ORIGINAL ARTICLE

Comparison of Friedewald Formula and Modified Friedewald Formula with Direct Homogeneous Assay for Low Density Lipoprotein Cholesterol Estimation

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ABSTRACT

Objective: To compare the Friedewald and modified Friedewald formulae with direct homogeneous assay for serum low-density lipoprotein cholesterol (LDL-C) levels estimation.

Study Design: Cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi, from June to December 2011.

Methodology: Healthy subjects of either gender, from Rawalpindi, aged 18-75 years were included by consecutive sampling. Patients with diabetes mellitus, chronic liver disease, chronic kidney disease, those taking lipid lowering drugs and samples with triglyceride (TG) > 4.52 mmol/l were excluded from the study. Total cholesterol, high-density lipoprotein cholesterol, TG and LDL-C were measured on Hitachi 912 chemistry analyzer (Roche). LDL-C levels were also calculated by Friedewald formula (FF) and Vujovic modified formula (VMF). Paired sample t-test and scatter plots were used for statistical analysis.

Results: Although both calculated methods showed good correlation with direct assay ($r > 0.93$) in 300 subjects, but the difference was statistically significant. The ffLDL-C were 0.12 ± 31 mmol/l ($p < 0.001$) lower and vmfLDL-C were 0.11 ± 26 mmol/l ($p < 0.001$) higher than dLDL-C. The difference was not significant between ffLDL-C and dLDL-C at TG levels < 1.70 mmol/l ($p = 0.58$) and between vmfLDL-C and dLDL-C at TG levels 2.26 – 4.52 mmol/l ($p = 0.38$). At all other TG levels, the difference between LDL-C calculated by both formulas and dLDL-C was statistically significant ($p < 0.001$). As compared to direct assay, 11% and 14% subjects were classified in wrong National Cholesterol Education Programm's cardiac risk categories by FF and VMF respectively.

Conclusion: LDL-C should be measured by direct homogeneous assay in routine clinical laboratories, as the calculated methods did not have a uniform performance for LDL-C estimation at different TG levels.

Key Words: *Low density lipoprotein cholesterol. Direct homogeneous assay. Friedewald formula. Vujovic modified formula.*

INTRODUCTION

Coronary Artery Disease (CAD) is the leading cause of death worldwide; its incidence is also increasing in Pakistan.[1] The National Cholesterol Education Programme's (NCEP) Adult Treatment Panel III (ATP III) recommended low density lipoprotein cholesterol (LDL-C) as the primary lipid agent for CAD risk prediction and therapeutic target, emphasizing the importance of accuracy and precision of LDL-C estimation.[2] The reference method for measurement of LDL-C concentration, ultracentrifugation-polianion precipitation / Beta Quantification (ßQ), is expensive, laborious and not available everywhere. During the last decade, direct homogeneous assays have been developed for measurement of LDL-C levels and have shown reasonable accuracy and precision as compared to reference method. Commercially available direct LDL-C kits have been certified by NCEP and Cholesterol Reference Method Laboratory Network of Centre for Disease Control and Prevention for use in routine clinical laboratories (labs).[3,4]

Friedewald Formula (FF) is the most commonly used method to calculate LDL-C in routine clinical labs. FF has several limitations including requirement for fasting, analytical variability and invalidity in samples with triglyceride (TG) > 4.52 mmol/l and certain type of hyperlipidemias. Studies have shown that the accuracy of FF declines as TG increases beyond 2.00 mmol/l, because assumption that Very Low Density Lipoprotein Cholesterol (VLDL-C) = TG/2.2 is not always true.[5] Discrepancies in results have been reported, when LDL-C was measured directly and calculated by FF. Some studies have reported that FF underestimated LDL-C as compared to direct assays and many patients were classified in lower cardiac risk categories by FF.[3,6] The total error of FF was greater than total allowable error goal (≤ 12%) for LDL-C estimation even in experienced lipid labs.[7] Others have reported that direct assays underestimated LDL-C as compared to FF and calculated methods are as good or even better for CAD

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risk stratification.[8,9] Many modifications of FF have also been reported, claiming better accuracy and precision than FF.[6,10] Recently, Vujovic *et al*. reported a modified formula for LDL-C calculation in Serbian population.[6] They claimed that LDL-C levels calculated by the new formula were more closely related to levels measured by direct homogeneous assay than FF and patients were correctly classified in their NCEP cardiac risk categories.[6]

This study was aimed to compare two different calculated methods (FF and Vujovic modified formula) with direct homogeneous assay to assess their validity, suggest most precise, accurate and suitable method for LDL-C estimation in clinical labs in Pakistan and to assess whether different methods affect the classification of patients for CAD risk.

METHODOLOGY

This cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, from June to December 2011 after approval from the Ethical Committee of the Institute. Subjects of either gender, aged 18 – 75 years were included by non-probability consecutive sampling. Patients with diabetes mellitus, chronic liver or kidney disease and patients taking lipid lowering drugs were excluded from the study. Fasting samples from 319 subjects were collected in vacutainer tubes (BD, NJ USA). The samples were allowed to clot, centrifuged at 3000 g for 5 minutes and analyzed within 2 hours of collection. 19 specimens with TG > 4.52 mmol/l were excluded from further analysis.

Serum total cholesterol (TC) was measured by enzymatic endpoint method with a coefficient of variation (CV) of 3.1%. Serum triglyceride (TG) was measured by enzymatic method with a CV of 3.6%. Serum high density lipoprotein cholesterol (HDL-C) was measured by direct homogeneous assay with a CV of 5.6%. Serum low density lipoprotein cholesterol (LDL-C) was measured by direct homogeneous assay with a CV of 4.9%. All biochemical lipid analysis was done on Hitachi 912 chemistry auto analyzer by using Roche kit (Roche Diagnostic). LDL-C levels were also calculated by Friedewald's formula (FF); LDL-C = TC - (HDL-C +TG/2.2) and Vujovic modified formula (VMF); LDL-C = TC - (HDL-C +TG/3).

All statistical analysis was done by Statistical Package for Social Sciences version 18 (SPSS Inc, Chicago, IL, USA). Descriptive statistics for qualitative variables like gender were shown in percentages, while mean values with standard deviation (SD), median, 25th and 75th percentile were calculated for quantitative variable like age, TC, HDL-C, TG, direct measured LDL-C (dLDL-C), Friedewald formula calculated LDL-C (ffLDL-C) and Vujovic modified formula calculated LDL-C (vmfLDL-C). Mean LDL-C measured by homogeneous assay and calculated by FF and VMF were compared by paired sample t-test and correlated by scatter plots and Pearson correlation. The performance of calculated methods at different TG levels was also compared with dLDL-C by paired sample t-test. Two tailed p-values < 0.05 were considered as statistically significant. Patients were also classified in NCEP cardiac risk categories according to the LDL-C obtained by different methods.

RESULTS

Out of 300 subjects, 65.4% were male. Age distribution and basic lipid measurements are shown in Table I. The ffLDL-C were 0.12 ± 31 mmol/l lower, and vmfLDL-C were 0.11 ± 26 mmol/l higher than dLDL-C. When compared by paired sample t-test, there was significant difference between dLDL-C and calculated LDL-C (p < 0.001), although both calculated methods showed good correlation to dLDL-C, with correlation co-efficient of 0.93

Table I: Distribution of age, basic lipid characteristics and LDL cholesterol measured by direct assay and calculated by different formulas in selected subjects (n = 300).

Parameters	Mean ± SD	Median	IQR
Age (years)	51±12	51	43-60
Total cholesterol (mmol/l)	4.73±0.97	4.69	3.9-5.3
HDL cholesterol (mmol/l)	1.06±0.26	1.01	0.87-1.21
Triglyceride (mmol/l)	1.78±0.92	1.59	1.11-2.11
Direct measured LDL-C (mmol/l)	2.93±0.81	2.92	2.34-3.39
Friedewald formula calculated LDL-C (mmol/l)	2.81±0.82	2.77	2.23-3.38
Vujovic modified formula calculated LDL-C (mmol/l)	3.04±0.83	3.02	2.43-3.58

HDL cholesterol = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; IQR = Inter quartiles.

Table II: Comparison of direct measured LDL-C and LDL-C calculated by different formulas in all samples and at different triglyceride levels.

	Mean ± SD mmol/l	Mean difference*	p-value
All samples (n=300)			
dLDL-C	2.93±0.81	–	–
ffLDL-C	2.81±0.82	-0.12±31	< 0.001
vmfLDL-C	3.04±0.83	0.11±26	< 0.001
At TG level <1.7 mmol/l (n=164)			
dLDL-C	2.77± 0.74	–	–
ffLDL-C	2.78± 0.78	0.01 ±0.23	0.58
vmfLDL-C	2.93±0.79	0.15±0.24	< 0.001
At TG level 1.7-2.25 mmol/l (n=69)			
dLDL-C	2.97±0.86	–	–
ffLDL-C	2.84±0.85	-0.13±0.20	< 0.001
vmfLDL-C	3.07±0.86	0.09±0.20	< 0.001
At TG level 2.26-4.52 mmol/l (n=67)			
dLDL-C	3.25±0.85	–	–
ffLDL-C	2.90±0.99	-0.35±0.38	< 0.001
vmfLDL-C	3.29±0.90	0.04±0.33	0.38

LDL-C = Low density lipoprotein cholesterol; dLDL-C = Direct measured LDL-C; ffLDL-C = LDL-C calculated by Friedewald formula; vmfLDL-C = LDL-C calculated by Vujovic modified formula.
** Calculated LDL-C by respective formula – dLDL-C.*

and 0.95 for ffLDL-C and vmfLDL-C respectively (Figure 1). As the TG levels increased the difference; between ffLDL-C and dLDL-C increased, while between vmfLDL-C and dLDL-C decreased (Table II). The difference between dLDL-C and calculated LDL-C was statistically significant at all TG levels except for ffLDL-C at TG levels < 1.7 mmol/l (p = 0.58) and for vmfLDL-C at TG levels 2.26 – 4.52 mmol/l (p = 0.38).

When subjects were classified in National Cholesterol Education Programme's cardiac risk categories, 11.3% and 14% subjects were classified in wrong cardiac risk categories by ffLDL-C and vmfLDL-C respectively (Table III).

Table III: Classification* of subjects in National Cholesterol Education Programme's cardiac risk categories according to LDL-C levels calculated by using different formulas (n=300).

	Wrong classified n (%)	Higher risk category n (%)	Lower risk category n (%)
ffLDL-C	34 (11.3%)	6 (2%)	28 (9.3%)
vmfLDL-C	42 (14%)	30 (10%)	12 (4%)

LDL-C = Low density lipoprotein cholesterol; ffLDL-C = LDL-C calculated by Friedewald formula; vmfLDL-C = LDL-C calculated by Vujovic modified formula.
** Direct measured LDL-C was considered as reference method.*

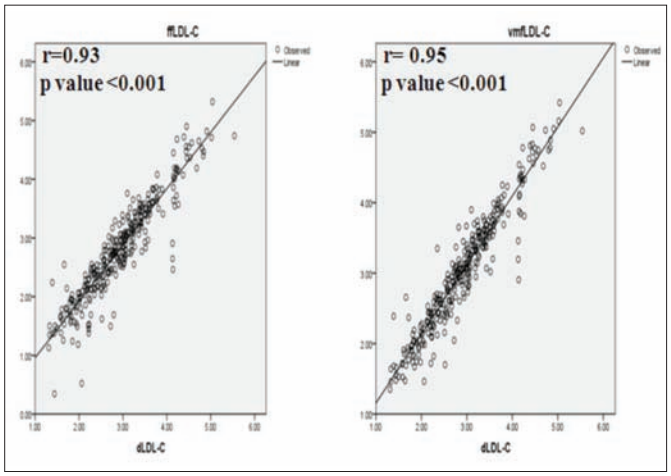

Figure 1: Scatter plot showing the correlation of direct measured LDL-C with LDL-C calculated by different formulas. Both calculated methods showed good correlation direct measured LDL-C.

dLDL-C = Direct measured LDL-C; ffLDL-C = LDL-C calculated by Friedewald formula; vmfLDL-C = LDL-C calculated by Vujovic modified formula.

DISCUSSION

Treatment strategies for lipid disorders are primarily based on low density lipoprotein cholesterol (LDL-C) levels. Therefore, to establish personal coronary artery diseases (CAD) risk for initiation of dietary adjustments, drug intervention and monitoring, LDL-C should be estimated accurately.[2,11]

Despite several limitations Friedewald 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,[6,10,12,13] but none of these modifications have provided sufficient evidence to replace original formula.[3,6] 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 β-quantification (βQ) method.[3,5,15]

Vujovic *et al*. evaluated FF, Anandaraja formula (AF) and Vujovic modified formula (VMF) by comparing with direct homogeneous assay.[6] 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.[16]

To the best of authors' knowledge this is the first study in which accuracy and reliability of Friedewald formula and 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*.[3,6,17] The mean VMF calculated LDL-C (vmfLDL-C) was 0.11 ± 26 mmol/l higher than dLDL-C, which was different as reported by Vujovic *et al*.[6] These results also showed that the calculated methods did not have a uniform performance for LDL-C estimation at different TG levels (Table II). This non-uniform performance of FF was also reported by De Cordova *et al*. in Brazil and Ahmadi *et al*. in Iran.[18,19] 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 (Table III).

Kamal and co-workers reported that LDL-C calculated by FF, Anandaraja formula and another modified formula were significantly lower than the dLDL-C (p < 0.001).[3] This underestimation of LDL-C by calculated methods increased as TG levels increased and many patients were classified in wrong cardiac risk categories. They recommended that as the direct assays are precise, accurate and not affected by TG levels, therefore, should be used to measure LDL-C.

Mora *et al*. compared FF and direct assay in specimens from healthy female subjects.[8] They reported that ffLDL-C were significantly higher than dLDL-C, although both methods were highly correlated (r 0.976) and the association of CAD with LDL-C levels estimated by both methods was almost identical in fasting specimens.

Non-fasting LDL-C estimated by either method was not associated with CAD risk. Sahu *et al*. also reported that ffLDL-C was significantly higher than dLDL-C.[9] FF classified 23.5% subjects in high cardiac risk category as compared to 17.58% by direct assay.

Differences in the results of different studies may be attributed to diversity in population, pathologies and kits used. Measurement uncertainty that arises from three independent parameters used to calculate LDL-C may have a major contribution to these differences. These differences not only arise from imprecision within laboratories, but also from lot to lot variation and assay to assay variations from different manufacturers.[20] Arderiu and colleagues in a multicentre study reported that measurement uncertainty of direct assay was 6.9% as compared to 19.4% of calculated method and total error of calculated method was greater than the total allowable error (≤ 12) for LDL-C estimation.[7] Miller and co-workers reported that 5 out of 8 LDL-C direct assays were able to meet the NCEP total error goals in healthy subjects, but none of these met these goals in diseased subjects.[21] This showed the non-specificity of these assays towards abnormal lipoproteins. Therefore, these assays also need more standardization and more research especially in subjects with altered lipoprotein composition.

Cost is another aspect that must be given due importance before adopting any method especially in countries like Pakistan. Earlier homogeneous assays were costly while ffLDL-C was calculated from routine lipid profile without additional cost,[5] but over the last few years, the cost of direct assays has reduced significantly. LDL-C plus kit (Roche diagnostic) used in this study costs only 11.25 PKR per test while the combined cost of TC, HDL-C and TG is 50.0 PKR. It may be more cost effective to monitor therapeutic response in patients who are on lipid lowering agents by measuring only LDL-C by direct assay. It will also increase throughput as only one direct assay will be needed instead of three assays.

One limitation of this study was that the methods were not compared with the reference method (βQ method). Although homogeneous assay kit used to measure LDL-C 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 βQ method. They reported that total error of this kit was 9.8% which was within the NCEP ATP III total allowable error goal.[15] They recommended that it can be used as an alternative to βQ method in routine clinical labs and research studies.

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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