Association between lipoprotein(a) and oxygen reactive metabolite in asymptomatic subjects

Kazuhiko Kotani¹,², Naoki Sakane¹

¹ Department of Preventive Medicine, Clinical Research Institute, National Hospital Organization Kyoto Medical Center, Kyoto, Japan
² Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi, Japan

KEY WORDS
atherosclerosis, hypercholesterolemia, lipoprotein(a), oxidative stress, reactive oxygen reaction

ABSTRACT
INTRODUCTION Lipoprotein(a) [Lp(a)] is generally considered a risk factor for cardiovascular disease (CVD). The coexistence of Lp(a) and oxidative status may be associated with the pathophysiology of the Lp(a)–CVD linkage.

OBJECTIVES The aim of the present study was to investigate the correlation between Lp(a) and oxidative status using the diacron Reactive Oxygen Metabolite (d-ROM) test as an oxidative stress-related marker in asymptomatic subjects.

PATIENTS AND METHODS The serum levels of Lp(a) and d-ROM were measured in 322 subjects (male/female 138/184; mean age 58.5 years) in addition to body mass index, blood pressure, glycemia, and lipid profile.

RESULTS The median levels of Lp(a) were 14.6 mg/dl (interquartile range 6.7–30.3) and the mean levels of d-ROM were 332 ±67 U. Carr. A simple linear regression analysis showed that the d-ROM level was significantly and positively correlated with Lp(a) (correlation coefficient = 0.22, P <0.01). Multivariate-adjusted analyses confirmed this weak but significant correlation, independent of confounding variables. This correlation appeared to be relatively stronger in men than in women.

CONCLUSIONS A positive correlation between Lp(a) and oxidative status, as assessed by d-ROM, in this population may be partially associated with the Lp(a)–CVD relationship.

INTRODUCTION Lipoprotein(a) [Lp(a)] contains low-density lipoprotein (LDL)-like moieties, in which the apoB-100 component is covalently linked to the unique glycoprotein apolipoprotein(a) [apo(a)]. Lipoprotein(a) (Lp(a)) is generally considered a risk factor for cardiovascular disease (CVD), while the precise mechanisms associated with the role of Lp(a) in atherogenesis remain to be elucidated. Apo(a) has a high degree of homology with plasminogen and can compete with plasminogen receptors on endothelial cells; therefore, it is possible that Lp(a) contributes to the development of CVD by inhibiting thrombolysis and fibrin clearance. In addition, Lp(a) is seen in the arterial wall at the sites of atherosclerotic lesions, where the interaction of Lp(a) with other lipids and lipoproteins, as well as various inflammatory molecules, is suggested to promote atherosclerosis. There is growing evidence that oxidative stress burden is associated with the development of CVD. Lipoprotein(a) undergoes oxidative modification in the subendothelium, and Lp(a) and its oxidized form can cause apoptotic endothelial cell death and oxidative stress. Furthermore, while oxidized phospholipids are involved in atherogenesis, there is a close correlation between Lp(a) and oxidized phospholipids in the circulation. This proposes a fascinating hypothesis that when oxidized phospholipids are transferred to the blood circulation from other sources associated with hyperoxidative environments (i.e., oxidized lipoproteins, vascular atherosclerotic lesions, or inflammatory tissues), Lp(a) may preferentially bind and transport oxidized phospholipids in a natural defense framework. Therefore, the coexistence of
Lp(a) and oxidative status may be relevant with the Lp(a)–CVD linkage.

However, clinical studies on the association between Lp(a) and oxidative stress-related markers have been limited. In addition to the findings of the connection between Lp(a) and oxidized phospholipids, a few studies report that Lp(a) is positively correlated with oxidized LDL antigen levels in a nonspecific population and that Lp(a) is positively correlated with malondialdehyde and protein-carbonyl levels in a specific population of subjects with familial hypercholesterolemia. More studies are thus required including various populations and various oxidative stress-related markers. The diacron Reactive Oxygen Metabolite (d-ROM) test (Diacron, Italy) can quantify the oxidative status by measuring the hydroperoxides of organic compounds (not only lipids but also proteins, nucleic acids, etc.), and has been recently used as a clinically-applicable marker. This test may thus lead to a better understanding of the association between Lp(a) and oxidative stress. The aim of the present study was to investigate the correlation between Lp(a) and oxidative status, as assessed by the d-ROM test, in asymptomatic subjects.

**PATIENTS AND METHODS**

A total of 322 subjects (male/female 138/184; mean age 58.5 years) were recruited during general check-ups in the health education classes and outpatient clinics. The characteristics of the study subjects are presented in Table 1. Subjects that were asymptomatic, non-smoking and not receiving current medication were included in the study. Patients with a history of cardiovascular, severe kidney, or liver diseases were excluded. The study was approved by the institutional ethics committee, and all subjects provided their informed consent.

Smoking was defined as the presence of current smoking habits on self-reports, and medical histories were checked based on an interview by physicians and a physical examination. In addition to the body mass index (BMI), the systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined in the seated subject’s right-arm with a mercury sphygmomanometer after 5 minutes of rest. The subjects fasted overnight, and the levels of plasma glucose and serum triglycerides (TG) were measured using enzymatic methods. The levels of serum LDL-cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured using homogeneous methods, according to a previous report. Lp(a) was measured by an automated latex agglutination assay system (Sekisui Medical Co. Ltd., Tokyo, Japan). The d-ROM test was performed using a kinetic spectrophotometric assay system (F.R.E.E. system; Diacron, Italy). In brief, serum samples were mixed with a buffered solution, and a chromogenic substrate was added to the mixture. The mixture was centrifuged, and then incubated in the thermostatic block of the system. The absorbance at 505 nm was recorded. The measurement unit is expressed as U. Carr. (1 U. Carr. corresponds to 0.08 mg/dl H2O2). The intra- and interassay coefficients of variation of this test were 2.1% and 3.1%, respectively.

The data were expressed as the means ± standard deviations, the medians (interquartile ranges) or subject numbers, as appropriate. The difference between sexes was examined by the unpaired t test. Significance level: a P < 0.05; b P < 0.01 Abbreviations: BMI – body mass index, DBP – diastolic blood pressure, d-ROM – diacron Reactive Oxygen Metabolite, FPG – fasting plasma glucose, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, Lp(a) – lipoprotein(a), SBP – systolic blood pressure

### Table 1 Clinical characteristics of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>Men (n = 138)</th>
<th>Women (n = 184)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, y</td>
<td>58.5 ± 11.6</td>
<td>56.2 ± 13.5</td>
<td>60.3 ± 10.0</td>
<td>0.002b</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.0 ± 3.6</td>
<td>24.8 ± 3.4</td>
<td>23.4 ± 3.7</td>
<td>&lt;0.0001a</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>139 ± 21</td>
<td>141 ± 22</td>
<td>137 ± 20</td>
<td>0.17</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>80 ± 11</td>
<td>83 ± 11</td>
<td>78 ± 11</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>6.58 ± 2.30</td>
<td>6.94 ± 2.50</td>
<td>6.32 ± 2.10</td>
<td>0.015a</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>4.14 ± 1.09</td>
<td>3.78 ± 1.17</td>
<td>4.41 ± 0.93</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>triglycerides, mmol/l</td>
<td>1.72 (1.14–2.50)</td>
<td>1.92 (1.40–2.88)</td>
<td>1.45 (1.03–2.14)</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.65 ± 0.49</td>
<td>1.43 ± 0.40</td>
<td>1.82 ± 0.49</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>d-ROM, U. Carr.</td>
<td>332 ± 67</td>
<td>316 ± 66</td>
<td>343 ± 66</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>Lp(a), mg/dl</td>
<td>14.6 (6.7–30.3)</td>
<td>10.9 (5.2–21.7)</td>
<td>17.8 (7.9–34.9)</td>
<td>0.001b</td>
</tr>
</tbody>
</table>
The results of the same correlation analyses by sex are presented in Table 2. A simple linear regression analysis showed that the d-ROM level in male subjects was significantly and positively correlated with Lp(a), along with SBP and LDL-C (Figure 2). A subsequent multivariate-adjusted analysis showed that the d-ROM level remained significantly and positively correlated with Lp(a), along with LDL-C. A simple linear regression analysis showed that the d-ROM level in female subjects was significantly and positively correlated with BMI, SBP, and TG, as well as significantly and inversely correlated with HDL-C; however, these significant correlations were lost in the subsequent multivariate-adjusted analysis. On the other hand, a simple linear regression analysis showed that the d-ROM level was positively correlated with Lp(a) at a nonsignificant level, but the subsequent multivariate-adjusted analysis revealed an independent, significant, and positive correlation between d-ROM and Lp(a) (Figure 2).

**RESULTS**

The median levels of Lp(a) in the entire population were 14.6 mg/dl (interquartile range 6.7–30.3) and the mean levels of d-ROM were 332 ±67 U. Carr., respectively (Table 1). The levels of BMI, DBP, glucose, and TG were significantly higher, while those of age, LDL-C, HDL-C, d-ROM, and Lp(a) were significantly lower in male subjects than in female subjects.

The results of correlation analyses for the entire population are presented in Table 2. The simple linear regression analysis showed that the d-ROM level was significantly and positively correlated with Lp(a) (Figure 1), along with female sex, SBP, and LDL-C. An analysis adjusted for all the measured variables revealed that the d-ROM was independently, significantly, and positively correlated with Lp(a).

**DISCUSSION**

The present study showed that there is an independent, significant, and positive correlation between Lp(a) and oxidative status, as assessed by the d-ROM test, in asymptomatic subjects. There was an independent, significant, and positive correlation between d-ROM and LDL-C (in male subjects in particular), as reported in a previous study. Although the correlation between Lp(a) and d-ROM was weak and the clinical relevance of this result should be further established, it is important to note the coexistence of Lp(a) and oxidative status in these subjects. This connection may be the key to better illustrate the pathophysiology of atherosclerotic formation in taking the Lp(a) functions into account.

The positive correlation between Lp(a) and the oxidative stress-related markers seems to be consistent with prior studies. It may be...
Abbreviations: see Table 1 log-transformed. Lp(a) values have been please refer to for correlation coefficients in men and women. For correlation coefficients please refer to TABLE 2. Lp(a) values have been log-transformed. Abbreviations: see Table 1 log-transformed. Lp(a) values have been please refer to for correlation coefficients in men and women. For correlation coefficients please refer to TABLE 2. Lp(a) values have been log-transformed. The data on smoking was based on self-reports, (as shown in view by physicians and a physical examination. Furthermore, the genetic and ethnic effects of Lp(a) could potentially affect the oxidative status. Exercise and dietary factors were not included in this study. Atherosclerosis. 1999; 143: 229-243. However, some biological mechanisms for the Lp(a)-oxidative status interaction are considered. For example, Lp(a) can cause oxidative conditions in vascular walls, and this subsequently induces a vicious cycle of hyperoxidative status and inflammation where Lp(a) may be induced as an acute reactant. Moreover, Lp(a) is postulated to be a carrier of oxidized phospholipids, although it is not necessarily clear whether the carrier function of Lp(a) is specific for oxidized phospholipids only. The environment where oxidized phospholipids are formed is thought to be hyperoxidative; namely, this environment can be accompanied with increased levels of the other oxidative stress markers. The accumulation of data with prospective/intervention designs and various markers is needed.

The sex-based subanalyses revealed a relatively greater correlation between Lp(a) and d-ROM in male subjects than in female subjects. The reason for this result was unclear. These results may be partially affected by the higher levels of Lp(a) and d-ROM in female subjects than in male subjects (as shown in Table 1), while there have been previous studies reporting that female subjects might have a higher level of Lp(a)25 and a high tendency of d-ROM level26 in the general Japanese population. Further research is therefore needed to confirm whether there is any sex difference in the relationship between Lp(a) and d-ROM.

There are additional limitations to the study. It is important to consider the factors that can potentially affect the oxidative status. Exercise and dietary factors were not included in this study. The data on smoking was based on self-reports, and the presence of CVD was based on an interview by physicians and a physical examination. Moreover, the genetic and ethnic effects of Lp(a) must be addressed in the future studies.27,28 Because the d-ROM test can be easily performed in clinical settings, this test will help us to overcome the above-mentioned study limitations.

In conclusion, a weak but significant, independent and positive correlation between Lp(a) and oxidative status, as assessed by the d-ROM test, was found in this population (this correlation appeared to be stronger in male subjects than in female subjects). Although the clinical relevance of this correlation is unknown, the coexistence of Lp(a) and oxidative status may be a hint to understand the pathophysiology of the Lp(a)-CVD linkage. Further studies are therefore necessary to clarify the clinical relevance and the biological mechanisms of the present findings.

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REFERENCES


Związek między lipoproteiną (a) a reaktywnym metabolitem tlenu u pacjentów bezobjawowych

Kazuhiko Kotani¹,², Naoki Sakane¹

¹ Department of Preventive Medicine, Clinical Research Institute, National Hospital Organization Kyoto Medical Center, Kyoto, Japonia
² Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi, Japonia

SŁOWA KLUCZOWE
hipercholesterolemia, lipoproteina (a), miażdżyca, reakcja wolnorodnikowa, stres oksydacyjny

STRESZCZENIE

Lipoproteina (a) [Lp(a)] jest powszechnie uważana za czynnik ryzyka chorób sercowo-naczyniowych (ChSN). Współstnienie Lp(a) i stresu oksydacyjnego może tłumaczyć patofizjologię powiązania Lp(a) z ChSN.

CELE

Celem niniejszego badania było określenie korelacji między Lp(a) a stanem oksydacyjnym ocenianym za pomocą testu oznaczającego reaktywny metabolit tlenu (diacron Reactive Oxygen Metabolite – d-ROM) jako markera stresu oksydacyjnego u pacjentów bezobjawowych.

PACJENCI I METODY

Poziom Lp(a) i d-ROM w surowicy określono u 322 osób (mężczyźni/kobiety – 138/184; średni wiek 58,5 lat). Oceniono również wskaźnik masy ciała, ciśnienie tętnicze krwi, profil lipidowy oraz glikemię.

WYNIKI

Mediana stężenia Lp(a) wynosiła 14,6 mg/dl (przedział międzykwartylowy 6,7–30,3), a średnie stężenie d-ROM 332 ±67 jednostek Carratelliiego. W prostej analizie regresji liniowej wykazano istotną dodatnią korelację między poziomem d-ROM i Lp(a) (współczynnik korelacji = 0,22; p <0,01). Analizy wieloczynnikowe potwierdziły tę słabą, ale znamienną korelację, niezależnie od zmiennych zakłócających. Korelacja była względnie silniejsza u mężczyzn niż u kobiet.

WNIOSKI

Dodatnia korelacja między Lp(a) a potencjałem oksydacyjnym określonym za pomocą d-ROM w badanej populacji może częściowo tłumaczyć związek między Lp(a) a ChSN.