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Establishment of chemiluminescence enzyme immunoassay for apolipoprotein B-48 and its clinical applications for evaluation of impaired chylomicron remnant metabolism

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ABSTRACT

Background: Apolipoprotein B-48 (apoB-48) is a constituent of chylomicron remnants synthesized in the small intestines. The serum concentration of apoB-48 at fasting has been reported to be a marker of postprandial hyperlipidemia, a presumed risk factor for atherosclerosis.
Methods: We evaluated the basal performance of a recently developed chemiluminescent enzyme immunoassay (CLEIA). We also examined the correlations between serum apoB-48 concentrations and other lipid concentrations or life style patterns, including smoking and drinking. We analyzed the data of 273 clinical samples by multiple regression analysis to examine the influence of other serum lipid values, age, sex, smoking, drinking status and BMI on serum apoB-48 values.
Results: Within-run and between-run precision was obtained with 1.7–2.7% and 1.2–7.3%, respectively. The correlativity of enzyme-linked immunosorbent assay was correlation coefficient $r = 0.953$, and regression $y = 1.02 \times -1.59$. Serum apoB-48 concentrations were higher in males than in females, and were correlated with the status of smoking as well as with remnant-like particle-cholesterol (RLP-C) concentrations. Patients with the metabolic syndrome showed higher values of serum apoB-48 compared with control subjects.
Conclusion: Serum apoB-48 measurement by CLEIA was satisfactory for clinical use to assess abnormalities in the chylomicron remnant metabolism.

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1. Introduction

In the postprandial period, we observe an increase of serum triglycerides (TG), which are mainly transported by chylomicrons (CMs) and their remnants (CM remnants). Recently, postprandial hyperlipidemia has been considered as an independent determinant of cardiovascular diseases [1,2]. Dietary fats are absorbed by the small intestines and transported as chylomicrons (CMs), which are macromolecules synthesized exclusively by the small intestines. After the excretion of CMs into the intestinal lymph and their entrance into the systemic circulation, the TG moiety of CMs is promptly hydrolyzed by lipoprotein lipase (LPL), resulting in the production of CM remnants. Thereafter, CM remnants are promptly taken up by the liver via CM-remnant receptors.

Postprandial hyperlipidemia is a state characterized by the impaired catabolism of exogenous triglyceride-rich lipoproteins (TRL), in which the number of CMs and CM remnants is increased. However, no method has so far been developed to quantitatively and accurately measure the serum concentrations of CMs and CM remnants. CMs and CM remnants have a characteristic apolipoprotein B48 (apoB-48), each with one apoB-48 molecule per particle. In contrast, very-low-density lipoproteins (VLDL) and their remnants (intermediate-density-lipoproteins,

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IDL, or VLDL remnants) contain one apolipoprotein B-100 (apoB-100) molecule per particle. CM remnants contain apoB-48, but not apoB-100 [3]. CM remnants are taken up by monocyte-derived macrophages leading to the foam cell formation. Therefore, it is assumed that the measurement of serum apoB-48 concentration can help evaluate the synthesis and metabolism of CM remnants [4]. We have established an enzyme-linked immunosorbent assay (ELISA) to measure serum apoB-48 concentrations [5]. Thus, it has recently become possible to conveniently measure serum apoB-48 concentrations, thereby estimating the number of CMs and CM-remnant particles [4,5].

2. Materials and methods

2.1. Analysis equipment and reagents

We evaluated the basal performance of a recently developed CLEIA for apoB-48 measurement kit (Fujirebio Inc., Tokyo, Japan), carried out on the Lumipulse *f* fully automated immunoassay analyzer (Fujirebio). An in-house ELIS [4,5] provided by Fujirebio was used to measure serum apoB-48 concentrations and confirm the correlativity. Cholestest CHO (Sekisui Medical Ltd., Tokyo, Japan) was used for the measurement of total cholesterol (T-CHO); Cholestest TG (Sekisui) for triglycerides; Cholestest LDL (Sekisui) for LDL-cholesterol; Cholestest N HDL (Sekisui) for HDL-cholesterol; and Metabolead RemL-C (Kyowa Medex Co, Tokyo, Japan) for remnant lipoprotein cholesterol, respectively. TBA-200FRneo fully automated chemical analyzer (Toshiba Ltd., Tokyo, Japan) was used for automated measurements.

2.2. Principle of measurement of serum ApoB-48 concentrations

Serum samples were incubated with a treatment buffer solution supplemented with surfactants for separation of apoB-48 from CMs and CM remnants. The pre-treated samples were incubated with ferrite particles coupled with murine monoclonal antibody against apoB-48 in a solid phase. After incubation for 10 min at 37 °C and washing, further incubation was carried out for 10 min at 37 °C with alkaline phosphatase-conjugated anti-apoB-48 monoclonal antibody as a second antibody. After washing, AMPPD [3-(2′-spiroadamantan)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane disodium salt] (Applied Biosystems, Bedford, MA) as a substrate was added to the test cartridge, and further incubation was performed for 5 min at 37 °C. Relative chemiluminescent intensity was measured and serum apoB-48 concentration was calculated by a standard curve.

2.3. Samples

We analyzed the data of 6 patients from Osaka University Hospital and 273 clinical samples from Health Care Center Osaka University and Minami-Osaka Total Health Screening Center by multiple regression analysis to examine the influence of other serum lipid values, as well as age, sex, smoking, drinking status and body mass index (BMI) on serum apoB-48 values. The experimental protocol was approved by the institutional ethics committee of the Osaka University Hospital and informed consent was obtained from all patients and healthy volunteers.

2.4. Storage of serum samples

Specimen preservation at room temperature, 4 °C, and −20 °C was investigated in sample sera from 6 control subjects. The preservation period for serum was for 1 week, 2 weeks, and 4 weeks, and collection day, respectively.

3. Methods

3.1. Precision

Within-run and between-run imprecision was evaluated with three concentrations of quality control (QC) material. Ten aliquots were analyzed in one analytical run. Briefly, three concentrations of QC material and three concentrations of human serum were prepared as individual pools. All samples were immediately stored at −80 °C. Duplicate apoB-48 analyses were performed on each pool in two separate runs per day for 10 days. Precision was evaluated as the coefficient of variation calculated from the date series mean and standard deviation.

3.2. Limit of blank and limit of detection

The limit of blank (LoB) and limit of detection (LoD) were determined in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP17-A requirement [6]. The LoD represents the 95th percentile value from measurements of analyte-free samples over several independent series.

3.3. Linearity

We assessed the dilution linearity by serial dilution of 3 human serum samples spiked with recombinant apoB-48 to concentrations covering the whole measuring range. Each sample was diluted 1:4 with analyte-free human serum as diluents in 5 consecutive steps. We measured all dilutions in duplicate with various assay applications and calculated linearity separately for each assay application and instrument.

3.4. Interference studies

The CLEIA for measuring serum apoB-48 concentration was evaluated for common interferences including those due to hyperlipidemia, hemolysis and bilirubinemia by using Interference Check A Plus (Sysmex Co, Hyogo, Japan). Each interference material was evaluated by supplementation of human serum with the indicated to create a high or low interference pool followed by serial dilution with the high to the low pool to create a dilution series.

3.5. Statistical analysis

Data were analyzed by using Stat Flex software (Ver.5.0, Artec Inc., Osaka, Japan), and Mann–Whitney test and two-way ANOVA were used to evaluate the between-group differences. A $p<0.05$ was considered statistically significant.

4. Results

4.1. Imprecision

Imprecision was evaluated as the coefficient of variation (CV%). Within-run and between-run variations were examined using 3 QC materials and 3 kinds of patient pool sera with different concentrations of serum apoB-48 (Low,Middle,High). The largest within-run CV% observed was 2.7% (apoB-48 = 29.0 μg/ml). The largest between-run CV% observed was 7.3% (apoB-48 = 3.2 μg/ml). ApoB-48 imprecision for all samples are summarized in Supplementary Table 1.

4.2. Limit of blank and limit of detection

LoB and LoD for the apoB-48 assay were determined to be 0.06 μg/ml and 0.125 μg/ml, respectively. Linearity was documented by dilution up

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