Original Article

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Comparison of Manual, Semi-automated and Fully-automated Enzymatic Methods for High Density Lipoprotein Estimation- A Cross-sectional Study

Biochemistry Section

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ABSTRACT

Introduction: Spherical particles with nonpolar neutral lipids at the center and more polar amphipathic lipids at their surface are called Lipoproteins. High Density Lipoprotein (HDL) aids in cholesterol homeostasis by removing it from cells by several different mechanisms. The results of HDL Cholesterol (HDL-C) should be accurate and reliable, especially for the patients with borderline values, where the variation resulting from the methodology used may interfere with the interpretation of clinical scenario by the doctors.

Aim: To compare the results of HDL values obtained by using three different technical methods.

Materials and Methods: The present study was conducted using 30 serum samples from the Biochemistry laboratory of KLE Society's Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, Karnataka, India for HDL values, each for the three different technical methods (fully-automated, semi-automated and manual method). The data obtained was analysed statistically by computing descriptive statistics the mean, standard deviation and correlation coefficient.

Results: Results of serum samples analysed for HDL-C values ranged from 31-79 mg/dL (mean 43.1) in fully-automated analyser, 28.06-75.18 (mean 39.6) in semi-automated and 27.06-62.7 mg/dL (mean 39.7) using manual method. There was significant difference in the mean values of HDL-C values obtained using the three different methods and positive correlation was established on comparing semi-automated and manual method (Semi-automated v/s manual method, r=0.827) and found to be statistically significant.

Conclusion: As HDL estimation is routinely done in many of the clinical laboratories, a sound knowledge of the HDL values obtained using different techniques can help the clinicians while ordering for the test and also for diagnosing and monitoring of the cases with various lipid abnormalities.

Keywords: Accurate, Atheroprotective, Lipoproteins, Reliable, Ultracentrifugation

INTRODUCTION

Lipoproteins are complex particles with nonpolar lipids in their core and more polar amphipathic lipids at their surface [1]. They contain different proportions of lipids and proteins and thus differ in their physical and chemical properties. On the basis of their differences in densities as determined by ultracentrifugation they are classified as Chylomicrons, Very Low Density Lipoprotein (VLDL), Intermediate Density Lipoprotein (IDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Lipoprotein (a).

HDL cholesterol plays an important role in reverse cholesterol transport during which it takes the cholesterol from peripheral tissues to liver. HDL also possess anti-oxidant property by preventing the oxidation of LDL, anti-inflammatory and anti-clotting properties, which are yet to be known clearly [2]. HDL increases the production of Nitric oxide (NO) which is a signaling molecule and atheroprotective in nature. NO synthesis is increased due to increased expression of endothelial NO synthase (eNOS) [3]. The patients with blood levels of HDL below 35 mg/dL are more prone for developing Coronary Artery Disease (CAD). There is an inverse relation between serum HDL levels and the risk of the CAD [4]. HDL is known as "anti-atherosclerotic factor" or cardio-protection factor [5]. It is also proved in some of the studies that significant vascular residual risk still exists even at the normal or high HDL levels also [6,7].

Manual methods used for estimation in laboratory rely on the technical skills of the technician while automated machines generate quick results and also have less scope for human errors [4]. It is emphasised that the results of HDL cholesterol determination should be accurate and reliable, especially for the patients with borderline values, where the variation resulting from the methodology used may interfere with the interpretation of clinical scenario by the doctors [5]. There is improvement in both analytical and biological variability when the HDL cholesterol is estimated by automatic direct method [8].

Different results are obtained with different methodologies available in the market. Hence, it is the sole responsibility of the laboratories to spell out the different methodologies which are available for HDL estimation which will help the clinicians in monitoring and correctly diagnosing the changes in serum lipids using the knowledge of available methodologies [9]. Considering the above points this study was undertaken to compare the values of HDL using three different technical methodologies.

MATERIALS AND METHODS

It was a cross-sectional study, conducted using 30 serum samples which were obtained from the Biochemistry laboratory of KLE Society's Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, Karnataka, India. The study was undertaken after obtaining the Institutional Ethical Committee approval (letter no. MDC/DOME/397). The study was conducted between January 2015 to December 2015. Convenient sampling was followed.

Study Procedure

The samples were subjected to analysis using fully-automated, semi-automated and manual method for estimating HDL cholesterol. The HDL-C was estimated by fully-automated technique using

modification of the Abell Kendall method [10]. Semi-automated estimation of HDL-C is based on Burstein M et al., and Roesclau P et al., and for manual method, precipitation by phosphotungstate-Magnesium chloride followed by Carr JJ and Drektor IJ Method was used [11-13].

The instruments used in this study were- Dimension^{R}Clinical Chemistry analyser by Siemens (fully-automated), Erba Chem 5 V2 manufactured by Erba Manheim (semi-automated) and EI spectrophotometer by Jayanti Scientific Instruments, New Delhi (manual). Semi-automated analysis was done by using commercially available Erba kit whereas for manual analysis reagents were prepared freshly in the laboratory.

STATISTICAL ANALYSIS

Descriptive statistics, the mean, standard deviation and Pearson correlation coefficient were the statistical methods used to analyse the obtained data using SPSS software version 20. The difference between each method was also calculated.

RESULTS

Results of serum samples analysed for HDL-C values ranged from 31-79 mg/dL (mean 43.1) in fully-automated analyser, 28.06-75.18 mg/dL (mean 39.6) in semi-automated and 27.06-62.7 mg/dL (mean 39.7) using manual method as shown in [Table/Fig-1].

Total samples	Fully-automated method range (mean) in mg/dL	Semi-automated method range (mean) in mg/dL	Manual method range (mean) in mg/dL	Difference (mg/dL)
HDL-C	31-79 (43.1±9.94)	28.06-75.18 (39.6±9.86)	27.06-62.7 (39.7±8.75)	----
Fully-automated and Semi-automated	----	----	----	0.14±5.49
Fully-automated and Manual method	----	----	----	3.37±6.42
Semi-automated and Manual method	----	----	----	3.51±5.58

[Table/Fig-1]: Range and mean values along with difference between HDL cholesterol levels by fully-automated, semi-automated and manual methods.

There was significant difference in the mean values of HDL-C values obtained using the three different methods and positive correlation was established on comparing semi-automated and manual method (Semi-automated v/s manual method, r=0.827) and found to be statistically significant.

The values obtained using manual method showed positive correlation when compared with fully-automated method (Manual v/s fully-automated r=0.769), and similarly there was also positive correlation of the results between semi-automated v/s fully-automated method (r=0.838) [Table/Fig-2].

Method	Semi-automated	Manual method
Fully-automated	r=0.838, p<0.001	r=0.769, p<0.001
Semi-automated		r=0.827, p<0.001

[Table/Fig-2]: Correlation analysis of HDL levels.

DISCUSSION

The morbidity associated with coronary vascular events has shown an increasing trend in the last decades due to physical inactivity, stressful life and consumption of junk food [8]. The ratio of LDL-C/HDL-C is one of the important predictors of cardiovascular risk [14-16].

Accuracy of reports reflecting correct HDL-C levels are of utmost significance during risk assessment strategies, while treating patients suffering from any cardiovascular illness. This study found a significant difference in HDL cholesterol values obtained using fully-automated, semi-automated and manual method.

Jabbar J et al., stated that there was no significant difference between precipitation and automated method for the analysis of HDL-C levels which was contradictory to the findings of this study [8]. The reason for this could be incomplete precipitation of apoB lipoprotein by the precipitation methods (manual method) when compared with the other two methods. The turbidity of supernatant seen in some of the conditions like hypertriglyceridemia, inflammatory conditions and cryopreservation may cause discordance between the methods, which can be overcome in fully-automated methods than manual method. Mulinge JM et al., concluded that the results obtained by direct (automated) and precipitation (manual) method for HDL-C measurement are comparable which is also not in accordance with the present study [17]. The findings of this study was also not in agreement with another study conducted by Arranz-Pena ML et al., that showed close correlation of HDL-C assay using direct method and precipitation method [18]. Nauck M et al., stated that precise and accurate HDL-C concentration can be obtained using homogenous assays even for samples with hypertriglyceridemia, thus offering an advantage on the use of direct techniques in patients with higher triglyceride levels [19]. HDL-C isolated and estimated by manual method was found to be lower than that estimated using the direct homogenous assay method as per a study done by Jensen T et al., which is similar to the findings of the index study [20]. This is because the fraction with a density of >1.063 includes some apolipoprotein (apo B)-containing lipoproteins and it seems that the automated methods which are direct, react with these lipoproteins; thus indicating that the results obtained by this method are more accurate than those obtained by precipitation or manual method.

Inspite of the sufficient evidence that has concluded that the CHD event rates are gradually lower in cohort participants with very high HDL levels, the risk for CHD still exists in these cohorts [21]. In most of the developing countries HDL-C analysis is performed using manual (precipitation) method. This method mainly relies on the accurate pipetting technique under the hands of a skilled medical technologist [8]. Fully-automated analysers have various advantages over manual methods like less human errors, time-saving, labour intensive and calibration reliability. They also offer laboratories substantial operational and productivity benefits. In addition, the results obtained from fully-automated method offer the promise of achieving the high degree of certainty required in clinical decision making based on HDL-C concentrations. Busy laboratories, with high work load and frequent analysis of multiple samples, should adopt automated methodologies. Hence, a clinician must have sound knowledge about the discrepancies in the results obtained using different technical methods for HDL-C estimation.

Limitation(s)

The major limitation of the present study was the small sample size. As it a cross sectional study hence casual relationship could not be established. Analytical studies can be taken up in future to establish the casual relationship.

CONCLUSION(S)

On comparing the HDL-C values using three methods, it was found that there was a significant difference in the values obtained using manual method (Carr JJ and Drektor IJ method) semi-automated method and fully-automated method. The desired method can be chosen depending on the laboratory workload and set up used. As HDL estimation is routinely done in many of the clinical laboratories, a sound knowledge of the HDL values obtained using different techniques can help the clinicians during interpretation of the test results and also for diagnosing and monitoring of the cases with various lipid abnormalities.

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