Original Article

Determination of Lipid Composition of Plasma Lipoproteins in Children with a Rapid Agarose Gel Electrophoresis Method

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The agarose gel electrophoresis and differential staining system is an easy and quick method for analyzing the serum lipid composition of each lipoprotein fraction. It has been reported in adults that measured values obtained by this method strongly correlated with those obtained by ultracentrifugation. The aim of this study was to examine the clinical application of this method for children, in comparison with the ultracentrifugation method.

The subjects were sixteen hyperlipidemic and twenty-five normolipidemic children, aged from two to eighteen years old. Cholesterol (C) and triglyceride (TG) levels were determined in serum very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) fractions by both methods. Correlation coefficients between the two methods for cholesterol levels were 0.937 (HDL), 0.983 (LDL) and 0.837 (VLDL), and for triglyceride levels were 0.735 (HDL), 0.621 (LDL) and 0.964 (VLDL). We confirmed the clinical application of this method to evaluate the lipoprotein lipid profile in children as well as in adults.


**Key words:** Hypertriglyceridemia, VLDL, HDL, LDL, REP method

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**Introduction**

The ultracentrifugation method is the standard method used to analyze lipoproteins, although it is complicated and time-consuming. Recently, an alternative easy and quick method for lipoprotein analysis using agarose gel electrophoresis and differential staining of lipids was developed. The correlation between the measured values obtained by this method and those obtained by ultracentrifugation was reported to be very strong in adults. This method may also benefit children, because it requires a shorter time and needs a smaller sample volume; however, children have different serum lipoprotein and lipid profiles from adults.

The aim of this study was to confirm the clinical application of this method for children, and to compare its values to those obtained by ultracentrifugation.

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**Subjects and Methods**

**Subjects**

The subjects were 39 children (26 boys and 13 girls) aged 10.3 ± 4.1 years (mean ± SD); 23 with normolipidemia (14 boys and 9 girls) and 16 with hyperlipidemia (12 boys and 4 girls) who attended our outpatient clinic with obesity or hyperlipidemia in 2001-2002. They had been found to have such health problems during screening for lifestyle-related disease in school health care. According to the criteria for normal serum lipid levels in Japanese children based on a recent nationwide study, the cut-off points for hypercholesterolemia, hypertriglyceridemia and low HDLC level were 220 mg/dL, 140 mg/dL and 40 mg/dL, respectively. Obesity was defined as a relative body weight greater than 120%, which was calculated according to the standard weight obtained for sex, age and height on the basis of data from the Ministry of Education, Science, Sports and Culture. Informed consent was obtained from each child and parents.

**Methods**

Blood sampling was performed from an antecu-
bital vein after overnight fasting. Serum total cholesterol (TC), high density lipoprotein cholesterol (HDLC) and triglyceride (TG) levels were determined by standard enzymatic methods. Low density lipoprotein cholesterol (LDLC) was calculated by the Friedewald formula.

**Agarose Gel Electrophoresis**

Cholesterol (C) and TG levels in each lipoprotein fraction were determined using a combination of agarose gel electrophoresis and differential staining, which was established by Kido et al. In agarose gel electrophoresis, three lipoprotein fractions were detected, which will henceforth be referred to as β (LDL), pre-β (VLDL), and α (HDL). Sample application (1 μL), electrophoresis (400 V , 15 min), staining, drying and densitometric scanning (570 nm) were performed automatically by a Rapid Electrophoresis System (REP, Helena Laboratories, Beaumont TX, USA). After electrophoresis, cholesterol and triglyceride in lipoprotein fractions were visualized with enzymatic staining reagents (CHOL/TRIG COMB, K.K. Helena Laboratories, Saitama, Japan). The visualized gel plate was scanned on a densitometer. The scanning patterns of C and TG were identified using analytical software (ELECTROPHORESIS DATA BANK, K.K. Helena Laboratories). The scanned patterns were divided into each lipoprotein fraction by each nadir of lipoprotein sequential curve. The C and TG levels in each lipoprotein were estimated from the area percentages and total concentrations.

**Ultracentrifugation**

Each lipoprotein was separated using a modified method of Havel et al., which is commercially available in BML Co. (Saitama, JPN). In order to obtain each sample with a prepared density (d=1.006 and 1.063), the individual serum (0.2 mL) was mixed equivalently with KBr solution (Wako Pure Chemical Industries, Osaka, Japan) which was prepared at density 1.006 and 1.120. Each mixed solution was then centrifuged by 100,000 x g for four hours at 4°C. Sequential ultracentrifugation of the prepared serum lipoproteins was performed at the following densities: d <1.006 g/mL for VLDL, 1.006<d<1.063 g/mL for LDL, and 1.063 g/mL<d for HDL.

**Statistical Analysis**

All statistical analyses were conducted using the statistical package STATVIEW (ver. 5.0; Abacus Concepts, Berkeley, CA, USA). The significance of differences in mean values was analyzed by Student’s-T test. Correlation coefficients were determined by single regression analyses. A p-value less than 0.05 was considered significant.

**Results**

The characteristics of the subjects are shown in Table 1. Twenty-four (M:F=17:7) children were obese and 16 (M:F=12:4) had hyperlipidemia.

A visualized gel plate and typical scanning pattern of a normolipidemic child are shown in Fig. 1. The distribution of C and TG in lipoprotein fractions is determined from the scanning figure.

The C and TG levels in each lipoprotein fraction determined by the ultracentrifugation method and

<table>
<thead>
<tr>
<th>Table 1. Characteristics of subjects</th>
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<tbody>
<tr>
<td>N (M/F)</td>
</tr>
<tr>
<td>Age, Years</td>
</tr>
<tr>
<td>Relative body weight, %</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Total CHOL, mg/dL</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
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mean ± SD

Serum lipids measured by enzymatic methods

![Figure 1](image.png)
Table 2. Comparison of serum lipoprotein contents between REP and ultracentrifugation methods

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>REP method</th>
<th>Ultracentrifugation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C, mg/dL</td>
<td>50.3 ± 15.7</td>
<td>49.1 ± 13.8</td>
</tr>
<tr>
<td>VLDL-C, mg/dL</td>
<td>13.7 ± 9.0</td>
<td>29.4 ± 11.0</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>133.9 ± 64.5</td>
<td>120.2 ± 56.2</td>
</tr>
<tr>
<td>HDL-TG, mg/dL</td>
<td>13.5 ± 4.9</td>
<td>13.6 ± 5.4</td>
</tr>
<tr>
<td>VLDL-TG, mg/dL</td>
<td>66.3 ± 65.9</td>
<td>63.9 ± 63.5</td>
</tr>
<tr>
<td>LDL-TG, mg/dL</td>
<td>32.7 ± 13.0</td>
<td>24.6 ± 9.6</td>
</tr>
</tbody>
</table>

Mean ± SD

Fig. 2. Determination of TG in VLDL fraction: comparison of electrophoresis and enzymatic staining (y-axis) with ultracentrifugation method (x-axis).

Correlation coefficients were determined by single regression analyses. The lines are regression lines obtained by the least squares method. $n = 39$.

The correlation coefficient of LDL-TG between the two methods was low in this study. If the serum level of TG is very low, as is often observed in children, it is difficult to clearly determine the nadir between the preβ and β bands in the TG-stained agarose gel, because the peak of LDL-TG fraction is low. Even if the examination is manually performed in this situation, it is difficult to distinguish preβ from β bands. This may contribute to the low correlation coefficient between the REP method and ultracentrifugation in LDL-TG measurement. A discrepancy in the VLDL-C level was also observed between the methods. The obtained values using the REP method in our study clearly and consistently revealed the characteristics of lipids of each lipoprotein, but there were somewhat unusual values of VLDL-C in ultracentrifugation. The mean value of VLDL-C was high in the ultracentrifugation method in this study and unfortunately, we could not find a clear cause in the measurement process. TG enrichment might alter the lipid core and surface of LDL and affect apo B conformation. Levels of lipids and lipoproteins among children vary by sex and race/ethnicity, and are correlated with age, obesity, and other characteristics. These alterations could then influence the net electric charge of LDL, as reported for HDL particles. These factors may affect the electric charge of plasma lipid particles in children, which may also contribute to the discrepancy between the methods in this examination.

In summary, the REP method showed some advantages for determining lipids of serum lipoprotein subfractions in this study: First, the technique is relatively easy and it needs only a small sample volume, giving faster results than the ultracentrifugation method; second, we can obtain exact values of VLDL-TG for evaluating hypertriglyceridemia in children, in routine examination. The REP method may therefore contribute to analyzing lipid metabolism in children, especially in infants.

Discussion

Hyperlipoproteinemia frequently involves not only quantitative changes of lipoproteins but also qualitative changes in particle size and chemical components. These findings may provide a better understanding of the role of various risk factors in the development of coronary heart disease in children. In this study, we demonstrated that C and TG levels in lipoproteins determined by the REP method strongly correlated with those determined by ultracentrifugation in children, as well as in adults. We confirmed that even in children, the REP method is applicable for the analysis of C and TG levels in each lipoprotein, as an alternative method to ultracentrifugation. Furthermore, the REP method may provide a potent analysis for the lipoprotein profile of infants, because the method needs only a small sample volume. In particular, it was very beneficial in children to achieve exact values of VLDL-TG using the REP method in routine examination.
Conclusions

It is possible to precisely assess the lipid composition of each serum lipoprotein fraction using the REP method in children. This method may also provide a better understanding of various cardiovascular risk factors in early life.

References