



Association of remnant-like lipoprotein particles cholesterol with "oketsu" syndrome

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Increasing evidence suggests that remnant-like lipoprotein particles cholesterol (RLP-C) is an independent cardiovascular disease (CVD) risk factor. High levels of RLP-C exhibit findings similar to those of "oketsu" syndrome on blood rheology studies and scanning electron micrographs. We measured levels of RLP-C in plasma samples and "oketsu" scores obtained in 199 randomly selected subjects. The coefficient of correlation between levels of RLP-C and "oketsu" score was highly significant ($r=0.581$, $P<0.0001$). This finding suggests that presence of excess RLP-C in the blood resembles closely "oketsu" syndrome which we have shown to be an independent risk factor for CVD.

Key words Remnant-like lipoprotein particles cholesterol, "Oketsu" syndrome, Cardiovascular disease.

Introduction

Postprandial hyperlipidemia is now believed to be an important risk factor for cardiovascular disease (CVD).^{1,2)} However, patients who were assessed to have CVD were not hyperlipidemic or hypertriglyceridemic before or after the meal.^{1,2)} The Japan Atherosclerosis Society has therefore recently established strict guidelines for classification of risk factors. In addition, increasing evidence suggests that remnant-like lipoprotein cholesterol (RLP-C) is an independent risk factor for CVD.^{3,4,5)} Remnant-like lipoprotein (RLP) is formed from very low density lipoprotein (VLDL) and chylomicrons (CM) upon partial metabolism of these lipoprotein particles by lipoprotein lipase. RLP is rich in triglyceride, cholesterol, and apolipoproteins, especially apolipoprotein E (apoE). "Oketsu" syndrome has also been related to microcirculatory disturbance.^{6,7)}

Assay of RLP-C has recently become a simple and reliable means of evaluating remnant lipoprotein levels in the clinical laboratory.^{8,9)} On the other hand, "oketsu" syndrome can be objectively evaluated using the "oketsu" score.¹⁰⁾ We used these methods to determine the association between RLP-C and the "oketsu" score.

Materials and Methods

Subjects. One hundred and ninety-nine subjects (19 years to 93 years of age (mean, 67.1 years): 95 males, 104 females) who visited the Department of Internal Medicine, Meimai Central Hospital, and had certain symptoms and

received informed consent were randomly selected and examined in this study. The plasma sample was extracted to at any time.

Analysis of RLP-C. The immunoaffinity mixed gel for separation of remnant-like particles (RLP) was provided by JIMRO, Co. Ltd, Takasaki, Japan. RLP isolation was based on the removal of lipoproteins containing apo A-I particles (HDL, nascent chylomicrons) and most lipoproteins containing apo B particles (LDL, nascent VLDL).^{8,9,11)} Briefly, monoclonal antibodies to apo A-I and apo B-100, which do not recognize partially hydrolyzed, apo E-enriched lipoprotein remnants, were conjugated with Sepharose 4B. Plasma was incubated with the gel for 2 hours by a RLP-Mixer J-100 using Hitachi microcups. Cholesterol concentration was measured in the unbound supernatants using two enzymatic reagents with sensitive chromophores (Kyowa Medex) for RLP-C measurement on an AU600 automatic chemical analyzer (Olympus).

Evaluation of "oketsu" syndrome. According to the diagnostic criteria ("oketsu" score) of Terasawa et al.,¹²⁾ the 199 subjects were scored and divided into three groups: a non-"oketsu" group ($n=78$, "oketsu" score 20 points or less), a mildly affected group ($n=97$, "oketsu" score 21 points or above, but less than 40 points), and a severely affected group ($n=24$, "oketsu" score 40 points or above). The "oketsu" score was determined before measurement of RLP-C, by one examiner.

Statistical analysis. The Statflex package (Ver. 5.0, release 2.15) was used for statistical analysis. All numerical values are the mean value \pm standard deviation. The unpaired t -test and χ^2 -test were used for examination of

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intergroup differences and frequency comparison, respectively. The level of significance was set at $p < 0.05$.

Results

RLP-C level was significantly correlated with "oketsu" score ($r=0.581$, $p < 0.005$, $n=199$; Figure 1a). There was a tendency for the direction of female group ($r=0.608$, $p < 0.005$, $n=104$; Figure 1b) to have the correlativity whose RLPC level is higher than male group ($r=0.576$, $p < 0.005$, $n=95$; Figure 1c).

After checking that a difference is among 3 groups in analysis of variance, it was authorized whether a difference would be among each 2 groups by post hoc test.

The result was RLP-C levels in three groups were significantly different from each other (the non-"oketsu" group: 5.4 ± 1.9 mg/dL; the mildly affected group: 7.5 ± 3.6 mg/dL; the severely affected group: 11.4 ± 4.1 mg/dL; $P < 0.005$; Figure 2) without significant difference in sex and age difference.

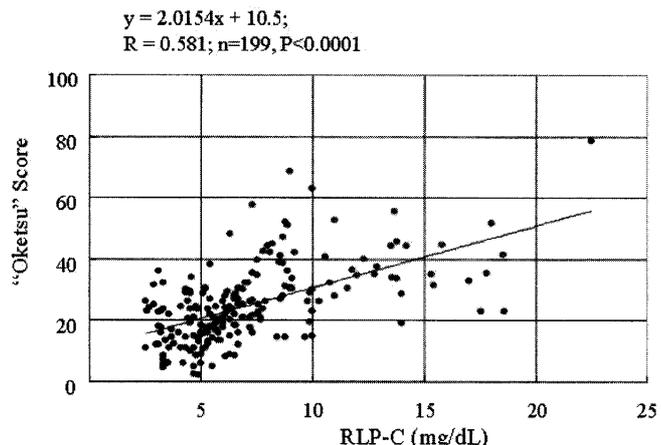


Fig 1a. Correlation between RLP-C levels and the "oketsu" score in the group of all subjects

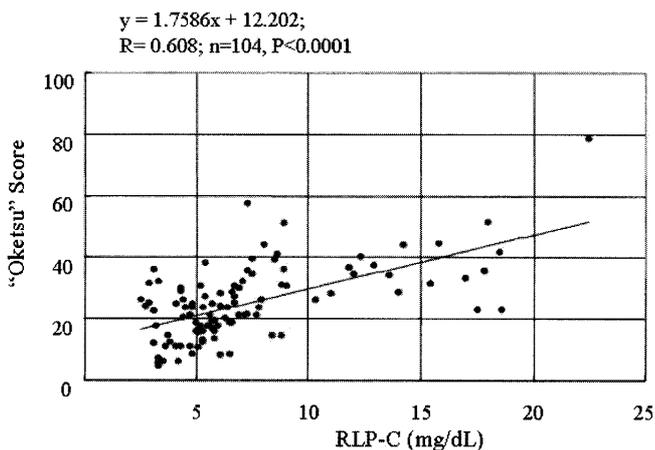


Fig 1b. Correlation between RLP-C levels and the "oketsu" score in the group of females

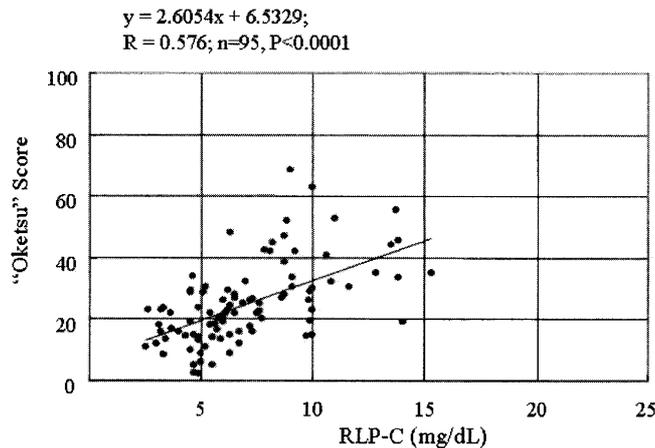


Fig 1c. Correlation between RLP-C levels and the "oketsu" score in the group of males

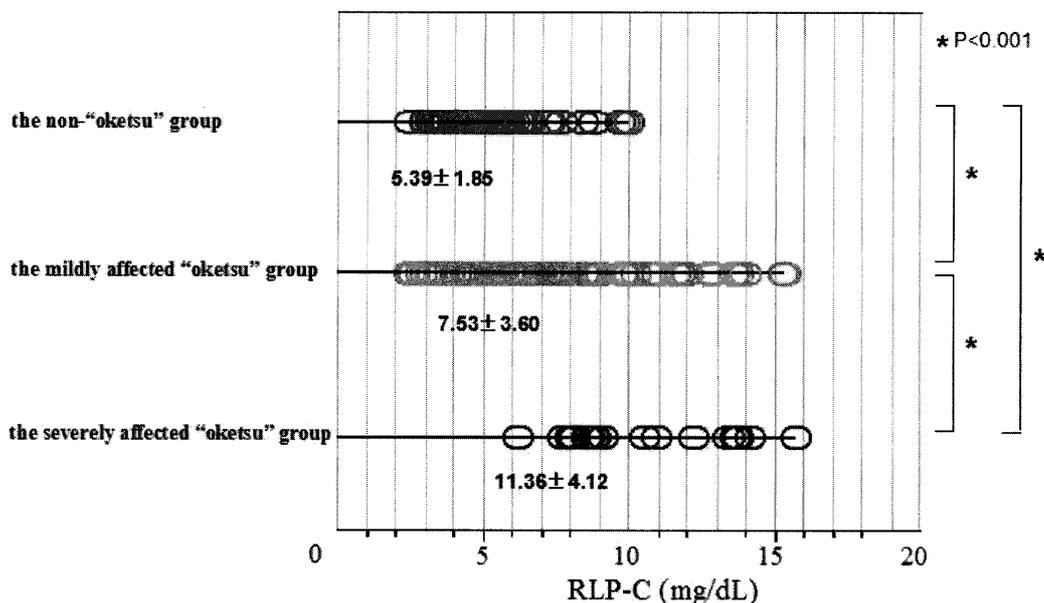


Fig 2. RLP-C levels in three group

Discussion

"Oketsu" syndrome is a systemic microcirculation disturbance with abnormal hemorheological studies, blood viscosity,¹⁰⁾ and erythrocyte aggregation.¹³⁾ Similarly, even if RLP-C levels were high, the blood viscosity and erythrocyte aggregation¹⁴⁾ were unusual value.

The correlation coefficient of "oketsu" score and RLP-C was almost equivalent to the erythrocyte deformability coefficient ($r = 0.506^{(6)} \sim 0.659^{(7)}$) to the "oketsu" score which was previously reported on. On the other hand, the correlation coefficient to RLP-C was almost equivalent to TG ($r = 0.302 \sim 0.893^{(9)}$), VLDL ($r = 0.50^{(15)} \sim 0.88^{(16)}$), and HDL ($r = 0.417^{(15)} \sim 0.60^{(17)}$), which are a serological parameter or less than those, but to T-Cho ($r = 0.544^{(16)}$), and LDL ($r = 0.410^{(15)}$) and IDL ($r = 0.20^{(15)} \sim 0.59^{(16)}$) were higher than those.

It was reported that RLP-C is a risk factor for CVD in women, based on the results of the Framingham Heart Study,⁵⁾ because the correlation with still higher especially "oketsu" score and RLP-C had been acquired from the results of this study. It was suggested that "oketsu" syndrome is related to being a risk factor of CVD.

It was reported that erythrocyte aggregability rises only in the severely affected group but not in the mildly affected group,⁷⁾ because erythrocyte aggregability did not rise in the mildly affected group from the report that RLP-C levels might be low. RLP-C induces white blood cells or red blood cells to mediate the platelet aggregation.¹⁸⁾ In mildly affected group, low levels of RLP-C might have difficulty to induce erythrocyte aggregability. Conversely, before erythrocyte aggregation, RLP-C was taken in by the macrophage, and it became a bubble cell to the present "oketsu" state in the stage which is not, and it carried out injury to the blood vessel. From the report of promoting arteriosclerosis, even if RLP-C was a low value, it was thought to be so because the state in which arteriosclerosis is advancing is caught in oriental medical-examination study.

It was reported that blood viscosity was high in "oketsu" syndrome, and it was thought that I was a possible. That the high molecular compound or a thing like cholesterol was involved with making blood viscosity high. Although it was reported to be possible that fibrinogen was participating in one as a bridge construction molecule erythrocyte becomes easy to condense. From this result, it was also suggested that it was possible that RLP-C was involved as a cholesterol which increased blood viscosity.

Although the normal maximum values of RLP-C were 7.5 mg/dL, the mean value of RLP-C in the mildly affected group was in agreement with 7.5 mg/dL from this result. On the other hand, although the mean value of RLP-C in non-"oketsu" group is 5.4 mg/dL, if RLP-C is regarded as a risk-factor of a coronary-arteries disease, it is not contradictory for it being reported that the value still lower than 7.5 mg/dL is recommended. If these are taken into consideration, I would think that the special-feature nature of the physician-of-the-Chinese-school study which thinks "mibyō", namely preventive medicine as important was shown.

RLP-C is made into the factor of arteriosclerosis, it is thought as a risk-factor of a blood vessel event, and it was thought that "oketsu" state might serve as a risk-factor of a blood vessel event from the result of correlation with RLP-C. Therefore, it was thought to it being desirable to treat bearing in mind that "oketsu" state serves as a risk-factor of a blood vessel event in a physician of the Chinese school, and RLP-C being a substitute as a diagnostic marker of "oketsu" syndrome in a general medical doctor, and bearing "oketsu" syndrome in mind for hyperlipemia medical treatment that control of a blood vessel event was to be expected. Between RLP-C and "oketsu" syndrome, it became clear that there is a significant correlation. Therefore, a possibility that "oketsu" syndrome would serve as a risk-factor of a blood vessel event on hyperlipemia medical treatment was suggested. On the other hand, it is difficult if traditional oriental-examination study-technique beyond a fixed grade is not mastered in order to evaluate the "oketsu" score. However, by measuring RLP-C, even if the general medical doctor had, it was suggested that "oketsu" syndrome, the risk of a CVD, etc. can be grasped in advance.

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Japanese abstract

レムナント様リポ蛋白コレステロール (RLP-C) が独立した心血管疾患 (CVD) の危険因子であることが明らかになりつつある。RLP-C が高値であると、血流動態上も電子顕微鏡像上でも瘀血病態と類似している。我々は無作為に選んだ199例より得られた血漿 RLP-C 値と瘀血スコアを測定した。RLP-C 値と瘀血スコアの間には有意な相関が認められた ($r=0.581$, $P<0.0001$)。このことより、CVD に対する独立危険因子である高RLP-C血症が瘀血病態と酷似していることを示唆する結果となった。

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