Serum levels of remnant lipoprotein cholesterol and oxidized low-density lipoprotein in patients with coronary artery disease

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Coronary artery disease; Lipoproteins; Lipoproteins; LDL

Summary
Background: Oxidized low-density lipoprotein (OxLDL) and remnant lipoprotein play a crucial role in the development of atherosclerosis. Recently, a novel method for measuring remnant cholesterol levels (remnant lipoproteins cholesterol homogenous assay: RemL-C) has been established. However, the correlation between OxLDL and remnant lipoprotein, including RemL-C, has not been fully investigated.

Methods: We enrolled 25 consecutive patients with documented coronary artery disease (CAD) and 20 controls. Remnant-like particle cholesterol (RLP-C) and RemL-C were used to determine the levels of remnant lipoprotein cholesterol. Serum levels of malondialdehyde-modified LDL (MDA-LDL) and OxLDL using a monoclonal antibody DLH3 (OxPC) were used to measure the concentration of circulating OxLDL.

Results: The CAD group had high levels of fasting glucose and glycosylated hemoglobin (HbA1c), and low levels of high-density lipoprotein cholesterol compared with the control group. Serum levels of total cholesterol or LDL cholesterol were not significantly different between the two groups. The levels of RemL-C (p = 0.035), MDA-LDL (p = 0.018), and MDA-LDL/LDL-C (p = 0.036) in the CAD group were significantly higher than those in the control group. The levels of RLP-C tended to be higher in the CAD group than those in the control group (p = 0.096). Positive correlations were demonstrated between remnant lipoprotein cholesterol and OxLDL (RLP-C and MDA-LDL/LDL-C, r = 0.45, p = 0.0024, RLP-C and OxPC, r = 0.51, p = 0.0005, RemL-C and MDA-LDL/LDL-C, r = 0.42, p = 0.0044, RemL-C and OxPC, r = 0.43, p = 0.0043).

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Similar trends were observed in non-diabetic subjects and in subjects without metabolic syndrome. Positive correlations were also observed between RLP-C and RemL-C ($r = 0.94$, $p < 0.0001$) and between MDA-LDL/LDL-C and OxPC ($r = 0.40$, $p = 0.0074$).

**Conclusions:** These results suggest that the association between high levels of remnant lipoprotein cholesterol and high OxLDL levels might be linked to atherogenesis in patients with CAD.

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## Introduction

Remnant lipoproteins, which are produced by hydrolysis of chylomicrons and very low-density lipoproteins, are thought to be atherogenic [1,2]. Remnant lipoproteins, not only activate surface molecules of monocytes and endothelial cells, but also induce foam cell formation and proliferation of smooth muscle cells [2]. Indeed, high levels of remnant-like particles cholesterol (RLP-C) determined by a widely used method, are considered to be a coronary risk factor and a predictor of cardiovascular events independent of high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in healthy subjects and patients with coronary artery disease (CAD) [3—6].

Oxidized low-density lipoprotein (OxLDL) plays a crucial role in atherogenesis via a number of initiative and accelerative functions, including adherence induction between endothelial cells and monocytes, recruitment of monocyte-derived macrophages into the vessel wall, and foam cell formation of macrophages [7—9]. Previous reports have shown that the level of circulating OxLDL is a marker for identifying patients with CAD or coronary spastic angina [10—12], and has a positive relationship with acute coronary syndromes [13,14]. Moreover, we and other groups demonstrated that high levels of OxLDL are independent predictors of future cardiovascular events in apparently healthy subjects and patients with CAD [15—18].

Until now, several methodologies have been available for determining the levels of remnant lipoprotein cholesterol as well as circulating OxLDL [8,9,19]. In the present study, we measured the levels of remnant lipoprotein cholesterol determined by RLP-C and a recently established method (remnant lipoproteins cholesterol homogenous assay: RemL-C), malondialdehyde-modified (MDA-LDL), and oxidized phosphatidylcholine (OxPC) in patients with CAD and control subjects. Moreover, we assessed each correlation between remnant lipoprotein cholesterol and OxLDL in those subjects.

## Methods

### Subjects

We enrolled 25 consecutive patients who underwent diagnostic angiography at Juntendo University between August 2006 and October 2006, and 20 controls who had no clinical history of CAD and hospitalization at the same period. All patients had documented CAD defined as more than 50% stenosis in at least one major coronary artery. Patients with acute coronary syndrome and/or ongoing congestive heart failure were excluded. Control subjects had no abnormal electrocardiographic finding and no evidence of coronary ischemia examined by stress cardiac testing at our outpatient clinic. Subjects who had liver and/or renal dysfunction, or were taking medications, including insulin, lipid-lowering drugs, and vitamin E were also excluded. All subjects gave written informed consent and the Ethical Committee of the Institution approved this study.

### Blood sampling and biochemical analysis

Whole blood samples were drawn after overnight fasting. Serum levels of total cholesterol, triglyceride (TG), HDL cholesterol, and high sensitivity C-reactive protein (hs-CRP), were measured by standard methods. LDL cholesterol values were measured by the direct assay (Sekisui Medical Co., Ltd., Tokyo, Japan). Serum levels of RLP-C were measured by widely using an immunoaffinity mixed gel containing anti-apolipoprotein A-1 and anti-apolipoprotein B-100 monoclonal antibodies method (JIMRO II, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), as previously described [3,20,21]. RemL-C, which has recently been established, was
also employed in this study (Kyowa Medex Co., Ltd., Tokyo, Japan). In brief, RemL-C utilizes a selective solubilizing and degradative method by using surfactant and phospholipase-D. After this reaction, released cholesterol was measured enzymatically [22]. The levels of malondialdehyde-modified LDL (MDA-LDL) recognized by the monoclonal antibody, ML25, and OxPC defined by the monoclonal antibody, DLH3, were measured by enzyme-linked immunosorbent assay, as we and other groups previously reported [8,16,17,23,24].

Statistical analysis

Statistical intergroup differences were analyzed by the Chi-square test and the Student’s t-test. Correlation between the two parameters was determined by simple linear regression analysis. A value of p < 0.05 was considered to be significant.

Results

Characteristics of the study subjects

The characteristics of the subjects are shown in Table 1. There was no significant difference in body mass index, prevalence of hypertension, smoking history, or family history, between the two groups. The CAD group had a higher prevalence of diabetes mellitus (p = 0.012) and metabolic syndrome (p = 0.005) defined by the Evaluation Committee of Diagnostic Criteria for Metabolic syndrome [25], and significantly lower levels of HDL cholesterol (p = 0.003) than the control group. The levels of fasting glucose and glycosylated hemoglobin (HbA1c) in the CAD group were significantly higher than in the control group (p = 0.019, p = 0.001, respectively). Total cholesterol and LDL cholesterol levels were not significantly different between the two groups.

Comparison of RLP-C, RemL-C, MDA-LDL, and OxPC between the two groups

As shown in Fig. 1, the CAD group had significantly higher levels of RemL-C than the control group (12.3 ± 9.0 mg/dl vs. 7.4 ± 5.1 mg/dl, p = 0.035). The level of RLP-C tended to be higher in the CAD group than that in the control group (9.1 ± 8.6 mg/dl vs. 5.7 ± 3.3 mg/dl, p = 0.096). As demonstrated in Fig. 2, the level of MDA-LDL and the ratio of MDA-LDL to LDL cholesterol (MDA-LDL/LDL-C) were significantly higher than those in the control group (181 ± 53 IU/l vs. 147 ± 36 IU/l, p = 0.018; 1.36 ± 0.36 vs. 1.16 ± 0.21, p = 0.036, respectively). There was no significant difference of Ox-PC level between the two groups (9.5 ± 6.9 IU/l vs. 8.0 ± 2.9 IU/l, p = 0.38).

The levels of RLP-C (6.6 ± 3.5 mg/dl vs. 4.5 ± 1.8 mg/dl, p = 0.050) and RemL-C (9.0 ± 5.2 mg/dl vs. 5.9 ± 3.7 mg/dl, p = 0.066) tended to be higher in the CAD group than in the control group even in subjects without metabolic syndrome. The levels of RLP-C (6.9 ± 3.1 mg/dl vs. 5.4 ± 3.2 mg/dl, p = 0.1) and RemL-C (9.9 ± 4.7 mg/dl vs. 7.0 ± 4.9 mg/dl, p = 0.085) tended to be higher in the CAD group than in the control group in subjects without diabetes. The levels of MDA-LDL were significantly higher in the CAD group than in the control group in the subjects without diabetes (193 ± 61 IU/l vs. 144 ± 36 IU/l, p = 0.0065).

Associations between remnant lipoprotein cholesterol and OxLDL

As shown in Fig. 3, the levels of RLP-C were positively correlated with MDA-LDL/LDL-C (r = 0.45, p = 0.0024) and OxPC (r = 0.51, p = 0.0005) in all subjects. The serum levels of RemL-C were also positively correlated with MDA-LDL/LDL-C (r = 0.42, p = 0.0044) and OxPC (r = 0.43, p = 0.0043) in all subjects. The positive correlations between RLP-C and RemL-C (r = 0.43, p = 0.030), RLP-C and OxPC (r = 0.53, p = 0.0062), RemL-C and MDA-LDL/LDL-C (r = 0.46, p = 0.019), and RemL-C and OxPC (r = 0.53, p = 0.0059) were also observed in the CAD group. The trends of positive correlations between RLP-C and MDA-LDL/LDL-C (r = 0.40, p = 0.08), and RemL-C and MDA-LDL/LDL-C (r = 0.31, p = 0.1) were observed in the control group.

The positive correlations between levels of RLP-C and OxPC (r = 0.34, p = 0.057), between levels of RLP-C and MDA-LDL/LDL-C (r = 0.47, p = 0.0048), between levels of RemL-C and OxPC (r = 0.32, p = 0.071), and between levels of RemL-C and MDA-LDL/LDL-C (r = 0.42, p = 0.013), were observed in non-diabetic patients as well as in all subjects (Fig. 4).

Correlations between each measurement of remnant lipoprotein cholesterol and OxLDL

As demonstrated in Fig. 5, a strong correlation was observed between RLP-C and RemL-C levels (r = 0.94, p < 0.0001). The MDA-LDL/LDL-C levels were positively correlated with OxPC (r = 0.40, p = 0.0074).
Table 1  Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CAD</th>
<th>p-Value</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td>20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 ± 12</td>
<td>64 ± 12</td>
<td>0.126</td>
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<tr>
<td>Male (%)</td>
<td>16 (80)</td>
<td>23 (92)</td>
<td>0.239</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>24.5 ± 4.8</td>
<td>24.6 ± 2.4</td>
<td>0.935</td>
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<tr>
<td>Hypertension (%)</td>
<td>11 (55)</td>
<td>19 (76)</td>
<td>0.138</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>1 (5)</td>
<td>10 (40)</td>
<td>0.012</td>
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<td>Metabolic syndrome (%)</td>
<td>3 (15)</td>
<td>14 (56)</td>
<td>0.005</td>
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<tr>
<td>Smokers (%)</td>
<td>10 (50)</td>
<td>16 (64)</td>
<td>0.448</td>
</tr>
<tr>
<td>Family history (%)</td>
<td>3 (15)</td>
<td>5 (20)</td>
<td>0.922</td>
</tr>
<tr>
<td>Number of diseased vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One (%)</td>
<td>—</td>
<td>9 (36)</td>
<td></td>
</tr>
<tr>
<td>Two (%)</td>
<td>—</td>
<td>10 (40)</td>
<td></td>
</tr>
<tr>
<td>Three (%)</td>
<td>—</td>
<td>6 (24)</td>
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<tr>
<td>Gensini score</td>
<td>—</td>
<td>52.9 ± 48.6</td>
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<tr>
<td>TC (mg/dl)</td>
<td>215 ± 28</td>
<td>220 ± 32</td>
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<td>TG (mg/dl)</td>
<td>125 ± 89</td>
<td>189 ± 130</td>
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<tr>
<td>HDL-C (mg/dl)</td>
<td>60 ± 14</td>
<td>46 ± 16</td>
<td>0.003</td>
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<tr>
<td>LDL-C (mg/dl)</td>
<td>127 ± 27</td>
<td>135 ± 27</td>
<td>0.343</td>
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<td>FBS (mg/dl)</td>
<td>99 ± 12</td>
<td>120 ± 36</td>
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<td>HbA1c (%)</td>
<td>5.2 ± 0.5</td>
<td>6.3 ± 1.2</td>
<td>0.001</td>
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<tr>
<td>hs-CRP (mg/dl)</td>
<td>0.097 ± 0.152</td>
<td>0.133 ± 0.118</td>
<td>0.409</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. CAD, coronary artery disease; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBS, fasting blood sugar; hs-CRP, high-sensitivity C-reactive protein.

Discussion

This study demonstrated that: (1) patients with CAD had high levels of remnant lipoprotein cholesterol, especially when measured by RemL-C method; (2) CAD patients had high levels of MDA-LDL; (3) the serum levels of remnant lipoprotein cholesterol were positively correlated with OxLDL, suggesting that the association between high levels of remnant lipoprotein cholesterol and high OxLDL levels might be linked to atherogenesis in patients with CAD.

Recent prospective studies and meta-analysis clearly demonstrated that a high level of TG is an independent predictor for CAD independent of other cardiovascular risk factors [6, 26–28]. Indeed, the deterioration of TG-rich lipoproteins, such as remnant lipoproteins, is frequently observed in high risk patients for CAD, such as patients with metabolic syndrome and/or diabetes [29–31]. Therefore, it is important to establish assays for the measurement of remnant lipoprotein. Four methods, including ultracentrifugation, polyacrylamide

Figure 1  Comparison of serum levels of remnant lipoprotein cholesterol between the control and the CAD groups: CAD, coronary artery disease; RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol.
Figure 2  Comparison of serum levels of OxLDL between the control and the CAD groups: CAD, coronary artery disease; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

Figure 3  Correlations between remnant lipoprotein cholesterol and OxLDL in all subjects: RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.
Figure 4  Correlations between remnant lipoprotein cholesterol and OxLDL in non-diabetic patients: RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

Figure 5  Correlations between each measurement of remnant lipoprotein cholesterol and OxLDL: RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

gel electrophoresis, RLP-C, and automated analysis of RemL-C, are available for determining remnant lipoproteins levels. The former two methods have some limitations for widespread use in the clinical setting, as they are time- and labor-intensive, complicated to perform, and are complex to quantify. In contrast, the latter two methods, especially RLP-C, are commonly used to measure remnant lipoprotein cholesterol. The correlation between RLP-C and RemL-C levels was consistent with the previous study \( r = 0.95 \) [22], however, serum levels of RemL-C, but not RLP-C, were significantly higher in patients with CAD than in the control subjects. One reason for this difference may be due to the small sample number of the present study. Another explanation might be derived from the difference in the method of measurement. The widely used RLP-C assay measures cholesterol levels in the heterogeneous unbound fraction of anti-apoA1 and anti-apoB-100 antibodies utilizing immuno-affinity methodology [3,20,21]. In contrast, the RemL-C assay, which can be carried out by an automated analyzer, utilizes selective agents, such as surfactant and phospholipase-D, for the determination of
remnant lipoproteins [22]. The RemL-C assay might be sensitive enough to identify high-risk subjects, such as patients with CAD.

Oxidative modification of LDL plays a crucial role in the pathogenesis of initiation and progression of atherosclerosis [7–9]. Until recently, several bioassay systems, such as MDA-LDL recognized by a monoclonal antibody, ML25, OxPC determined by the monoclonal antibody, DLH3, and OxLDL utilizing monoclonal antibodies, 4E6 and E06, for the measurement of circulating OxLDL have been developed [8,19,23,32,33]. In the present study, the serum levels of MDA-LDL, but not OxPC, were significantly higher in patients with CAD than in the control subjects. This finding may be caused by the difference in OxLDL determination measured from the use of different antibodies. ML25 for detecting MDA recognizes part of a lipid peroxide product [23], and DLH3 for determining OxPC levels specifically recognizes oxidized phosphatidylcholine [34]. Indeed, a modest correlation between MDA-LDL and OxPC levels was observed in this study (Fig. 3). Further analyses, including a large number of subjects, are needed to investigate the differences and clinical significance of each method.

The result of positive correlations between remnant lipoprotein cholesterols and OxLDL levels was a novel finding in the present study. Although the precise mechanism of these correlations is uncertain, the following possibilities are raised. Holvoet et al. reported that metabolic syndrome was associated with higher levels of OxLDL [35]. These authors also demonstrated the OxLDL levels positively correlated with waist circumstances, serum TG, insulin, and glucose levels, and were negatively correlated with levels of HDL cholesterol [35]. This study found positive correlations not only between body mass index and remnant cholesterol levels, but also between body mass index and OxLDL levels in the present subjects (data not shown). The levels of total cholesterol and LDL cholesterol in the present study were identical between the two groups. The features of the current patients with CAD might be represented by metabolic syndrome [36]. In addition, the high levels of small dense LDL, which is more susceptible to oxidative stress, appear to be proportional to the degree of deterioration of TG rich lipoproteins, such as remnant particles, in metabolic syndrome [37]. However, the results of the present study have consistency regardless of diabetes or metabolic syndrome. Further studies are needed to clarify the reason of these correlations. Another possibility is that enhanced oxidative stress could be induced by low-grade inflammation in subjects with abdominal obesity [38,39]. These indirect mechanisms may be linked to positive correlations between remnant lipoprotein cholesterols and OxLDL levels.

The mechanics and kinetics of OxLDL in the blood stream remain unclear. It is possible that OxLDL might be partly released from atherosclerotic plaques in not only the coronary arteries, but also in the systemic arteries. The levels of OxLDL correlated with plaque morphology, especially macrophage-rich plaque [40], and the amount of OxLDL in the coronary plaque [41]. These results suggest that circulating OxLDL may be released from the atherosclerotic lesions. In the present study, there were no significant associations between remnant lipoprotein cholesterol, OxLDL, and number of diseased vessels. Then, we assessed correlations between remnant lipoprotein cholesterol, OxLDL, and extent of CAD defined by Gensini score [42]. The levels of OxPC were positively correlated with Gensini score. In addition, the trend of positive associations between RLP-C and Gensini score and between RemL-C and Gensini score were observed in the CAD group (data not shown). These results could explain the possibility that OxLDL might be partly released from atherosclerotic lesions.

There are several limitations to the current study. First, this investigation was a small sample-size study. However, this is, to the best of our knowledge, the first report to demonstrate the correlations of remnant lipoprotein cholesterols and OxLDL determined by different measurement methods. Studies with larger sample sizes are required to confirm these results. Secondly, the serum levels of other oxidative and inflammatory markers except hsCRP were not measured. It has been proposed that LDL oxidation may be part of local and systemic inflammatory reaction [43]. Thirdly, there were no significant differences in total or LDL cholesterol between the CAD patients and the control subjects. Therefore the results of the present study may not be representative of all patients with CAD.

In conclusion, this study showed that the association between high levels of remnant lipoprotein cholesterol and high OxLDL levels might be linked to atherogenesis in patients with CAD.

References

Remnant lipoprotein cholesterol and oxidized LDL in CAD


