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Original Article

Comparison of two methods (Precipitation Manual and Fully Automated Enzymatic) for the Analysis of HDL and LDL Cholesterol

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Abstract

Objective: To compare accuracy and throughput time for the measurement of HDL and LDL cholesterol by manual precipitation and fully automated enzymatic methods.

Methods: Fifty, serum samples collected over a 4 months period (February - May 2004) were analyzed for HDL and LDL cholesterol by two different methods i.e. precipitation manual and automatic enzymatic method in the section of chemical pathology, Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi Pakistan.

Results: The mean standard deviation for HDL Cholesterol by precipitation method and automated method were 43.12±8.97mg/dl and 43.86±10.34mg/dl respectively (p-value = 0.301). The mean standard deviation for LDL cholesterol by precipitation method and automated method were 111.76±25.57mg/dl and 111.8±28.41mg/dl respectively (p-value = 0.981). The calculated "t" and "F" value for HDL-C was 0.0172 and 0.75 respectively, and calculated "t" and "F" values for LDL-C were 0.047 and 0.809 respectively. Average time for manual method was 45 minutes and automation 20 minutes.

Conclusion: Both the precipitation (manual) method and the automated method provide reliable, precise and accurate results. In both the methods "t" and "F" values were less than critical. Automated method provide high throughput and are less labor intensive. The choice of method can depend on laboratory facilities and workload (JPMA 56:59;2006).

Introduction

Cholesterol is transported in plasma via lipoprotein; there are five classes of lipoprotein. High-Density Lipoprotein (HDL), low-density lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), Intermediate Density Lipoprotein (IDL) and chylomicrons. HDL-C and LDL-C are major lipoproteins of cholesterol in human plasma[1], and major transporters of cholesterol in human plasma.[2] The proatherogenic role of LDL and the antiatherogenic role of HDL have found their clinical relevance through epidemiological evidence that circulatory concentration of LDL-cholesterol (LDL-C)[3] and HDL cholesterol (HDL-C) are high biological predictors of cardiovascular disease.[2,4,5]

Measurements of these markers have been proposed as primary tools for risk assessment and monitoring of patients with risk of developing cardiovascular disease.[2]

There are two methods available for estimation of HDL-C and LDL-C, first precipitation method and secondly fully automated method.[6-8]

Measurements of HDL-C and LDL-C by automatic direct method offer the potential to improve both analytical and biological variability. The precision of HDL and LDL measurement would not depend upon the analytical variability in measurement of total cholesterol and low levels cholesterol in supernatants after precipitation.[6]

Manual precipitation method depends on the skills of medical technologist while automated method has the advantage of shorter time and less chances of human error. This paper describes a comparative analysis of HDL-C and LDL-C estimation by manual precipitation and automatic enzymatic in terms of accuracy and throughput times.

Material and Methods

This comparative cross sectional study was done at the section of Chemical Pathology, Aga Khan University Hospital (AKUH) Karachi.

Sample Size

Fifty selected samples in four months (February to May 2004) duration, received at the AKUH laboratory for the analysis of lipid profile were included in the study. Haemolysed sample, icteric samples (unconjugated bilirubin >40mg/dl and unconjugated bilirubin >30mg/dl, and lipaemic samples with triglyceridy >1000mg/dl) were excluded. Samples were collected after 10-12 hrs. fast and allowed to clot at room temperature. Serum was separated immediately after centrifugation at 3000rpm for 8-10 minutes. Separated serum was divided in two and analyzed for HDL-C and LDL-C by precipitation (reference) method and automated (test) method simultaneously.

The precipitation method was done on "SELECTRA 2"

using reagent, calibrator and recommendations of "MERCK". While the automated method was done on "HITACHI 912" using reagent, calibrator and recommendations of "ROCHE". In addition our laboratory met the regular national and international quality control requirements for the analysis of serum HDL-C and LDL-C.

Manual method of HDL-C estimation

Separated serum was precipitated by adding precipitating reagent (phosphotungstic acid and dextran sulfate-magnesium chloride) after centrifugation at 3000rpm, the supernatant was estimated for HDL-C by using cholesterol reagent (cholesterol esterase and cholesterol oxidase). In the presence of peroxidase it gives purple blue dye, the concentration measured colorimetrically at a wavelength of 500nm.The color intensity of this dye is directly proportional to concentration of HDL-C.[7,8]

Manual method of LDL-C estimation

LDL-C is precipitated using heparin as a precipitating agent. After centrifugation at 3000rpm, supernatant is used to estimate the cholesterol other than LDL-C. Its concentration is measured colorimetrically at a wavelength of 500nm.The color intensity is directly proportional to concentration of cholesterol (other then LDL). Calculation of LDL-C is obtained by subtracting this cholesterol (cholesterol in the supernatant) from total cholesterol value.[7,8]

Fully automated method of HDL-C estimation

HDL-C was estimated by homogenous enzymatic colorimetric method. In the first step it forms water soluble complexes with LDL, VLDL and chylomicrons in presence of magnesium sulfate and dextrans sulfate. These complexes are resistant to PEG (polyethylene glycol). The HDL-C is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to amino groups. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to concentration of HDL-C and is measured photometrically at 585nm.[9,10]

Fully automated method of LDL-C estimation

Automated method for the direct determination of LDL-C takes advantage of the selective micellary solubilization of LDL-C by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase and oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL-C < Chylomicron < VLDL < LDL-C. In the presence of magnesium, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of sugar compound with detergent enables the selective determination LDL-C in serum, measured photometrically at absorbance of 585nm.[11]

Statistical analysis included mean, standard deviation, paired t test and linear regression, analyzed by SPSS version 11.5. To asses significant difference a p value of 0.05 was taken as significant.

The accuracy and precision was calculated by the following formulae: -

" $t = [\text{bias}] / \sqrt{n/\text{standard deviation (difference)}}$

" $F = [\text{standard deviation (precipitation)}]^2 / [\text{standard deviation (automation)}]^2$

Results

Result of the 50 samples analyzed by manual precipitation methods of HDL-C ranged from 29 to 69mg/dl (mean 43.12) and LDL-C from 58 to 171mg/dl (mean 111.76). In automated method, the HDL-C ranged from 28 to 71mg/dl (mean 43.86) and LDL-C from 56 to 175mg/dl (mean 111.8).

There was no significant difference between precipitation and automated method for the analysis of HDL and LDL-C (Table 1).

Table 1. Results of HDL-C and LDL-C by precipitation and automated methods.

	Precipitation (reference method)	Automated (test method)	p-value
Total samples	50	50	
HDL-Cholesterol	43.12 ± 8.97	43.86 ± 10.34	0.301
LDL -Cholesterol	111.76 ± 25.57	111.80 ± 28.41	0.981

The accuracy and precision of these two methods was calculated by "t" and "F" values (Table 2).

It was observed that calculated "t" and "F" values were less then the critical "t" and "F" values in HDL and LDL cholesterol, which concludes that there was no difference between these two methods for the analysis of HDL-C

Table 2. Precession and accuracy for HDL-C and LDL-C.

	HDL-C	LDL-C
Critical "t" value	2.09	2.09
Calculated "t" value	0.0172	0.047
Critical "F" value	2.12	2.12
Calculated "F" value	0.75	0.809

and LDL-C with respect to their accuracy and precision.

An estimate of time was undertaken for both tests. On an average HDL-C and LDL-C by manual precipitation method required 45 minutes, and by fully automated methods 20 minutes.

Discussion

In last decade as a result of sedentary life style, junk foods and stressful life there has been an increase in cardiac events. Each increase by 1 unit of the LDL-C/HDL-C ratio increases the risk of myocardial infarction by 53%.[12] Cardiologists rely on lipid profile specially LDL-C and HDL-C to predict chances of cardiac event, since dyslipidemia could be a major determinant of premature atherosclerosis. The severity of atherosclerosis is strongly related to the LDL-C concentration.[13] The LDL-C/HDL-C ratio is a high predictor of cardiovascular risk.[2,4,5]

In our country most of the laboratories are performing precipitation (manual) method for the analysis of HDL-C and LDL-C. In precipitation method, the supernatant is used for the lipoprotein analysis. Quality and accuracy of these depends upon the centrifugation speed and time of centrifugation. Furthermore precipitation method requires multiple accurate pipetting and the test has to be performed by skilled medical technologists. Automatic method provides the advantage of less human error, reliability of calibration and time saving. In a busy laboratory setup with multiple analyses on each sample and high workload these methods are of great advantage.

Both the methods i.e. precipitation and automated are precise and accurate for the analysis of HDL and LDL cholesterol. However automated method has the advantage of saving time and being less labour intensive, making the chances of error to a bare minimum. The direct assessment of HDL-C or LDL-C appears attractive for their simplicity and their potential high throughput and analytical performance because of full automation.[2]

It is recommended that different laboratories should select their technique on the basis of their current and future predicted workload.

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