Original Research Article

Comparison between direct estimation of LDL and Friedewald's formula

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Abstract

Background: The reference method for determining LDL-C is b-quantification. It requires ultracentrifugation, uses large volumes of samples and is a time consuming and expensive technique. Therefore, this method is not suitable for routine laboratory testing. The Friedewald's formula use in routine practice for LDL has many limitation as it is not suitable for TG values >400 mg/dl. Also it tends to underestimate the LDL values.

Aim: The aim of this study was to compare the results obtained by direct homogenous assay for LDL-C to those obtained by Friedewald's formulas with the assumption that the results obtained by direct assay are the most accurate.

Materials and methods: Outpatient fasting complete lipid profile (including directly measured LDL) for patients >18 years of age performed between October 2014 and January 2015 was included in the study. A total of 1768 separate fasting lipid profiles were analyzed. Calculated LDL was derived using FF, and directly measured using homogenous assay using liquid selective detergent. Fasting heparin samples were collected

Results: It was found that the level of LDL estimated by Friedewald's formula was significantly lesser than that by direct estimation of LDL. There was a direct positive correlation between LDL by direct method and Friedewald's formula.

Conclusion: Novel and innovative direct homogeneous assays are accurate, precise, fully automated and cost effective. Therefore, for correct cardiac risk classification, direct homogeneous assay should be the method of choice to estimate LDL-C in routine clinical laboratories.

Key words

Cholesterol, Chylomicron, Lipoprotein, Friedewald's formula (FF), Cardiac, Dysbetalipoproteinemia.

Introduction

Coronary artery disease accounts for the greatest number of deaths of adult individuals worldwide [1]. Several studies have shown the correlation existing between the increased levels of cholesterol in low-density lipoproteins (LDL-C) and the risk of developing that disease [2, 3]. According to the National Cholesterol Education Program (NCEP) Adult Treatment Panel, LDL-C concentration is the primary basis for treatment and appropriate patient's classification in risk categories [4]. The reference method for determining LDL-C is b-quantification [5]. It requires ultracentrifugation, uses large volumes of samples and is a time consuming and expensive technique. Therefore, this method is not suitable for routine laboratory testing [6]. In 1972, Friedewald, et al. published a landmark report describing a formula to estimate LDL-C as an alternative to tedious ultra centrifugation. Because VLDL (very low density lipoprotein) carries most of the circulating triglycerides (TG), VLDL-C can be estimated reasonably well from the measured TG divided by 5 for mg/dl units. LDL-C is then calculated as total cholesterol (TC) minus high density lipoprotein cholesterol (HDL-C) minus estimated VLDL-C [7]. Although this estimation formula correlates highly with beta quantification, it has certain limitations: it is not valid for samples with chylomicrons, with TG >400 mg/dl or in patients with dysbetalipoproteinemia. This formula assumes the ratio of total TG to VLDL-C to be constant in all samples. The formula will overestimate VLDL-C and underestimate LDL-C as a consequence if TG rich chylomicrons and chylomicron remnants are present in the serum sample (hence the requirement for a fasting sample) [8].

Recently, several homogeneous methods have been developed by different manufacturers for the direct measurement of LDL-C levels, expecting that the NCEP criteria are met, as well as that the medical community's need to prevent coronary artery disease and myocardial infarction are fulfilled. These methods seem to be better than the previous ones that use selective chemical precipitation or immunoprecipitation, which are laborious and have a significant bias as compared with the reference method [8, 9]. However, mainly due to the costs of the reagents, their use in clinical laboratories has not been largely disseminated, resulting in scarcity of data about the performance and validation of those methods. This study aimed at assessing the performance of a direct homogeneous method for measuring LDL-C and comparing it with the estimation of LDL-C levels using the Friedwald's formula, analyzing a large sample obtained over 2 years of experience with those reagents.

The association between total cholesterol (TC) and risk of developing coronary heart disease (CHD) has been well established by studies such as the Framingham Heart Study. Most of the cholesterol in circulation is carried by LDL, which has been conclusively shown by many prospective studies and randomized clinical trials to be primarily responsible for the association with CHD risk [10, 11]. Intervention studies performed in patients with (secondary prevention) [12, 13] and without (primary prevention) clinically manifested CHD [14–16] clearly demonstrated the efficacy of lipid-lowering therapies even at relatively low LDL-cholesterol (LDL-C) concentrations. In spite of the technical disadvantages of FF, it is difficult to displace it from clinical practice unless a method with clear advantages in performance and overall cost effectiveness is developed. Recently a new formula for calculation of LDL-C has been proposed by Anandaraja, et al. [17]. This formula uses only two analytes, TG and TC for calculation which may decrease the total error when compared to the FF in which analytical errors of three analytes get added in calculus.

Since the formula does not require HDL-C result for calculation, it can prove to be more economical also. Anandaraja's formula has been approved for use in Brazilian and Greek population [18, 19]. There are no studies reporting use of this new formula in India. The formula needs to be validated before approval for routine use in clinical laboratories. The aim of this study is to compare the results obtained by direct homogenous assay for LDL-C to those obtained by Friedwald's formulas with the assumption that the results obtained by direct assay are the most accurate.

Material and methods

Outpatient fasting complete lipid profile (including directly measured LDL) for patients >18 years of age performed between October 2014 and January 2015 was included in the study. A total of 1768 separate fasting lipid profiles were analyzed. Calculated LDL was derived using FF, and directly measured using homogenous assay using liquid selective detergent. Fasting heparin samples were collected The plasma was separated by centrifugation and the following parameters estimated.

- Total Cholesterol (TC) by Enzymatic endpoint CHOD- PAP method [20]
- Triglycerides (TG) by Enzymatic Glycerol Phosphate Oxidase/ Peroxidase method [21, 22]
- HDL-Cholesterol (HDL-C) by Direct Homogenous Assay [23]
- LDL-Cholesterol (LDL-C) by Direct Enzymatic Assay [24]
- LDL-Cholesterol (LDL-C) obtained by Friedewald calculation [25]
- LDL-Cholesterol (LDL-C) obtained by Anandaraja calculation

The analysis was done on Beckmen Coultor DXC800 fully automated analyzer using modular and cartridge chemistry with dedicated reagents. Patients with TG >400 (n = 228) were excluded from study.

The TG values were divided into five strata (<100-I, 101-150-II, 151-200-III, 201-400-IV, and >401-V), HDL values were divided into three strata (<35-a, 35-70-b, >70-c), and LDL was stratified into five levels (<100-i; 100-129-ii; 130-159-iii; 160-189-iv; and >190-v).

Results

Application of Student 't' test

P value and statistical significance: The two-tailed P value equals 0.0304 By conventional criteria, this difference is considered to be statistically significant.
Confidence interval: The mean of Group One minus Group Two equals 16.3200 95% confidence interval of this difference: From 1.5743 to 31.0657
Intermediate values used in calculations: t = 2.1963, df = 98, standard error of difference = 7.431
Mean for Direct LDL 144.78 mg/dl SD for Direct LDL 39.749362721 mg/dl
Mean for FF LDL 128.464 mg/dl SD for FF LDL 34.356152406 mg/dl

Correlation between FF and direct LDL was as per **Graph – 1**. Mean and SD of direct and FF calculated LDL was as per **Graph – 2**.

Discussion

We found that calculated LDL by FF can underestimate LDL (in comparison to directly measured LDL) at lower levels of LDL and higher levels of TG. Comparison of LDL-C results at different levels of TGs showed statistically significant difference (P<0.001) between measured values and those calculated by Friedewald's Despite several limitations Friedewald's formula (FF) is most commonly used method in routine clinical laboratories to estimate LDL-C. In order to improve the accuracy of FF, many modifications of original formula have been proposed [26, 27, 28, 29], but none of these modifications have provided sufficient evidence to replace original formula [27, 30]. After the recommendations of National

Cholesterol Education Program's (NCEP) working group on lipoprotein measurements, 14 many direct assays have been developed. These assays are precise, accurate, easily automated and have shown good correlation with b-quantification (bQ) method [31, 32, 33]. Vujovic, et al. evaluated FF, Anandaraja formula (AF) and Vujovic modified formula (VMF) by comparing with direct homogeneous assay [26]. There was no significant difference between VMF calculated and direct measured LDL-C (dLDL-C), but FF calculated (ffLDL-C) and Anandaraja formula calculated LDL-C were significantly lower than dLDL-C. Mean absolute bias between calculated LDL-C and dLDL-C were -0.06 ± 0.37 mmol/l for VMF, -0.27 ± 0.31 mmol/l for FF and -0.18 ± 0.51 mmol/l for AF. They recommended VMF for LDL-C estimation, because it was cost effective and better in performance than FF and AF. Paz and colleagues performed systematic analysis of the accuracy of FF and Anandaraja formula by comparing with electrophoretic estimation of LDL-C and reported that there was no advantage of Anandaraja formula over FF [33]. Vujovic modified formula was evaluated in Pakistan. There was significant difference between calculated LDL-C and dLDL-C (p < 0.001), although both methods showed good correlation (r > 0.93). The mean ffLDL-C was 0.12 ±31 mmol/l lower than dLDL-C. This underestimation by FF was also reported by Kamal, et al.; Vujovic, et al. and Chen, et al. [26, 30, 34]. These results also showed that the calculated methods did not have a uniform performance for LDL-C estimation at different TG levels (**Graph - 2**) [35]. This non-uniform performance of FF was also reported by De Cordova, et al. in Brazil and Ahmadi, et al. in Iran [36, 37]. They reported that at lower TG levels FF overestimated and at high TG levels FF underestimated LDL-C than the direct assay. Many subjects were classified in wrong NCEP cardiac risk categories by calculated methods. One limitation of this study was that the methods were not compared with the reference method (bQ method). Although homogeneous assay kit used to measure LDLC in this study, is certified

by Cholesterol Reference Method Laboratory Network (CRMLN) and it was also validated by Esteban, et al. in a multicentre study in Spain by comparing with bQ method. They reported that total error of this kit was 9.8% which was within the NCEP ATP III total allowable error goal.

Strategies for the treatment of lipid abnormalities are primarily based on the concentrations of LDL-C. Therefore, LDL-C must be accurately determined to establish CHD risk profile in order to initiate dietary adjustments, drug therapy and to monitor their effects. Beta quantification, which is the reference method [38] or LDL-C estimation is time consuming and expensive and is not suitable for routine laboratory testing [5]. Most of the studies have claimed to be falsely overestimates LDL level by direct homogeneous assay. However if we take a closer look at the cholesterol metabolism cycle we can see that, the total cholesterol is not just a total of High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol, Very Low Density Lipoprotein Cholesterol; there are some other components which also contains remarkable amount of cholesterol like Intermediate Density Lipoprotein Cholesterol and chylomicrons (**Table - 1**).

These components may interfere with the total cholesterol analysis. So the total cholesterol values may be in fact lower than the sum of actually estimated HDL-C, LDL-C and VLDL-C. Also the VLDL-C value is dependent on triglyceride concentration which varies markedly with recent diet intake. The principle for LDL-C estimation is: this method depends on a unique detergent which solubilizes only the non-LDL lipoprotein particles and releases cholesterol to react with cholesterol esterase and cholesterol oxidase to produce a non-color forming reaction. A second detergent solubilizes the remaining LDL particles, and a chromogenic coupler allows for color formation. This test principle eliminates the possibility of non LDL cholesterol interference in the estimation process. In Our study also though there was significant f\difference between directly estimated LDL-C

and calculated LDL-C, we have found that the LDL-C direct estimation fairly correlates with the calculated LDL-C by Friedwald's Formula at lower triglyceride levels; while at high triglyceride level though it shows a very strong positive correlation between these two values, the values of directly estimated LDL-C are higher than calculated LDL-C by Friedewald's formula. We recommend use of direct estimation of LDL-C method as these methods have shown to give comparable result with the reference method (bQ) in many other studies. Patients who have their LDL underestimated may lead to delay in initiation of adequate lipid lowering therapy in high risk patients as the practitioner is led to believe that the calculated LDL is indeed low, when it is not. On the other hand, when LDL is overestimated at higher levels, placing the patient in a higher risk strata, it results in unnecessary pharmacological therapy. Rechecking the LDL by standardized, direct assay techniques, particularly in patients with TG >200 and LDL <70 or >130 can correctly stratify the risk.

Graph - 1: Correlation between FF and direct LDL.
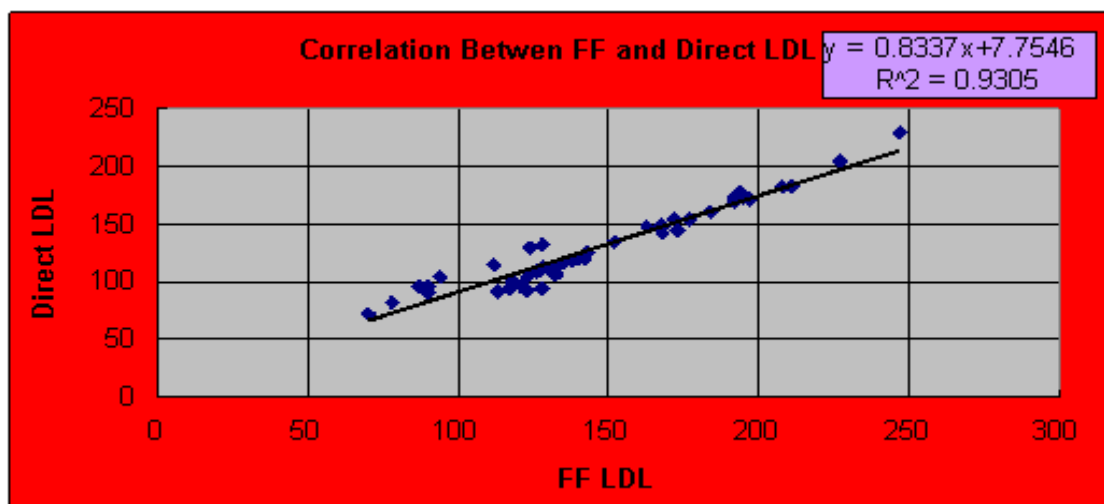
Graph - 2: Mean and SD of direct and FF calculated LDL.
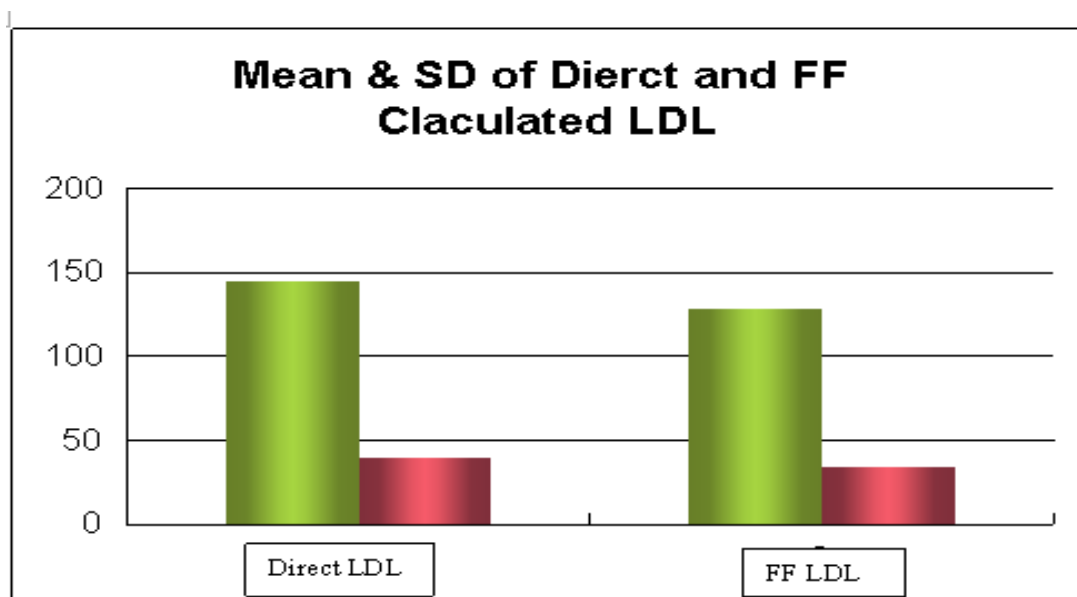
Table - 1: Composition of Lipoproteins.

For young healthy research subjects, ~70 kg, 154 lb, the following applies:

Density (g/mL)	Class	Diameter (nm)	% protein	% cholesterol	% phospholipid	% triacylglycerol & cholesterol ester
>1.063	HDL	5–15	33	30	29	4
1.019–1.063	LDL	18–28	25	50	21	8
1.006–1.019	IDL	25–50	18	29	22	31
0.95–1.006	VLDL	30–80	10	22	18	50
<0.95	Chylomicrons	100-1000	<2	8	7	84

Cost was one of the issue which was hindering the use of direct estimation of LDL-C previously. Earlier homogeneous assays were costly while ffLDL-C was calculated from routine lipid profile without additional cost, but over the last few years, the cost of direct assays has reduced significantly making it affordable to use direct estimation of LDL-C.

Conclusion

The performance of calculated methods was not uniform at different TG levels and many subjects were classified in wrong NCEP cardiac risk categories by calculated methods. Novel and innovative direct homogeneous assays are accurate, precise, fully automated and cost effective. Therefore, for correct cardiac risk classification, direct homogeneous assay should be the method of choice to estimate LDL-C in routine clinical laboratories.

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