Abnormal Lipoprotein Patterns in Human Serum as Determined by Agarose Gel Electrophoresis

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Two groups of abnormal electrophoretic patterns of serum lipoproteins are reported here. One demonstrates a deficiency or absence of lipoprotein fractions, which is characteristic of patients with abeta-lipoproteinemia, hypo-beta-lipoproteinemia, or Tangier disease. The other shows the presence of extra lipoprotein fractions, as found in cholestasis and multiple myeloma. These patterns, together with those of hyperlipoproteinemia phenotypes previously reported (Clin. Chem. 24: 227, 1978), form a reference record and a basis for the detection and evaluation of lipoprotein abnormalities in normal and dyslipo-proteinemic subjects, as determined by a sensitive, accurate, rapid, and inexpensive electrophoretic technique.

Additional Keyphrases: dyslipoproteinemia · screening · diagnostic aids

Cholesterol and triglycerides are major constituents of tissues, cells, and fluids and are transported in the blood in the form of lipoproteins. Determination of the circulating lipoproteins in plasma provides information useful for diagnosis and treatment of lipoprotein abnormalities in health and disease.

An electrophoretic test system in which pure agarose gel has been used for the first time (1) clearly separated the lipoprotein fractions beta (β), pre-beta (pre-β), and alpha (α), and demonstrated additional subfractions. The significance of the subfractions is illustrated:

(a) By the demonstration of two pre-β bands in 35% of normal subjects and in over 90% of patients with cardiovascular disease (2). This finding indicated that the extra pre-β band may represent a risk factor in the development of cardiovascular disease, and has stimulated further investigations.

(b) By the detection of a beta doublet in patients with type 3 hyperlipoproteinemia for the first time (3). This unique feature allows the diagnosis, by electrophoresis alone, of this important abnormality, which readily responds to treatment (4). This new information, together with the refinement of the original technique, enabled improvement of identification and expansion of the classification of the lipoproteinemia phenotypes into two normolipidemic and six hyperlipidemic types (5).

The purpose of this report is to demonstrate serum lipoprotein patterns characterized by absence or marked deficiency of lipoprotein fractions as found in abeta-lipoproteinemia, hypo-betalipoproteinemia, and Tangier disease (familial high-density-lipoprotein deficiency). Two additional

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weak $\alpha_2$-band, and $\beta$- and pre-$\beta$-lipoproteins absent. The values for cholesterol and triglycerides in this sample were 0.45 g/L and 0.27 g/L, respectively. This condition is characterized by lack of apoB-containing lipoproteins in plasma and presumably by a lack of synthesis of apoB protein, which is necessary for the formation of $\beta$- and pre-$\beta$ lipoproteins (7). No chylomicrons at the origin could be demonstrated in the fasting or non-fasting state or after a high fat intake.

$D$ is the hypo-beta-lipoprotein pattern with decreased $\alpha$- and $\beta$-lipoproteins and absence of pre-$\beta$-lipoproteins. The values for cholesterol and triglycerides were 0.8 g/L and 0.3 g/L, respectively. This sample was obtained from a heterozygote of familial hypo-beta-lipoproteinemia. The condition is characterized by decreased synthesis of apoB protein, resulting in a decreased concentration of $\beta$- and pre-$\beta$-lipoproteins (8). Homozygotes of this condition demonstrate lipoprotein patterns that phenotypically are indistinguishable from the above abeta-lipoprotein patterns. Because this latter condition is often characterized by less-severe disability than the true abeta-lipoproteinemia, differentiation is made clinically and by family studies.

Abnormal lipoproteins associated with immunoglobulin abnormalities have been previously described (9) and recently reported in multiple myelomatosis (10). Figure 2 shows an example of serum protein and lipoprotein patterns from a normolipidemic patient with a monoclonal $\gamma$-globulin (MM), as obtained by the agarose gel electrophoretic system. These patterns demonstrate an extra lipoprotein

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**Methods**

Blood samples were obtained from patients whose cases were being followed in the Molecular Disease Branch Clinic. Blood was sampled after a 12-h fast and allowed to clot. The serum, separated by centrifugation, was analyzed the same day by use of the agarose-gel electrophoretic system previously reported (1, 5).

**Results**

Typical examples of patterns are shown in Figure 1.

$A$ in Figure 1 is the lipoprotein pattern from a normolipidemic subject with two pre-$\beta$ bands, a cholesterol concentration of 2.1 g/L, and triglycerides concentration of 1.3 g/L.

$B$ is the serum lipoprotein pattern that attends Tangier disease. It is distinctive for the absence of $\alpha$-lipoproteins. Two pre-$\beta$ lipoproteins are present, and a weak but discrete $\beta$-lipoprotein that migrates a little faster than the normal beta. The patient was not fasting when specimen was drawn, to demonstrate the presence of chylomicrons. The cholesterol concentration was lower than normal, 0.75 g/L, and the triglycerides concentration was 1.5 g/L. The major apolipoproteins (apo) of high-density lipoproteins (apoA-I and apoA-II) are synthesized in this condition, and the defect appears to be one of abnormal and rapid catabolism of $\alpha$-lipoprotein particles containing apoA-I and apoA-II lipoproteins (6).

$C$ is the abeta-lipoprotein pattern with a broad $\alpha_1$-zone, a

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**Fig. 1. Lipoprotein electrophoretic patterns of serum from a normolipidemic subject with two prebeta bands (A), a patient with Tangier's disease (B), a patient with abetalipoproteinemia (C), and a patient with hypo-beta-lipoproteinemia (D).**

**Fig. 2. Serum electrophoretic patterns of proteins (P), and lipoproteins (L) of a patient with multiple myeloma**

The protein pattern shows a monoclonal band in the $\gamma$-globulin area and the lipoprotein pattern shows an extra lipoprotein zone with the same electrophoretic mobility as the monoclonal band. Also shown are lipoprotein electrophoretic patterns of serum from a normolipidemic subject with two prebeta bands ($N$) and of a patient with cholestasis demonstrating an LP-X ($X$).
fraction with the same electrophoretic mobility as the \( \gamma \)-globulin monoclonal band. A lipoprotein electrophoretic pattern from a normolipidemic subject with two pre-\( \beta \) bands is included for comparison. The same figure also shows a serum lipoprotein pattern from a patient with obstructive liver disease. It demonstrates an extra lipoprotein band between the origin and the \( \beta \)-lipoprotein zone, a broad \( \beta \)-lipoprotein fraction, weak pre-\( \beta \)-liproteins, and only traces of \( \alpha \)-lipoproteins. Cholestasis is characterized by the formation of abnormal lipoproteins such as LP-X (11), variously demonstrated by different electrophoretic methods.

**Discussion**

Clinical disorders and diseases of lipid metabolism are manifested by qualitative and quantitative changes in serum lipoproteins. Electrophoretic determination of serum lipoproteins has been a useful procedure for detecting these changes. I show examples of serum lipoprotein patterns of hypolipidemia cases and two other varieties with abnormal lipoprotein bands, as demonstrated by agarose gel electrophoresis. These patterns, together with the hyperlipoproteinemia phenotypes previously reported (5), form a reference record and a basis for the evaluation of lipoproteins of normal subjects and patients with dyslipoproteinemia.

Epidemiological studies have associated lipoprotein abnormalities with atherosclerotic cardiovascular disease (12). Metabolic studies have assigned specific roles on the circulating lipoproteins (13). Experimental studies have shown that if humans eat a diet high in cholesterol it may or may not affect the total cholesterol concentration in serum, but it definitely does produce changes in the lipoprotein fractions (14).

These studies emphasize the importance of determining serum lipoprotein in programs for screening asymptomatic subjects and in the clinical evaluation of patients. Recognition of abnormal patterns leads to appropriate intervention with established dietary regimens and therapeutic procedures. It also indicates further investigation of the genetic origin or association with other diseases.

Ultracentrifugation has been extensively used for the analysis of plasma lipoproteins, but it is not routinely available. Because the lipoprotein particles are large and somewhat unstable, artifacts might be produced by the addition of salts to plasma, the centrifugal forces, and the lengthy analysis. Furthermore, it is difficult to demonstrate or exclude such changes by ultracentrifugation.

In contrast, electrophoretic techniques are simpler and faster. Their reliability and sensitivity has improved from the original paper-electrophoresis to the present use of gels, acrylamide and agarose. The agarose-gel electrophoretic system used in these studies separates and clearly displays the serum lipoprotein fractions on a microscope slide, which can be visually evaluated and kept as a record for further comparisons. Furthermore the very low concentration of agarose (5 g/L) and the very rapid electrophoresis (10 min) sharply resolve the serum lipoproteins into multiple \( \beta \) and pre-\( \beta \) bands. The lipoprotein pattern thus obtained demonstrates the lipoprotein fractions in the natural form in which they circulate in blood, and the distribution of lipids and apoproteins in them, more accurately than any other system. It has been observed that lipoprotein patterns of normal normolipidemic subjects vary. In fact the lipoprotein patterns evidently are peculiar to each individual, reflecting his hereditary make-up and lifestyle.

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**References**