Original Article

Correlation of Friedewald's calculated low-density lipoprotein cholesterol levels with direct low-density lipoprotein cholesterol levels in a tertiary care hospital

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Abstract

Background: One of the risk factors for the development of coronary heart disease is high low-density lipoprotein (LDL) cholesterol levels. National Cholesterol Education Program ATP III guidelines suggest drug therapy to be considered at LDL-cholesterol levels >130 mg/dl. This makes accurate reporting of LDL cholesterol crucial in the management of Coronary heart disease. Estimation of LDL cholesterol by direct LDL method is accurate, but it is expensive. Hence, We compared Friedewald's calculated LDL values with direct LDL values. **Aim:** To evaluate the correlation of Friedewalds calculated LDL with direct LDL method. **Materials and Methods:** We compared LDL cholesterol measured by Friedewald's formula with direct LDL method in 248 samples between the age group of 20–70 years. Paired *t*-test was used to test the difference in LDL concentration obtained by a direct method and Friedewald's formula. The level of significance was taken as $P < 0.05$. Pearsons correlation formula was used to test the correlation between direct LDL values with Friedewald's formula. **Results:** There was no significant difference between the direct LDL values when compared to calculated LDL by Friedewalds formula ($P = 0.140$). Pearson correlation showed there exists good correlation between direct LDL versus Friedewalds formula (correlation coefficient = 0.98). The correlation between direct LDL versus Friedewalds calculated LDL was best at triglycerides values between 101 and 200 mg/dl. **Conclusion:** This study indicates calculated LDL by Friedewalds equation can be used instead of direct LDL in patients who cannot afford direct LDL method.

Key words: Coronary heart disease, direct low-density lipoprotein, Friedewalds formula

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Introduction

One of the major risk factors for the development of coronary heart disease is high low-density lipoprotein (LDL) cholesterol.[1-4] Reduction in LDL cholesterol levels decreases the risk of development of coronary heart disease.[5,6] LDL is a lipoprotein made up of outer phospholipids, apolipoproteins, free cholesterol and inner triglycerides (TGs) and cholesterol ester. It carries cholesterol from the liver to the peripheral tissues. The apolipoprotein present in LDL is Apo B 100.[7] LDL fractionn has a hydrated density ranging from 1.006 to 1.063 kg/L.[8]

β-quantification is the standard method for estimating LDL, which includes ultracentrifugation and chemical

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precipitation. β-quantification requires ultracentrifuges and takes time, delaying the turnaround time and hence cannot be employed in day to day practice. Automated methods are available for direct LDL estimation which is expensive and requires significant time for analysis.[8,9] Friedewald *et al*. came up with a formula for estimating LDL using total cholesterol, high-density lipoprotein (HDL) cholesterol and TGs.[10] According to Friedewalds formula, TGs divided by five gives the value of very-LDL (VLDL). TGs >400 mg/dl, disorders related to lipoproteins (Type III hyperlipoproteinemia) and secondary hyperlipoproteinemias are conditions wherein Friedewalds equation for calculating LDL cannot be employed since these conditions decrease the accuracy of Friedewalds equation in estimating LDL.[10] The accuracy and targets to be achieved regarding the analytical performance of LDL cholesterol were issued by National Cholesterol Education Program (NCEP) panel. As per NCEP guidelines, precision should be <4%, bias < 4% and total analytical error should be < 12%.[11] Falsely, low values of LDL cholesterol has been reported in conditions such as diabetes mellitus, advanced renal disease and liver failure due to elevated TGs in these conditions.[8,12-14] In spite of these well-established limitations of Friedewalds equation in estimating LDL cholesterol, it remains to be widely employed. Many studies have been published questioning the accuracy of Friedewalds equation in measuring LDL cholesterol especially at levels of TGs above the normal range.[11,15-18]

This study was taken up to study the accuracy of Friedewalds calculated LDL in comparison to direct LDL method.

Materials and Methods

The current study was a validation study. Assuming a paired mean difference of 12.39 ± 67.0 between the LDL measured by the direct and Friedewald method, the sample size required for the study was estimated to be 234 to achieve 80% power of the study and 5% significance level.

Inclusion criteria

Lipid profile was estimated in 248 samples which were estimated in venous blood drawn after 12 h of fasting between the age group of 20–70 years. The blood was collected in plain tubes centrifuged at 3000 rpm for 15 min. The serum liberated was analyzed for lipid profile.

Exclusion criteria

Patients with TGs more than 400 mg/dl, diabetes mellitus, advanced renal disease and patients with liver failure, patients receiving lipid-lowering drugs were excluded from the study. The period of the study was from February 2015 to June 2015.

The following tests were done in Cobas Integra 400 plus from Roche Diagnostics. The principle of the methods were total cholesterol - cholesterol oxidase-peroxidase method; TGs -enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone; HDL-cholesterol - homogeneous enzymatic colorimetric assay; and direct LDL cholesterol - the homogeneous enzymatic colorimetric assay: This automated method for direct LDL-cholesterol estimation is based on micellar solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). Biorad internal and external controls were run for total cholesterol, TGs, HDL and LDL cholesterol. Friedewalds calculated LDL was calculated by the formula: LDL cholesterol = Total cholesterol − HDL cholesterol − TGs/5 (VLDL cholesterol).

Statistical analysis

Data were entered into Microsoft Office Excel 2007. Categorical data were reported as frequency and continuous data were reported as mean and standard deviation. Paired *t*-test was used to test the difference in LDL concentration obtained by the direct method and Friedewalds calculated method. The level of significance was taken as $P < 0.05$ and two-sided. Scatter plot was used to represent the correlation between the two methods.

Data were categorized into three groups based on the TG levels [Table 1].

Results

The study group consisted of 248 patients, 151 were males and 97 were females. Table 2 indicates there was no statistical difference between direct LDL values and Friedewalds calculated LDL values ($P = 0.140$). Table 3 shows there is no statistical difference between direct LDL values and Friedewalds calculated LDL at different ranges of TGs,

Table 1: Categorization of groups based on Triglyceride values

Groups	Triglyceride levels
I	<200 mg/dl
II	201 to 300 mg/dl
III	301 to 400 mg/dl

Table 2: Comparison between direct LDL method and Friedewalds calculated LDL (*n*=248)

Method	Mean±SD	Mean difference±SD	*P* value
Direct LDL	112.02±39.34	0.82±8.66	0.140
Friedewalds calculated LDL	111.21±39.48		

≤ 200 mg/dl (P = 0.47), 201–300 mg/dl (P = 0.32) and 301–400 mg/dl (P = 0.22). Table 4 shows Pearson correlation which indicates good correlation between direct LDL and Friedewalds calculated LDL (correlation coefficient – 0.98). Figure 1 indicates Scatter plot representing good correlation between direct LDL and Friedewalds calculated LDL with correlation coefficient of 0.98. Figure 2 indicates Scatter plot which indicates good correlation between direct LDL and Friedewalds calculated LDL at TGs < 200 mg/dl with a correlation coefficient of 0.982. Figure 3 indicates Scatter plot representing good correlation between direct LDL and Friedewalds calculated LDL at TGs 201–300 mg/dl with correlation coefficient of 0.964. Figure 4 indicates Scatter plot representing good correlation between direct LDL and Friedewalds calculated LDL at TGs 301–400 mg/dl with correlation coefficient of 0.968.

Person correlation showed that there exists good correlation between direct LDL versus Friedewalds formula (correlation coefficient = 0.98) [Table 4].

Discussion

The primary target for diagnosis and management of hypercholesterolemia is LDL cholesterol as per NCEP Adult Treatment Panel report.[19] Hence, accurate reporting of LDL cholesterol is utmost important. In this study, there was no significant difference between the overall mean of direct LDL cholesterol with that of Friedewalds calculated LDL cholesterol [Table 2]. There was no significant difference between direct LDL and Friedewalds LDL at different TG levels ranging below 200 mg/dl, 201–300 mg/dl, and 301–400 mg/dl [Table 3]. Sudha *et al.* observed that Friedewalds calculated LDL were lower when compared to direct LDL with TG more than 180 mg/dl.[18] Boshtam *et al.* observed in their study that Friedewalds method overestimated LDL cholesterol by 7 mg/dl when compared to direct LDL method.[15] Kapoor R *et al.* observed that Friedewalds LDL method underestimated LDL cholesterol by 10.39% in comparison with direct LDL method.[20] Martin *et al.* observed that Friedewalds formula underestimated LDL cholesterol especially when TG value is >150 mg/dl.[16] Knopfholz *et al.* observed no significant difference between direct LDL and Friedewalds LDL at TGs below 150 mg/dl and above 150 mg/dl which was comparable to findings of this study.[21] Kannan *et al.* observed Friedewalds calculated LDL underestimated LDL cholesterol in comparison to direct LDL method.[17]

Direct LDL homogeneous assays are not free from limitations. They exhibit a negative bias as observed in studies done by Rifai *et al.* and this may result in placing a patient into low risk who actually belongs to high-risk hypercholesterolemia.[22,23] Nauck *et al.* in their study observed, direct LDL method has no advantage when compared to calculated LDL method and recommended further validation for direct homogeneous methods.[8] Miller *et al.* in their study observed no advantage of direct LDL method in comparison to calculated LDL method at TG value below 400 mg/dl.[24] Mora *et al.* observed in their study the nonassociation of direct LDL with

Table 3: Comparison between direct LDL method and Friedewalds calculated LDL at different serum level of TG (mg/dl)

TG (mg/dl)	*n*	Direct LDL Mean±SD	Friedewalds calculated LDL Mean±SD	*P* value
<200	183	111.40±37.97	111.06±38.34	0.47
201-300	52	118.10±44.80	116.15±43.78	0.32
301-400	13	96.46±32.05	93.60±34.60	0.22

Table 4: Pearson correlation between Direct LDL and Friedewalds calculated LDL

Method	Mean±SD	Pearson's correlation
Direct LDL	112.02±39.34	0.98
Friedewalds calculated LDL	111.21±39.48	

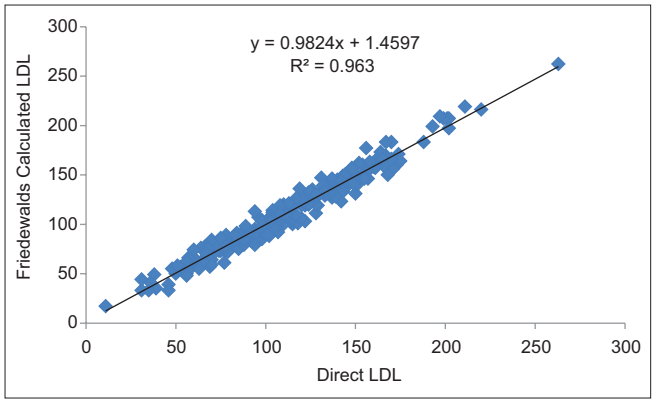

Figure 1: Scatter plot representing the correlation between Direct LDL and Friedewalds calculated LDL at Triglycerides Less than 400 mg/dl (r = 0.981, r^2 = 0.963)

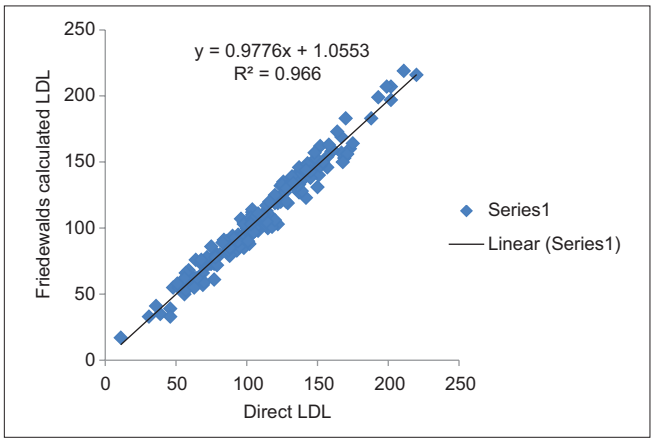

Figure 2: Scatter plot representing the correlation between Direct LDL and Friedewalds calculated LDL at Triglycerides < 200 mg/dl (r = 0.982, r^2 = 0.966)

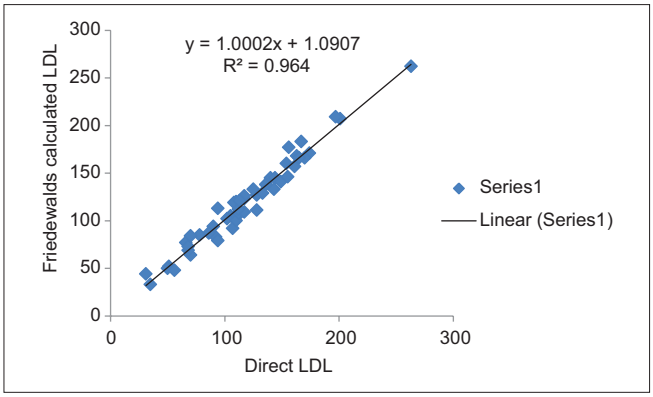

Figure 3: Scatter plot representing the correlation between Direct LDL and Friedewalds calculated LDL at Triglycerides 201 to 300 mg/dl ($r = 0.981$, $r^2 = 0.964$)

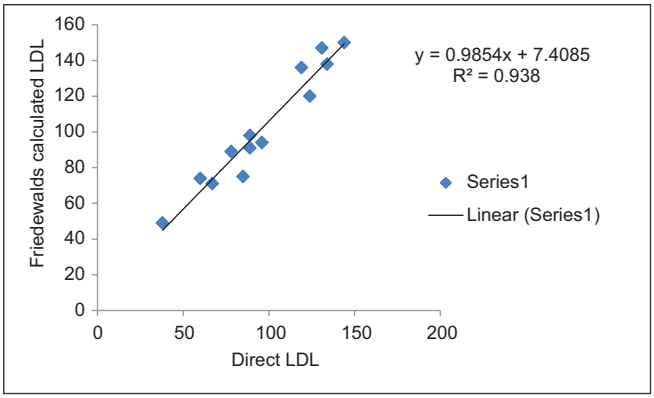

Figure 4: Scatter plot representing the correlation between Direct LDL and Friedewalds calculated LDL at Triglycerides 301 to 400 mg/dl ($r = 0.968$, $r^2 = 0.938$)

Friedewalds LDL in nonfasting samples and they could not demonstrate any advantage of direct LDL in comparison to Friedewalds calculated LDL. They also stated using direct LDL may misclassify the patients into low-risk NCEP category because the results of direct LDL were 5–10 mg/dl lower when compared to Friedewalds calculated LDL.[25] Gazi *et al*. observed Friedewalds calculated LDL was accurate for any value of TG below 400 mg/dl.[26] In this study, we observed a similar finding since the LDL cholesterol calculated by Friedewalds formula correlated well with direct LDL at TGs below 400 mg/dl [Figures 1-4]. Choi *et al*. observed that direct LDL values were 5% higher than calculated LDL and in diabetics, the difference was much higher.[27] Sudha *et al*. observed Friedewalds calculated LDL method underestimated LDL levels in comparison to direct method and they concluded that direct LDL method is better than Friedewalds calculated LDL in diabetics.[28] Chatterjee and Mendez observed there was a good correlation between Friedewalds calculated LDL and direct LDL method which is in agreement with the findings of the present study.[29] Lindsey *et al*. observed Friedewalds calculated LDL underestimated LDL levels by 20 mg/dl when compared to direct LDL method.[30] Kaur observed there was no significant difference between the LDL values measured by direct LDL method and Friedewalds calculated method in patients with metabolic syndrome.[31]

Friedewalds calculated LDL cannot be employed in individuals with TG value more than 400 mg/dl. Dansethakul *et al*. derived a new formula which correlated well with direct LDL values at TGs higher than 400 mg/dl.[32] Chaudhari *et al*. observed 38.2% of the study group were classified as high-risk group when LDL was estimated by direct LDL method and 24.9% were classified as high risk when LDL was calculated by Friedewalds calculated LDL.[33] Cordova *et al*. observed Friedewalds calculated LDL showed a positive bias at TG level <150 mg/dl and at TG level between 301 and 400 mg/dl, Friedewalds calculated LDL showed a negative bias in comparison to direct LDL.[34] Krishnaveni and Gowda observed Friedewalds calculated LDL correlated well with direct LDL except at TG level below 100 mg/dl and they observed at TG below 100 mg/dl, Anandaraja's calculated LDL performed better than Friedewalds calculated LDL.[35] Charuruks and Milintagas observed in their study, direct LDL to be more accurate and precise than Friedewalds calculated LDL and they suggested direct LDL method to be used in individuals with TG level > 200 mg/dl.[36] Ashour *et al*. observed in their study, there was no significant difference between Friedewalds calculated LDL and direct LDL at TG concentrations below 100 mg/dl but the LDL values obtained by Friedewalds equation was significantly lower when compared to direct LDL method at TG concentrations between 101–200 mg/dl and 201–300 mg/dl which is not in agreement with the findings of this study.[37] Mohan *et al*. observed in their study underestimation of LDL of 20–25 mg/dl by Friedewalds calculated LDL when compared to direct LDL method at TG between the range of 300–400 mg/dl.[38] Lee *et al*. observed LDL cholesterol measured by direct LDL method was significantly lower than Friedewalds calculated LDL and differences in the LDL cholesterol concentrations had no relation with TG concentrations.[39] Gasko observed Anandaraja's calculated LDL correlated better than Friedewalds calculated LDL with direct LDL in a Brazilian population.[40] Nakanishi *et al*. observed the original Friedewalds calculated LDL correlated best with direct LDL levels in comparison to modified Friedewalds formula and they suggested the chances of error in Calculated LDL increases with increase in TGs.[41] Teerakanchana *et al*. observed a high bias of 20.9 mg/dl at TGs between 301 and 400 mg/dl and a lower bias at TGs below 300 mg/dl with Friedewalds calculated LDL in comparison to direct LDL.[42] Sahu *et al*. observed a significant difference between direct LDL and Friedewalds calculated LDL at TG values below 300 mg/dl and there was no signifi cant difference at TG values above 300 mg/dl. According to Sahu *et al*., the possible explanation for such a result could be the masking

or removal of LDL cholesterol due to the detergent used in direct method.[43] The sample size of this study was only 248 which is the limitation of the present study.

Conclusion

Friedewalds Formula can be used to estimate LDL cholesterol, and direct LDL should be employed only in those cases wherein Friedewalds formula cannot be used like nonfasting samples, patients with TGs more than 400 mg/dl, disorders related to lipoproteins (Type III hyperlipoproteinemia) and secondary hyperlipoproteinemias.

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Conflicts of interest

There are no conflicts of interest.

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