed as the mean ± SD. A one-way analysis of variance (ANOVA) and an analysis of covariance (ANCOVA) were used to compare mean values among groups. Chi-square tests were also employed. Multiple regression analyses were performed to evaluate the relationship between small,dense LDL-cholesterol and other clinical parameters employing Excel ver. 6. A significant difference was defined as $p < 0.05$.

Results

Baseline characteristics of the subjects are listed in **Table 1**. The non-metabolic diabetic subjects were older than the other three groups of subjects. Fasting blood glucose levels and systolic blood pressure were highest in the metabolic diabetic group. The subjects with metabolic syndrome (both with and without diabetes) had significantly high total-cholesterol (**Fig. 1**), LDL-cholesterol (**Fig. 2**), and triglyceride (**Fig. 3**), and suppressed HDL-cholesterol (**Fig. 4**) levels compared to the non-metabolic, non-diabetic group. Concentrations of small,dense LDL-cholesterol were significantly higher among the subjects with metabolic syndrome (with and without diabetes) than in the non-metabolic, diabetic group or non-metabolic, non-diabetic group (**Fig. 5**). The ratio of small,dense LDL-cholesterol to LDL-cholesterol was significantly higher among subjects with metabolic syndrome (with and without diabetes) than members of the non-metabolic, non-diabetic group (**Fig. 6**). hs-CRP levels were highest in the metabolic diabetic group (**Fig. 7**). The frequency of increased small,dense LDL-cholesterol levels (higher than 30 mg/dL) was only 4.3% in the non-metabolic syndrome group, compared to 70.1% in the metabolic syndrome. This difference was significant ($p < 0.01$) according to the Chi-square test. A multiple regression analysis revealed the most significant determinant of plasma small,dense LDL-cholesterol levels to be LDL-cholesterol, followed by HDL-cholesterol, total-cholesterol, the presence of metabolic syndrome (MS), an association with type 2 diabetes mellitus (DM), and triglyceride (TG) (**Table 2**). Thus, the presence of metabolic syndrome is one of the most significant determinants of the concentration of small,dense LDL-cholesterol in plasma. Further-

Table 1. Characteristics of study subjects

group	MS DM	(−) (−)	(−) (+)	(+) (−)	(+) (+)
(n)		110	77	58	46
male/female		58/52	62/15	25/33	29/17
		a	b	a	a
age (year)		51.9 ± 15.8	63.8 ± 11.0	47.5 ± 15.1	51.4 ± 15.5
		a	a	b	b
BMI		21.9 ± 3.6	22.6 ± 2.1	30.2 ± 5.6	30.1 ± 4.2
		a	b	c	d
WC (cm)		75.8 ± 11.6	82.3 ± 7.4	96.6 ± 10.5	105.8 ± 15.4
		a	b	c	d
FPG (mg/dL)		91.1 ± 15.6	153.4 ± 40.0	106.6 ± 13.6	153.6 ± 55.8
		a	b	b, c	d
SBP (mmHg)		119.0 ± 14.7	118.7 ± 22.1	129.4 ± 16.7	137.2 ± 21.2
		a	a	b	c
DBP (mmHg)		71.9 ± 11.3	74.6 ± 10.6	78.6 ± 12.1	82.7 ± 13.4

MS: metabolic syndrome, DM: type 2 diabetes, n: number of subjects, BMI: body mass index, WS: waist circumference,
FBG: fasting blood glucose, SBP: systolic blood pressure, DBP: diastolic blood pressure
Data are expressed as the mean ± SD   a, b, c, d: data not
sharing the same superscript are significantly different ($p < 0.05$ or less)
One-way ANOVA was employed for estimating the differences between age, BMI, and WC.
The differences in data between FPG, SBP and DBP were analyzed by ANCOVA with adjustmens for age and gender.
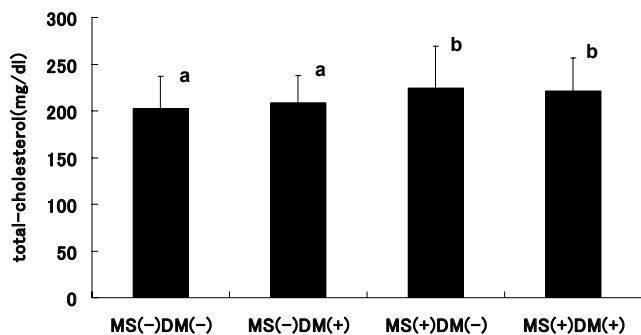
Fig. 1. Plasma total-cholesterol levels in the four study groups.

MS: metabolic syndrome, DM: type 2 diabetes mellitus. M: male,
Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.
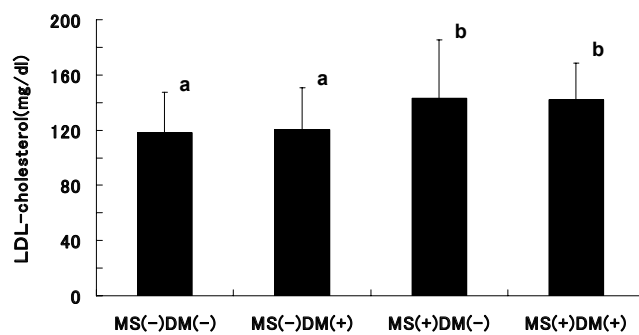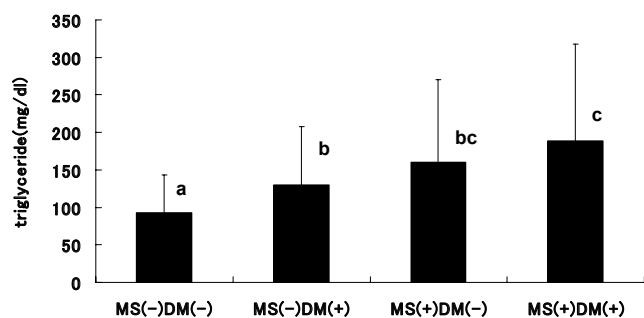

Fig. 2. Plasma LDL-cholesterol levels in the four study groups.

MS: metabolic syndrome, DM: type 2 diabetes mellitus. Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.

more, levels of small,dense LDL-cholesterol and hs-CRP were elevated in the patients with metabolic syndrome. Systolic blood pressure and hs-CRP were highest in the metabolic syndrome group and increased in association with type 2 diabetes.

Discussion

The visceral accumulation of fat has been recog-

nized as an important risk factor for CAD[17-20] since Fujioka *et al.* reported the contribution of intra-abdominal fat (visceral obesity) to the metabolism of glucose and lipid in human obesity[21]. Metabolic syndrome is a clinical entity characterized by visceral obesity, hypertension, hypertriglyceridemia, low HDL-cholesterol and glucose intolerance, although there is some incompatibility of diagnostic criteria among countries. In Japan, a definition and diagnostic criteria
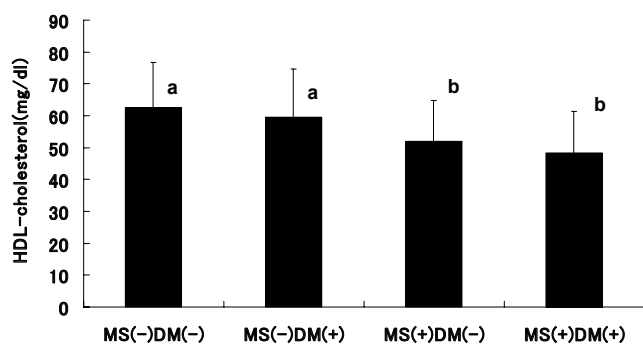
Fig. 3. Plasma triglyceride levels in the four study groups.
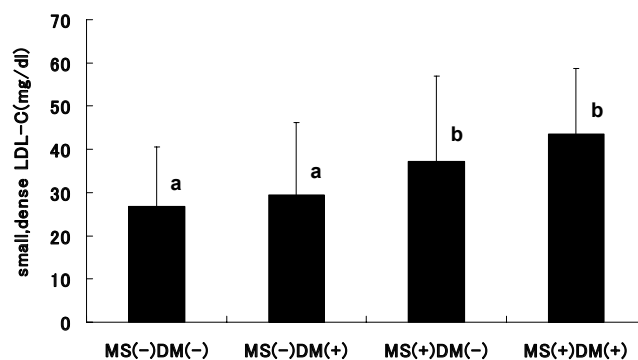
MS: metabolic syndrome, DM: type 2 diabetes mellitus. Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.

Fig. 4. Plasma HDL-cholesterol levels in the four study groups.

MS: metabolic syndrome, DM: type 2 diabetes mellitus. Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.
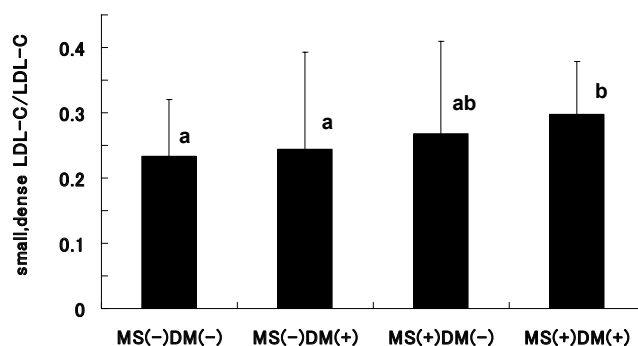

Fig. 5. Plasma small,dense LDL-cholesterol levels in the four study groups.

MS: metabolic syndrome, DM: type 2 diabetes mellitus. Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.
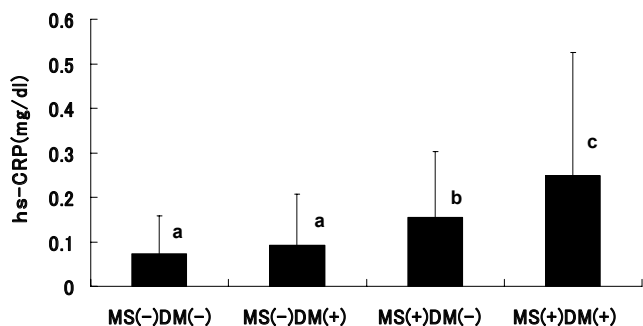

Fig. 6. Plasma small,dense LDL-cholesterol/LDL-cholesterol ratios in the four study groups.

MS: metabolic syndrome, DM: type 2 diabetes mellitus. Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.

for metabolic syndrome were established in April, 2005, in which increased waist circumference was emphasized as the most important component reflecting increased intra-abdominal accumulation of fat[1]. The presence of metabolic syndrome is associated with an increased frequency of CAD events, cardiovascular mortality, or reduced survival[2-4]. Metabolic syndrome is also reported to be predictive of subclinical atherosclerosis[22]. Since metabolic syndrome involves glucose intolerance, not intervening may result in type 2 diabetes. Thus, the purpose of diagnosis and intervention for metabolic syndrome is not only the early detection of subclinical atherosclerosis but also the prevention of type 2 diabetes mellitus. As patients with diabetes mellitus are at increased risk for CAD[5, 6], if patients with metabolic syndrome develop type 2 diabetes, their risk for CAD will increase further.

The LDL in plasma comprises multiple discrete subclasses, differing in size and density. Initial experiments conducted by Krauss and Burke[7] demonstrated that two distinct LDL subclass phenotypes can be distinguished on the basis of the distribution of LDL particles separated by gradient gel electrophoresis (GGE). In pattern A, the major peak is greater than 25.5 nm, whereas in pattern B, it is less than 25.5 nm. An investigation of LDL subfractions in normotriglyceridemic males revealed small LDL to be a coronary risk factor in subjects without apparent hyperlipidemia[23]. LDL composition was examined in diabetic, myocardial infarction survivors. Decreased cholesterol-loading in LDL and an increased number of LDL par-

Fig. 7. Plasma hs-CRP levels in the four study groups.

MS: metabolic syndrome, DM: type 2 diabetes mellitus. Data are expressed as the mean ± SD. There were significant differences between columns not sharing the same superscript ($p < 0.05$ or less). The data were analyzed by ANCOVA with adjustments for age and gender. The post-hoc test was followed by the Tukey test.

Table 2. Independent determinants of small,dense LDL-cholesterol levels in the multiple regression analysis

	R^2	r
LDL	0.35606	0.5360
TC	0.2774	0.4457
MS	0.1291	0.3579
DM	0.1267	0.2777
TG	0.1267	0.2046
FPG	0.0690	0.1853
HDL	− 0.3209	− 0.3777

R^2 = standard partial regression coefficient
r = single correlation coefficient

ticles were also remarkable in this group of patients, indicating the predominance of small,dense LDL in myocardial infarction survivors with type 2 diabetes[24].

A previous study based on gradient gel electrophoresis demonstrated subjects with metabolic syndrome to have small,dense LDL[22, 25]. In a recent cross-sectional study, the number of small,dense LDL particles determined by nuclear magnetic resonance spectroscopy was found to be greater in patients with metabolic syndrome and to increase with the number of components of metabolic syndrome[26]. In the present study, the subjects with metabolic syndrome (both with and without diabetes) had significantly higher levels of small,dense LDL-cholesterol (**Fig. 5**) than the non-metabolic, non-diabetic subjects. However, there was no significant difference in either small,dense LDL-cholesterol or small,dense LDL-cholesterol per LDL-cholesterol ratio between the metabolic syndrome patients with type 2 diabetes and those without. Increased small,dense LDL-cholesterol levels (higher than 30 mg/dL) were found in only 4.3% of subjects in the non-metabolic syndrome group versus 70.1% of those in the metabolic syndrome group.

The multiple regression analysis revealed the most significant determinant of plasma small,dense LDL-cholesterol levels to be LDL-cholesterol, followed by HDL-cholesterol, total-cholesterol, presence of metabolic syndrome, presence of type 2 diabetes mellitus, and triglyceride (**Table 2**). Thus, the small,dense LDL-cholesterol level in plasma is strongly affected by the presence of metabolic syndrome.

It is a matter of some debate how the presence of metabolic syndrome increases the amount of small,dense LDL. There is substantial evidence that the accumulation of intra-abdominal fat in individuals with type 2 diabetes and insulin resistance may increase the amount of free fatty acid released from adipocytes. The liver may increase triglyceride production by utilizing this free fatty acid from the plasma. As a consequence, there may be an increase in the production of triglyceride-rich (large) very low density lipoprotein (VLDL). This triglyceride-rich VLDL can be a precursor of small, dense LDL. ie; triglyceride-rich VLDLs are converted to triglyceride-enriched LDLs, the substrate favored by hepatic lipase, and transformed into smaller LDLs by lipase-mediated hydrolysis of triglycerides[27].

The atherosclerotic process is now recognized as an inflammatory disease in which immune mechanisms, triggered by endothelial injury, interact with metabolic risk factors, resulting in the initiation, propagation and activation of lesions in the arterial wall. It is therefore plausible to consider inflammatory-response proteins as predictors of cardiovascular events. CRP has been defined as a sensitive, but not specific, marker of inflammation. Several investigators reported that not only liver but also human atherosclerotic lesions, coronary artery smooth muscle cells, aortic endothelial cells and adipocytes express CRP[12-14]. This may indicate that persistent local production of CRP in atherosclerotic lesions, or coronary artery smooth muscle cells, leads to chronically evelated CRP levels of 0.1 to 0.3 mg/dL, which are detectable by hs-CRP assay and may be useful in predicting coronary risk[8-10]. In the present study, hs-CRP levels were highest in the metabolic diabetic group and significantly higher than those in the non-metabolic diabetic group, indicating the metabolic syndrome subjects who develop type 2 diabetes to be at further risk for CAD.

Conclusion

Metabolic syndrome increases the risk for CAD

in termes of LDL size and plasma hs-CRP levels. In this study, the presence of type 2 diabetes increased systolic blood pressure and hs-CRP levels in the metabolic syndrome subjects. Thus, not only prevention of metabolic syndrome but also intervention to stop the developement of type 2 diabetes is an important target for prevention of coronary atherosclerosis.

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