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Comparison of direct versus Friedewald estimation of LDL cholesterol: Experience in Indian hyperlipidemic patients

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ABSTRACT

Background and Objectives: LDL cholesterol is routinely calculated by the Friedewald equation to guide the treatment of dyslipidemia; however, Friedewald equation has certain limitations especially with high triglyceride levels. Direct methods are available for LDL estimation but have received relatively little scrutiny in India. Very limited data is available on comparison of these 2 methods in Indian patients. This study was aimed at comparing the calculative and direct methods of LDL Cholesterol estimation in Indian hyperlipidemic patients. **Materials and Methods:** In this observational study, data from 380 consecutive lipid profiles was analysed. CHOD PAP Method was used to estimate Total Cholesterol. Enzymatic Colorimetric Method was used to estimate Triglycerides, Enzyme selective protection method was used to estimate HDL, Homogenous Enzymatic Colorimetric Assay was used to estimate direct LDL and VLDL was calculated whereas Friedewald's formula was used to derive calculated LDL. **Results:** Total Cholesterol values correlated positively with LDL values measured by both the methods. However, a statistically significant difference ($p=0.0418$) was noted between the correlation coefficients of both the methods. Triglyceride values correlated weakly with LDL levels measured by both the methods. A weak negative correlation was observed with LDL-C whereas a weak positive correlation existed between TG and LDL-D values. The difference between the correlation coefficients was statistically significant. **Conclusion:** Both the direct and calculated methods of LDL estimation have their limitations. Need a robust study with larger sample size to further investigate whether the differences in LDL estimation methods are translated into "clinical relevance" in Indian settings.

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KEYWORDS

Direct;
Friedewald;
LDL cholesterol;
Hyperlipidemia.

INTRODUCTION

Robust clinical evidence supports the fact that elevated level of low-density lipoprotein cholesterol

(LDL-C) is an independent risk factor for coronary artery disease (CAD)^[1-3]. This has led to the understanding that lowering LDL-C is one of the key therapeutic targets in patients with CAD or those at a risk of

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developing it. Dietary changes, lifestyle modification and drug therapy to lower LDL-C can considerably reduce the morbidity and mortality associated with cardiovascular disorders, particularly CAD^[4-6]. Given the crucial role played by LDL-C in the etiopathogenesis and clinical management of CAD, laboratorial measurements of LDL-C have assumed paramount importance in its diagnosis and monitoring; particularly in patients presenting with hyperlipidemia or dyslipidemia^[7].

Different methods have been established for the measurement of LDL-C; each having their own pros and cons. LDL-C measured by; ultra centrifugation is recommended by Lipid Research Clinic^[8]. Bioquantification (LRC-BQ) has also been recommended as a standard technique for LDL-C estimation for measuring LDL-C. However, this method could not gain popularity at a ground level due to several shortcomings. As a laboratory method, BQ-LDL is expensive, labour intensive and is not freely available^[9,10]. Therefore, most laboratories prefer to use the indirect method of LDL-C estimation also called as the Friedewald method^[11,12]. Under this method, laboratory values for Triglycerides (TG) and Total Cholesterol (TC) are utilized to arrive at an indirect estimation of LDL-C. The TG and TC values are fed into the Friedwald formula (FF) to yield LDL-C values. This method is widely used for LDL-C estimation even today. However, several concerns have been expressed with the use of this method as well^[11,12].

To begin with, this method is based on the postulate that a constant non-dynamic correlation exists between TG / TC and LDL-C. Hence, TG and TC values can be extrapolated for LDL-C calculations. However, evidence has shown that this may not hold true for all clinical situations and scenarios and might adversely impact LDL-C calculations^[12-14]. Besides, combining TG, TC and LDL-C values has shown to give rise to significant analytical variability^[12-14]. Clinically, the most noteworthy limitation of the indirect method is that FF cannot be applied to samples with triglyceride levels above 400 mg/d. Also FF cannot be used in patients with dysbetalipoproteinemia (type III hyperlipoproteinemia) and when chylomicrons are present.

Hence, if LDL-C is to be estimated by the indirect method, the clinician is left with no choice but to opt for

a fasting sample. This limits post prandial assessment and is also cumbersome for the patient^[12-14].

Given these limiting factors of the indirect method of LDL estimation, a need was felt to improvise the laboratory technique for LDL-C measurements. Hence, several commercially available assays have been developed for direct measurement of LDL-C. Numerous such commercial assay kits are available and currently in use. Direct estimation of LDL-C represents the third generation of laboratory techniques for LDL-C estimation^[12]. However, discrepancies have been reported between LDL-C values calculated using the FF and those obtained by direct assays^[15-18]. These discrepancies are of notable concern as some laboratories continue to use the FF method whereas others have shifted to the direct method. The discrepancy of LDL-C estimates between the two methods is further augmented if the two methods are used interchangeably. This can trigger off confusions and misinterpretations particularly while stratifying patients into high and low risk groups, during the process of therapy decision making and therapeutic monitoring^[19,20].

There is very limited data comparing the direct method for LDL estimation with FF method particularly in Indian patients with hyperlipidaemia. Hence this study was conducted to compare the calculative (FF Method) and direct methods of LDL Cholesterol estimation at given total cholesterol and triglyceride values in selected Indian population.

MATERIALS AND METHODS

This is an observational data from 380 consecutive lipid profiles done at International Organization for Standardization (ISO) certified, College of American Pathologists (CAP) and National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratory in Mumbai, Maharashtra. There were no specific inclusion or exclusion criteria. Institutional Ethics Committee permission was obtained prior to the study.

Most of the parameters in Lipid profile were estimated by photometric technology. Photometry is the science of measuring visible light and is based on a relationship between absorption of light and the properties of the material through which the light is traveling

(Beer Lambert's law). Whenever light of a particular wavelength enters a solution of a substance, it comes out with a reduced intensity; this is because a part of it is absorbed by the solution. If this property needs to be exploited for the analytical work or biochemical assays, the phenomenon of absorption of light should obey the Beer-Lambert's Law. It can be stated as that the intensity of light decreases exponentially with the increase in the concentration of the solution and the depth or thickness of the solution through which the light passes.

This technology is integrated into various instruments which enables the detection of analytes. Extreme laboratory automations with world class chemistry analysers like Olympus AU 2700, Siemens Advia 1800 and Roche P800 in modular system were used.

CHOD PAP Method was used to estimate Total Cholesterol^[21]. Enzymatic Colorimetric Method (GPO PAP) was used to estimate Triglycerides^[22]. Enzyme selective protection method was used to estimate HDL^[23]. Homogenous Enzymatic Colorimetric Assay was used to estimate direct LDL^[23].

VLDL was calculated^[11] as follows:

$$\text{VLDL} = \text{Triglyceride} / 5$$

Calculated LDL readings were derived by Friedewald's formula^[11] as follows:

$$\text{LDL-Cholesterol} = [\text{Total Cholesterol}] - [\text{HDL-Cholesterol}] - [\text{Triglycerides}/5]$$

Statistical analysis

Descriptive statistics [means, standard deviations (SD) and CVs] were calculated with Microsoft Excel (Microsoft). Data was reported as mean \pm SD. Linear regression and paired t-test was used.

Mean values for LDL-C by the two methods were compared by paired student's t-tests. Linear relationships were determined from standard Pearson correlation coefficients by linear regression analyses using SPSS (VER 10.0).

RESULTS

For the purpose of data analysis, TG values of study patients were stratified into 3 ranges: 1-100, 101-200 and 201-400 (mg/dL). Similarly, TC values were also stratified into the following 3 ranges: 100-200, 201-250 and >250 (mg/dL).

The correlation of TC and TG values with LDL measured by both the methods was also analyzed without categorizing the TC and TG values into different ranges. In this case the TC and TG values were considered as whole un-stratified data sets.

TABLE 1: Correlation of TG levels with LDL values measured through the direct and calculated methods

TG range (mg/dL)	n	Mean \pm SD LDL-C (mg/dL)	Mean \pm SD LDL-D (mg/dL)	p-value (95% CI)
1-100	123	143.90 \pm 20.27	137.71 \pm 19.16	0.0146* (1.22 – 11.13)
101-200	195	148.77 \pm 20.85	144.27 \pm 17.26	0.0208* (0.68 – 8.31)
201-400	62	142.47 \pm 25.68	145.67 \pm 19.80	0.3829 (10.42 – 0.43)

2-tailed p values have been calculated. Both p-values marked with * are statistically significant as per conventional criteria; CI confidence interval; LDL calculated (LDL-C); LDL-Direct (LDL-D); TG triglyceride

TABLE 2 : Correlation of TC levels with LDL values measured through the direct and calculated methods

TC range (mg/dL)	n	Mean \pm SD LDL-C (mg/dL)	Mean \pm SD LDL-D (mg/dL)	p-value (95% CI)
100-200	62	116.60 \pm 12.61	118.52 \pm 12.41	0.3933(-6.37–2.52)
201-250	270	147.45 \pm 13.92	143.68 \pm 12.57	0.0010* (1.52–6.01)
>250	42	177.15 \pm 17.74	165.88 \pm 18.60	0.0031* (3.89–18.62)

2-tailed p values have been calculated. Both p-values marked with * are statistically significant as per conventional criteria; CI confidence interval; LDL calculated (LDL-C); LDL-Direct (LDL-D), TC Total cholesterol

DISCUSSION

The study data presented here explores as to how the dynamics of the clinical correlation between triglyc-

eride (TG) or total cholesterol (TC) with LDL is impacted; with a change in the method of measurement of LDL (calculated or direct).

In the TG ranges of 1-100 and 101-200 mg/dL, a statistically significant difference was noted in the cor-

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relation of TG values with LDL- values depending upon the method of LDL measurement. This difference was not seen in the TG value range above 201 mg/dL. Similarly, in the TC range of 100-200 mg/dL, a statistically significant difference was not noted in the correlation of TC with LDL-C and LDL-D values. However, TC values above 200 mg/dL correlated in a statistically significantly different manner with LDL-C and LDL-D. A statistically significant difference was also noted between the overall mean LDL values obtained through the direct and calculated methods.

The discrepancy in the LDL-C measurements between the two methods was also statistically significant ($p=0.0098$) when the entire study data was analyzed as a single un-stratified dataset. TC values correlated positively with LDL values measured by both the methods. However, a statistically significant difference ($p=0.0418$) was noted between the correlation coefficients of both the methods. TG values correlated weakly with LDL levels measured by both the methods. A weak negative correlation was observed with LDL-C whereas a weak positive correlation existed between TG and

TABLE 3 : Correlation between TC and LDL values when LDL is measured by the direct as well as calculated method

Correlation between TC and LDL by direct versus calculated method		
Type of LDL measurement	Correlation co-efficient (r)	p-value
LDL-C	0.86074	0.0418
LDL-D	0.81708	

LDL calculated (LDL-C); LDL-Direct (LDL-D); r Co-efficient of correlation; TC Total cholesterol

TABLE 4 : Correlation between TG and LDL values when LDL was measured by the direct as well as calculated method

Correlation between TG and LDL by direct versus calculated method		
Type of LDL measurement	Correlation co-efficient (r)	p-value
LDL-C	-0.0506*	0.009424
LDL-D	0.13758*	

LDL calculated (LDL-C); LDL-Direct (LDL-D); r Co-efficient of correlation; TG triglyceride. Weak correlation marked with *

LDL-D values. The difference between the correlation coefficients was statistically significant.

Nevertheless, the present study had its own limitations. The study data tested the statistical significance of

the difference between the direct and indirect methods of LDL-C estimation. However, the study did not further investigate whether this “*statistical significance*” also translated into “*clinical relevance*”. This leaves us with a couple of unanswered questions: Is the statistically significant difference between the two methods of LDL-C estimation; a clinically meaningful or clinically relevant difference? Does a statistically significant difference between the two methods also imply that this difference could have a cognizable impact on therapy decision making, monitoring and prognostication? Perhaps statistically significant differences between two arms of a clinical study should be further investigated to understand their clinical impact; in order to make a clinical recommendation in

TABLE 5 : The mean LDL values obtained through both the methods

LDL type	n	Mean \pm SD LDL-C (mg/dL)	p-value (95% CI)
LDL-C	380	146.17 \pm 21.64	0.0098
LDL-D	380	142.38 \pm 18.56	(0.92 to 6.66)

LDL calculated (LDL-C); LDL-Direct (LDL-D); n: Number of observations

favour of any one of the study arms.

With respect to study design, the sample size of the study was not large enough to arrive at a confirmatory consensus; as to which of the two methods is superior for LDL-C estimation. Besides, in order to ascertain which of these two methods is more robust, it is imperative to compare both of these with an accepted standard method. The current study involved a comparison between the two methods only and did not compare the two methods with a third standard reference method; and thus a comment cannot be made vis-à-vis the accuracy of the rate of detection, sensitivity and specificity of the two methods being compared.

These limitations need to be taken into account while designing future clinical studies for this comparison. Future clinical studies need to involve a larger sample size and be adequately powered to test the difference between the two methods. A third reference standard needs to be incorporated into the study design so that the direct and indirect methods of LDL-C estimation can be compared against this standard technique. The study population should perhaps involve more heterogeneous subgroups of dyslipidemic patients; for example those with mild, moderate and severe

hypertriglyceridemia and hypercholesterolemia. Perhaps, a prospective study with a larger sample size and heterogeneous patient subgroups may yield more robust information.

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