A COMPARATIVE STUDY FOR ESTIMATION OF SERUM HIGH-DENSITY LIPOPROTEIN CHOLESTEROL USING THREE DIFFERENT METHODS WITH REFERENCE TO BECKMAN AIRFUGE METHOD

THESIS

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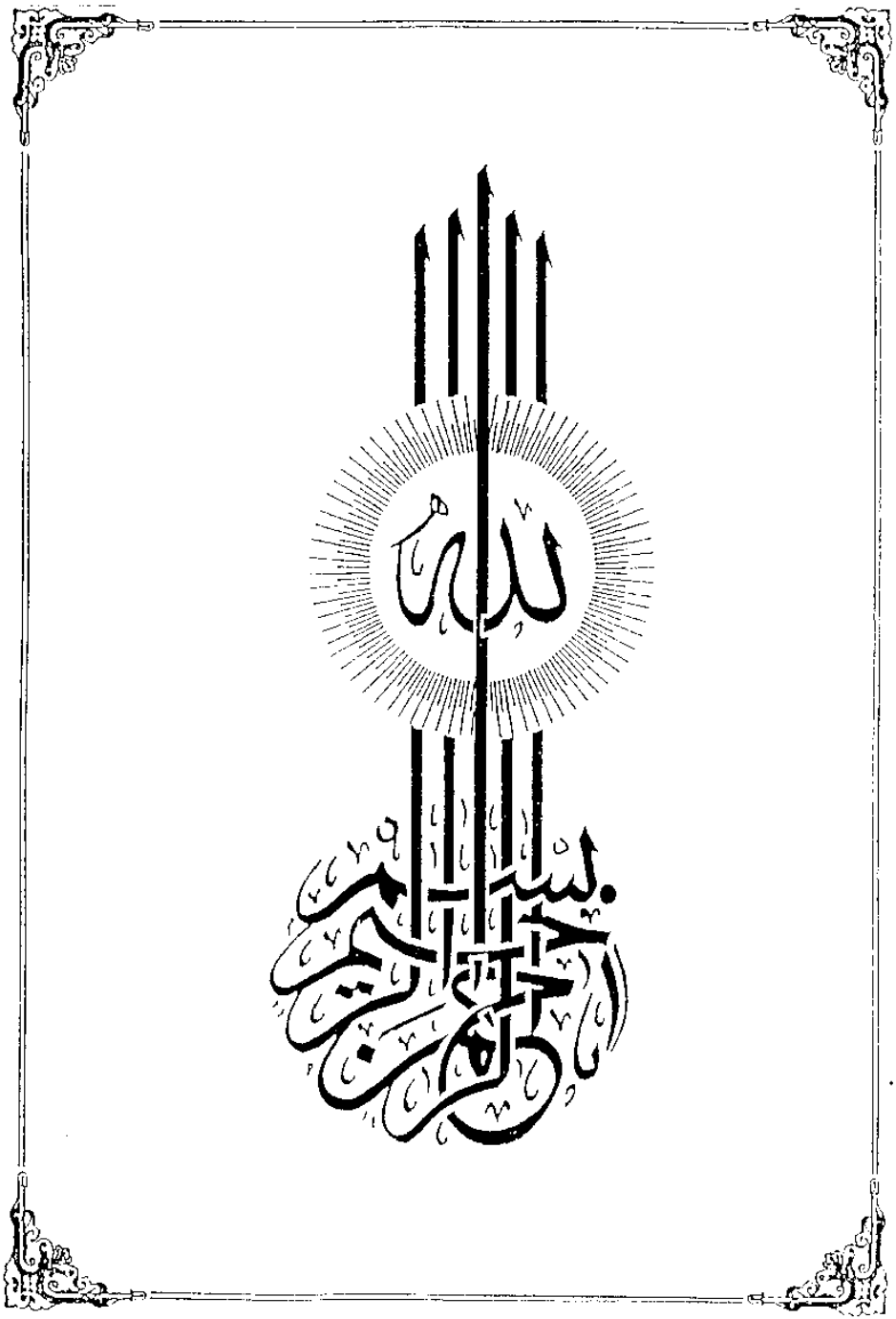

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1. INTRODUCTION

A- GENERAL INTRODUCTION AND AIM OF THE WORK

Multiple factors have been identified as important in predicting risk of coronary heart disease (CHD). Recent work has been focused on the importance of high-density lipoprotein (HDL) cholesterol in assessing risk of CHD.

As early as 1951, Barr and his colleagues described an inverse relationship between HDL cholesterol levels and CHD. Their findings were largely ignored until 1975, when Miller and Miller published a review of epidemiological studies on HDL cholesterol where they specifically emphasized on the protective role of HDL in the development of coronary artery disease. This was further supported by several extensive epidemiological studies which have reached similar conclusions (Kuller et al., 1977; Castelli et al., 1977; Gordon et al., 1977; Turner et al., 1978 and Knuiman et al., 1986).

Determination of plasma lipoprotein fractions and their cholesterol content provides the clinician with the prognostic, genetic, and therapeutic information necessary for the treatment of patients with lipid disorders (Fredrickson et al., 1967 and Stone and Levy, 1972). This had led to a

need for a method capable of handling large workloads conveniently and economically.

Several methods have been proposed for measuring lipoprotein fractions and their cholesterol content. The first widely used method was preparative ultracentrifugation (Havel et al., 1955). This method was time consuming and required both expensive equipments and highly trained personnel (Stein et al., 1978).

Next, came precipitation methods which depend on measuring HDL-cholesterol in supernates of serum or plasma after precipitation of other lipoproteins with a polyanion-divalent-cation combination (Warnick et al., 1979).

The most commonly used precipitation methods are sodium phosphotungstate -magnesium (Burstein et al., 1970), heparin manganese (Bachorik et al., 1976), and dextran-sulfate-magnesium (Finley et al., 1978).

A different approach for HDL-cholesterol separation and quantitation is through the use of electrophoresis. Lipoproteins are separated by routine electrophoresis and the cholesterol is made visible by the addition of an enzyme reagent directly on the electrophoresis membrane. The electrophoresis supporting media may be cellulose acetate (Cobb and

Sanders, 1978), agarose gel (Conlon et al., 1979), or polyacrylamide gel (Muniz , 1977).

The Beckman* "HDL-Centrifi Kit" follows the principle of separation of HDL from other lipoproteins in the presence of phosphotungstate and magnesium salts. The tubes in this kit are designed to precipitate and pellet serum low density lipoprotein (LDL) and very low density lipoprotein (VLDL) on centrifugation in the Beckman Airfuge Ultracentrifuge so that the serum HDL-cholesterol concentration can be measured in the supernatant. According to the manufacturer's operating manual, this method is efficient and simple.

In the present work, a comparative study will be done between three methods of HDL-cholesterol estimation, namely; sodium-phosphotungstate-magnesium chloride ($NaPhT-MgCl_2$) precipitation method (Bullock et al., 1981), cellulose acetate electrophoresis and the "HDL-Centrifi Kit' method using the Beckman Airfuge. We will evaluate the accuracy and precision of each method in an attempt to reach the most suitable method for routine use in our clinical chemistry laboratories.

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* Beckman Instruments International S.A.,
Rue des Pierres-du-Niton 17, 1207 Geneva, Switzerland.

B- REVIEW OF LITERATURE

1- PLASMA LIPOPROTEINS

Lipids are carried in the plasma combined with proteins forming complexes called lipoproteins (Zilva and Pannall, 1984). The protein moiety is called apoprotein or apo-lipoprotein, often abbreviated as apo- or apo-lp. All lipoproteins contain cholesterol, phospholipids, and triglycerides (Tietz, 1982). The lipid component causes lipoproteins to have densities that are less than the densities of albumin and of the globulins. The difference in the relative proportions of lipids and proteins among the several lipoproteins give them different densities and permit their separation by ultracentrifugation.

The lipoproteins also vary in density of electrical charge. This property, in combination with differences in size, also permits separation of lipoproteins by electrophoresis (Henry et al., 1974).

2- CLASSIFICATION OF LIPOPROTEINS BY PHYSICAL AND CHEMICAL PROPERTIES

a. Ultracentrifugation :

Lipoproteins show different rates of floatation when centrifuged in solutions of sodium chloride with different specific gravities. This is expressed as Svedberg (S_f) units of floatation. One S_f unit is equal to 10^{-13} cm/sec/dyne/g at 26 °C (Harper et al., 1977). Levy et al. (1966) classified lipoproteins according to this principle into :

i- Chylomicrons :which float in a medium of specific gravity < 0.96 gm/ml.

ii- Very low density lipoprotein (VLDL) : floats at densities 0.96 - 1.006 gm/ml.

iii- Low density lipoprotein (LDL) : floats in the density range 1.006 - 1.063 gm/ml.

iv- High density lipoprotein (HDL) : floats in the density range 1.063 - 1.21 gm/ml.

Table (I) : Shows a sum up of liporpotein classification by ultracentrifugation as given in Beckman Application Data, 1970.

Family Name	Synonyms (based on electrophoretic mobility)	Density Range (g/ml)	Floatation Rate Range (S_f)*	Composition			Major Function
				Protein (%)	Lipid (%)	Major Lipid	
Chylomicrons	-	< 0.95	> 400	0.5-2.0	98-99.5	Triglyceride	Transport exogenous triglycerides
Very low density lipoproteins (VLDL)	Pre-beta lipoproteins	0.95-1.006	20-400	12	88	Triglyceride	Transport endogenous triglycerides
Low density lipoproteins (LDL)	Beta lipoproteins	1.006-1.063	0-20	25	75	Cholesterol	Transport cholesterol and phospholipids to peripheral cells
High density lipoproteins (HDL)	Alpha lipoproteins	1.063-1.21	**	50	50	Cholesterol, Phospholipid	? Trasnport cholesterol from peripheral cells to the liver

* Expressed in Svedberg units for solvent density of 1.063.

** HDL sediment at 1.063 g/ml salt solution.

b. Electrophoresis :

The principle of electrophoresis is based upon the fact that when a charged particle is placed in an electric field, it will migrate towards one of the electrodes of the field. This depends upon the electrical charge, pH of the buffer and its ionic strength, size of the particle, the strength of the electrical field, the nature of the medium used to support the particle during the migration process and many others (Tietz, 1982).

Electrophoresis of lipoproteins had been performed by the relatively simple technique of Jencks and Durrum (1955). A major improvement in technique was developed by Lees and Hatch (1963), the importance of which became established when Fredrickson et al. (1967) classified clinically distinguishable disorders of lipid metabolism according to the pattern formed by classes of lipoproteins separated by this technique.

Accordingly, four classes of lipoproteins have been identified :

i- Alpha lipoprotein (α-lipoprotein) : the fastest migrating fraction towards the anode. This band corresponds to HDL separated by ultracentrifugation.

ii- Prebeta lipoprotein (pre B-lipoprotein) : after electrophoresis, preB-lipoprotein would be found

anodic to the beta fraction. It is often referred to as VLDL.

iii- Beta lipoprotein (B-lipoprotein) : it is the slowest moving band and often referred to as LDL. After electrophoresis, B-lipoprotein band will be found anodic to chylomicrons.

iv- Chylomicrons : if present, it remains stationary at the origin. This band does not appear in normal fasting state (Gurr and James, 1975).

3- BIOCHEMICAL ASPECTS OF LIPOPROTEINS

a. Chylomicrons

These are large particles consisting mainly of exogenous triglycerides, formed of long chain fatty acids, resynthesized in the intestinal epithelial cells after ingestion and absorption of dietary fat. They are transported in the lymphatic system draining the intestine and enter systemic circulation via the thoracic duct (Gray and Howorth, 1983).

Triglycerides constitute 80 - 95% of dry weight of this fraction. It also contains some cholesterol (2 - 5%), while phospholipids form 3 - 6%. The protein components of chylomicrons constitute only 1 - 2%. This is mainly in the form of apo C-I, II, III (66%), apo B (22%) and apo A-I, II (12%),

when using the alphabetical classification of apolipoproteins by Alaupovic in 1971 (Tietz, 1982).

Chylomicrons differ from other serum lipoproteins in being water-insoluble. Its function is to transport resynthesized triglycerides from the intestinal mucosa to the blood and then to the muscles, adipose tissue or the liver. There, they are hydrolysed by lipoprotein lipase in the capillary walls into glycerol and free fatty acids which pass into the cells. It was suggested that the liver does not remove intact chylomicrons in this way, but liver cells take up, by pinocytosis, chylomicron remnants enriched in phospholipid and cholesterol (Gurr and James, 1975).

Normally, chylomicrons are absent in a normal fasting plasma, and when present the plasma appears creamy. However, it takes up to 1 hours or little more in some individuals to disappear. That is why, when preparing a patient for lipoprotein studies, he should be fasting for 12 - 14 hours. After storage of creamy plasma overnight at 4 °C, chylomicrons float on the top (Brunzell et al., 1973).

b. Very low density lipoprotein (VLDL) :

VLDL transports mainly endogenous triglycerides. These triglycerides are formed of fatty acids either transported to or synthesized from carbohydrates or glucogenic amino acids in