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Serum apolipoprotein B-48 levels are correlated with carotid intima-media thickness in subjects with normal serum triglyceride levels

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ABSTRACT

Background: Postprandial hyperlipidemia (PPHL) is an independent risk factor for coronary heart disease (CHD) which is based on the accumulation of chylomicrons (CM) and CM remnants containing apolipoprotein B-48 (apoB-48). Since atherosclerotic cardiovascular diseases are frequently observed even in subjects with normal serum triglyceride (TG) level, the correlation between fasting apoB-48 containing lipoproteins and carotid intima-media thickness (IMT) was analyzed in subjects with normal TG levels.

Methods: From subjects who took their annual health check at the Osaka Police Hospital ($n = 245$, male), one-hundred and sixty-four male subjects were selected to take part in this study; the excluding factors were: systolic blood pressure $\geq$140 mmHg, intake of antihypertensive or antihyperlipidemic drugs, or age >65 years. The association between biochemical markers and IMT was analyzed and independent predictors of max-IMT were determined by multiple regression analysis in all subjects and in groups N-1 (TG < 100 mg/dl, $n = 58$), N-2 ($100 \leq$ TG < 150 mg/dl, $n = 53$) and H ($150 \leq$ TG mg/dl, $n = 53$), respectively.

Results: Fasting total cholesterol, LDL–cholesterol, HDL–cholesterol, apoB-100 and ln RemL–C (remnant lipoprotein–cholesterol) levels were not correlated with max-IMT, but ln TG and ln apoB-48 were significantly correlated with max-IMT in all subjects. Ln apoB-48 and apoB-48/TG ratio were significantly correlated with max-IMT in group N-2. By multiple regression analysis, age and ln apoB-48 were independent variables associated with max-IMT in group N-2.

Conclusion: Serum apoB-48 level might be a good marker for the detection of early atherosclerosis in middle-aged subjects with normal-range levels of blood pressure and TG.

1. Introduction

Hypercholesterolemia, including high serum LDL–cholesterol (LDL–C) level, is strongly correlated to the development of atherosclerotic cardiovascular diseases [1]. Statins significantly decrease LDL–C levels and the morbidity of atherosclerotic cardiovascular diseases; however, they cannot completely prevent the occurrence of these diseases yet [2]. Epidemiologic studies have revealed that fasting hypertriglyceridemia is also associated with atherosclerosis, independent of other coronary risk factors such as high LDL–C level [3,4]. A case-control study showed that fasting and non-fasting TG levels were also superior among patients with coronary heart disease (CHD) as compared with control subjects [5]. A Japanese prospective study demonstrated that not only fast-

Abbreviations: BMI, body mass index; apoB-48, apolipoprotein B-48; PPHL, postprandial hyperlipidemia; CM, chylomicrons; CMR, chylomicron remnants; RemL–C, remnant lipoprotein–cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment as an index of insulin resistance; IRI, immuno-reactive insulin; IMT, intima-media thickness.

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ing but also non-fasting TG levels were significantly correlated with CHD morbidity [6]. In this study, the authors also showed that an increase in TG levels was significantly correlated with an increase in CHD morbidity even though TG levels remained below 150 mg/dl, a level which has been recognized as borderline of high risk status for atherosclerotic cardiovascular diseases on the basis of Framingham Study [7]. Therefore, we need to evaluate the emerging risk of atherosclerotic cardiovascular diseases even in subjects with normotriglyceridemia (TG < 150 mg/dl).

Postprandial hyperlipidemia (PPHL) is caused by the impaired metabolism of lipoproteins, which is mainly characterized by a postprandial accumulation of intestine-derived lipoproteins, chylomicrons (CM) and their hydrolyzed lipoproteins, chylomicron remnants (CM-R). In subjects with normal lipoprotein metabolism, CM and CM-R are promptly hydrolyzed, diminished in size and cleared from the circulation by the liver within a few hours after a meal. PPHL does not indicate the postprandial increase of lipids and lipoproteins which are promptly cleared from the circulation in subjects with normal lipoprotein metabolism. However, in patients with PPHL, CM-R continue to accumulate for over 6–8 h after a meal, penetrating into the vessels to form foam cells. Many recent studies have proved that PPHL is an independent risk factor for the development of CHD and atherosclerosis of carotid arteries [8–10]. Many basic studies have suggested that accumulated CM-R particles may promote atherogenicity in the arterial wall [11]. An oral fat loading (OFL) test is sometimes used to assess PPHL levels; however, this it is not a suitable testing option for routine clinical use because it requires a lot of time (6–8 h). Further, consensus has not yet been reached regarding the indication and the interpretation of data from this test. We developed a novel enzyme-linked immuno-sorbent assay (ELISA) to measure serum levels of apolipoprotein B-48 (apoB-48) [12]. Since one apoB-48 molecule is included in one CM and CM-R particle up to the clearance by the liver, serum apoB-48 level represents the number of both CM and CM-R particles and is suitable for the quantitative evaluation of postprandial changes. In patients with suspected accumulation of CM and CM-R, serum apoB-48 levels are significantly higher at the fasting state and increased after OFL in normolipidemic subjects [12]. High levels of fasting serum apoB-48 suggest the existence of PPHL, without performing an OFL test [13], and are reportedly related to the development of atherosclerotic cardiovascular diseases [14–17]. These results suggest that fasting apoB-48 level is a good marker for the evaluation of atherogenic risk in patients with hypertriglyceridemia. However, very few studies have so far investigated the correlation between fasting serum apoB-48 levels and the development of atherosclerosis among subjects with normal fasting TG levels.

In the current study, we have investigated the correlations between profiles of apoB-48-containing lipoproteins and the progression of atherosclerosis in subjects with normal TG levels. For the evaluation of atherosclerosis progression, intima-media thickness (IMT) of carotid arteries was measured using a diagnostic ultrasound, which was shown to be significantly correlated with the development and prognosis of CHD and cerebrovascular diseases [18,19].

2. Subjects and methods

2.1. Subjects

A consecutive series of subjects ($n = 245$, male) who came to Osaka Police Hospital for the annual health checkup were picked up serially. One-hundred and sixty-four male subjects were finally enrolled by the following exclusion criteria: systolic blood pressure $\geq$140 mmHg, age over 65 years and intake of any drugs affecting lipid metabolism and blood pressure. This study was approved by the Ethical Committee of Osaka Police Hospital, and all participants gave their written informed consent.

2.2. Biochemical analyses

Height, weight, and waist circumference were measured in the standing position. Systolic and diastolic blood pressures were measured at rest in the sitting position. Blood samples were collected after an overnight fast, followed by an immediate separation of serum and plasma. Total cholesterol (TC), triglycerides (TG), HDL–C, fasting plasma glucose (FPG) and uric acid (UA) levels were measured by enzymatic methods, LDL–C levels by direct method, and serum apoB levels by immunoturbidity method, respectively (Sekisui Medical Co., Ltd., Tokyo, Japan). Hemoglobin A1c (HbA1c) levels were determined by high performance liquid chromatography (HPLC) method and immunoreactive insulin (IRI) levels by the immunoturbidity method (SRL Inc., Tokyo, Japan). Serum apoB-48 levels were measured by the chemiluminescent enzyme immunoassay (CLEIA) using anti-human apoB-48 monoclonal antibodies, which we developed previously with minor modification (Fujirebio Inc., Tokyo, Japan). Remnant lipoprotein–cholesterol (RemL–C) levels were measured by the homogenous assay (Kyowa Medex, Tokyo, Japan) [12]. ApoB-100 levels were calculated by subtracting the value of apoB-48 from the value of serum apoB. Plasma adiponectin levels were determined by the human adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokyo, Japan). Subjects were divided into 3 groups by serum TG level: group N-1 ($n = 58$), TG < 100 mg/dl; Group N-2 ($n = 53$), 100 $\leq$ TG < 150 mg/dl and Group H ($n = 53$), 150 $\leq$ TG mg/dl.

2.3. Ultrasound measurements

The IMT of carotid arteries was determined using ultrasonography in the supine position. High-resolution B-mode ultrasound images were obtained (Toshiba Nemio, Toshiba Corp., Tokyo, Japan) with a 12 MHz linear array transducer. Three arterial wall segments in each carotid artery were imaged from a fixed lateral transducer angle at the far wall. All segments, including both sides of common carotid artery, the carotid bifurcation, and the internal carotid artery, were scanned. The thickest part of the IMT was recorded as max-IMT, and the IMT of the far wall was measured at 3 continuous sites at a 1.0-cm interval proximal to the thickest part of IMT in each side and then averaged to obtain mean-IMT. The mean-IMT value and greater max-IMT value obtained from scans of the right and left carotid arteries in each subject were used for statistical analyses.

2.4. Statistical analysis

Values were expressed as mean $\pm$ SD. ApoB-48 levels were normalized by logarithmic transformation. Between-group comparisons of the means and median were performed by Tukey's HSD test among group N-1, group N-2 and group H. The correlations between metabolic parameters and mean-/max-IMT were calculated by Pearson's correlation coefficients. Stepwise multiple regression analysis was used to determine independent predictors of max-IMT measurement with P value-to-enter set at 0.20. Age, sBP, dBP, total cholesterol, ln TG, LDL–C, HDL–C, apoB-48, apoB-100, ln RemL–C, FPG, HbA1c, ln HOMA-IR, and IRI were included as explanatory variables in the method. Data were analyzed with JMP8 software (SAS Institute, Cary, NC). All statistical significance was accepted at $P < 0.05$.