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Correlations between Direct and Calculated Low-Density Lipoprotein Cholesterol Measurements in Children and Adolescents

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Abstract

Background: Low-density lipoprotein cholesterol (LDL-C), as a modifiable risk factor for atherosclerotic cardiovascular disease, should be assessed and monitored. This study compared directly measured and Friedewald-estimated LDL-C values in children and adolescents.

Methods: Blood samples were collected from 464 children and adolescents. Calculated LDL-C (CLDL-C) levels were estimated using the Friedewald formula for any triglyceride value below 4.6 mmol/L. Direct LDL-C (DLDL-C) levels were measured on an ARCHITECT c8000 Abbott Clinical Chemistry Analyzer. The differences in LDL-C were then calculated.

Results: The correlation coefficients (R) between DLDL-C and CLDL-C were 0.978 ($P = .148$) and $R = 0.970$ ($P = .052$) for children and adolescents, respectively. Children with LDL-C values above 4.92 mmol/L had a correlation value of 0.971 ($P = .419$). The correlation and agreement between DLDL-C and CLDL-C in adolescents were moderate for LDL-C below 2.85 mmol/L ($R = 0.806$; 84.1%) and improved above 2.85 mmol/L ($R = 0.978$; 91.5%). In children, good correlations between DLDL-C and CLDL-C were observed for normal (<0.85 mmol/L), borderline (0.85-1.12 mmol/L), and abnormal (≥1.13 mmol/L) triglyceride levels ($R = 0.9782$, 0.990, and 0.951,

respectively). The rates of agreement were better for normal (80.5%) and borderline (82.9%) but not abnormal (68.2%) triglyceride levels.

Conclusion: We observed good agreement between DLDL-C and CLDL-C in both children and adolescents. The Friedewald formula provided an adequate estimate of LDL-C for most fasting specimens. LDL-C difference percentage can also be used as a quality indicator to check laboratory analyzer performance in healthy subjects.

KEYWORDS
adolescents, children, Friedewald, low-density lipoprotein cholesterol

1 | INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) concentrations are widely recommended to be determined in individuals with risk factors for coronary heart disease (CHD). The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines for hyperlipidemia, which are the most commonly referred, were recently updated to ATP IV.[1,2] These guidelines suggest that calculated LDL-C (CLDL-C) assessment using the Friedewald formula should be the primary lipid target for reducing CHD risk. Beta quantification is the reference method for measuring LDL-C.[3,4]

LDL-C estimated by the Friedewald equation in routine patient care is a central focus of clinical practice guidelines worldwide, including the United States,[2] Europe,[5] and Canada.[4] Friedewald-estimated LDL-C is used in clinical practice because it is more convenient and less expensive than the more complicated and time-consuming beta quantification.[6] The Friedewald equation estimates LDL-C as the total cholesterol minus high-density lipoprotein cholesterol (HDL-C) minus triglycerides (TGs)/5 in milligrams per deciliter (mg/dL).[6] However, for millimoles per liter (mmol/L) calculations, the Friedewald equation is LDL-C = TC − HDL-C − TG/2.2.[7] The equation was introduced into clinical practice in 1972 because of the additional time and financial costs associated with ultracentrifugation to directly measure LDL-C.[6] Friedewald et al[6] recognized the inaccuracy of very-low-density lipoprotein cholesterol (VLDL-C)-based estimates. The shortcomings are (a) combining three measurements increases analytical imprecision and (b) it is unreliable for TG concentrations >400 mg%[7] and can be used only in the fasting state. Recently, DLDL-C assay kits using novel surfactants (homogenous methods) based on different principles have become commercially available and are widely used. These assays are reportedly suitable even for serum with high TG levels.[8,9]

Agreement between direct and calculated LDL-C has been reported in various adult populations but is poorly addressed in children and adolescents. Therefore, this study compared and correlated the determination of LDL-C concentrations by a direct assay and Friedewald calculation in child and adolescent populations.

2 | MATERIALS AND METHODS

2.1 | Study population

This study included 464 healthy children (n = 206) and adolescents (n = 235) who attended the pediatrics and primary clinic at King Abdulaziz Medical City, Riyadh, Saudi Arabia, and were screened for lipid profile (HDL-C, LDL-C, and TG). The inclusion criteria were any healthy Saudi male/female between 6 and 17 years of age. Children and adolescents with any pathological conditions or chronic disease or who were not Saudi were excluded from the study. The 206 children (age 6-12 years) included 96 girls and 110 boys. The 258 adolescents (age 12-17 years) included 139 girls and 119 boys. Blood samples were collected after 12 hours of fasting into serum separator tubes to analyze lipid profiles. The samples were transported in controlled conditions to the main laboratory, centrifuged, and analyzed immediately. The lipid profile, including cholesterol, HDL-C, LDL-C, and TG levels, was calculated for all serum samples using an ARCHITECT c8000 Abbott Clinical Chemistry Analyzer. The analytical methods were controlled according to the manufacturer's instructions by preventive maintenance, function checks, calibration, and quality control. All tested samples underwent automated interference analysis for hemolysis, icterus, and turbidity.

The LDL-C was calculated using the Friedewald equation, $LDL-C = TC - HDL-C - TG/2.2$ expressed in mmol/L, excluding samples with TG concentrations >4.52 mmol/L. No children or adolescents with TG concentrations lower than 4.52 mmol/L reported any chronic conditions. HDL-C was measured either by precipitation with dextran sulfate Mg^{2+} assay or by the direct assay.[7] The LDL difference was calculated by using the formula $LDL\ difference\ \% = [(LDL\ direct - LDL\ calculated)/LDL\ direct] \times 100$.

2.2 | Statistical analysis

Statistical and correlation analyses were performed using Microsoft Office Excel 2010. Values were expressed as means ± SD. Pearson correlation tests were used to analyze the correlations between parameters. Paired *t* tests were also performed to compare the means.

P-values < .05 were considered statistically significant. Statistical analyses were performed using SPSS version 16.0. Bland-Altman graphical plots were used to examine the degree of agreement between the values obtained by the two methods. The limit for total allowable error for LDL-C was equal or <20%, as recommended by the College of American Pathologists (CAP). Differences were considered statistically significant when *P* < .05.
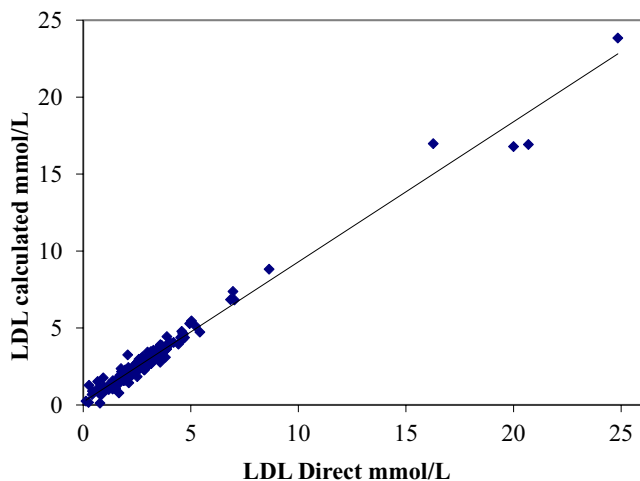
3 | RESULTS

The estimated correlation coefficients (*R*) between DLDL-C and CLDL-C for children (Figure 1) and adolescents (Figure 2) were 0.978 (*P* = .148) and 0.9703 (*P* = .05), respectively. The percent differences between direct and calculated LDL were in the ranges of ± 30% (*P* = .007) for children and ± 20% (*P* = .008) for adolescents (Figure 3).

We also assessed the correlations at different LDL-C concentrations. The correlation between DLDL-C and CLDL-C for children and adolescents with LDL-C levels below 2.6 mmol/L was 0.806 (*P* = .485), while that for those with LDL-C levels above 2.6 mmol/L was 0.805 (*P* = .062). The correlation for children with LDL-C values above 4.92 mmol/L was 0.971 (*P* = .4197; Figure 4). Both calculation methods showed good correlations with directly measured LDL-C values.

Based on the National Cholesterol Education Program (NCEP) guidelines for different LDL cutoff points, the correlation between DLDL-C and CLDL-C was reasonable (*R* = 0.844) for LDL below 2.85 mmol/L and improved (*R* = 0.980) for concentrations above 2.85 mmol/L, with improvement in agreement from 71.4% to 81.1%, respectively (Table 1).

The correlation and agreement between DLDL-C and CLDL-C in adolescents were moderate for LDL-C below 2.85 mmol/L (*R* = 0.806; 84.1%) and improved for value above 2.85 mmol/L (*R* = 0.978; 91.5%). We also observed that the correlation and agreement between DLDL-C and CLDL-C decreased for values below 2.0 mmol/L (*R* = 0.659; 83.7%).

However, they were better than the values observed in children (Table 2). The results for all groups are summarized in Table 3.

Good correlations were observed between DLDL-C and CLDL-C in children at normal (<0.85 mmol/L), borderline (0.85-1.12 mmol/L),
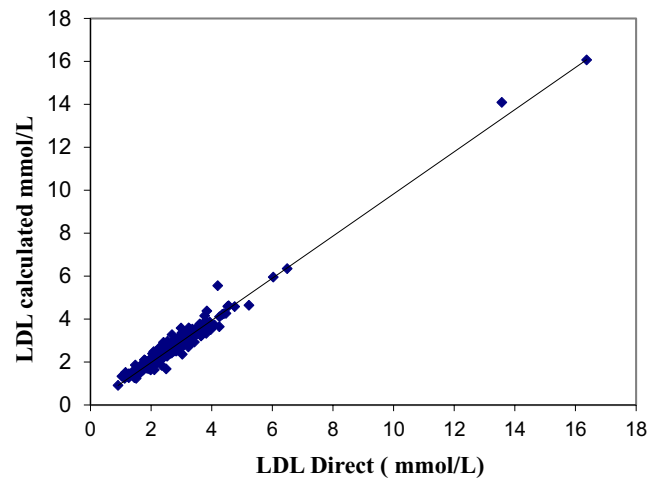
FIGURE 2 Direct LDL-C vs calculated LDL-C in adolescents (>12 y). Correlation of direct LDL-C and calculated LDL-C. Y = 0.9814x + 0.0166, R^2 = 0.9703, *P* < .05

FIGURE 3 Differences (%) of direct LDL-C vs calculated LDL-C. The percentage of differences between direct and calculated LDL-C in children (A) and in adolescent (B)
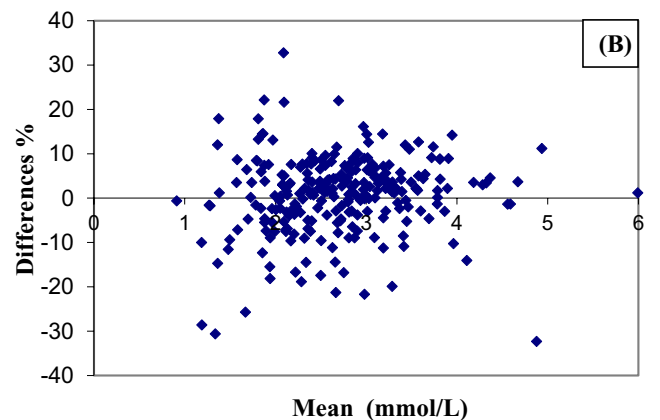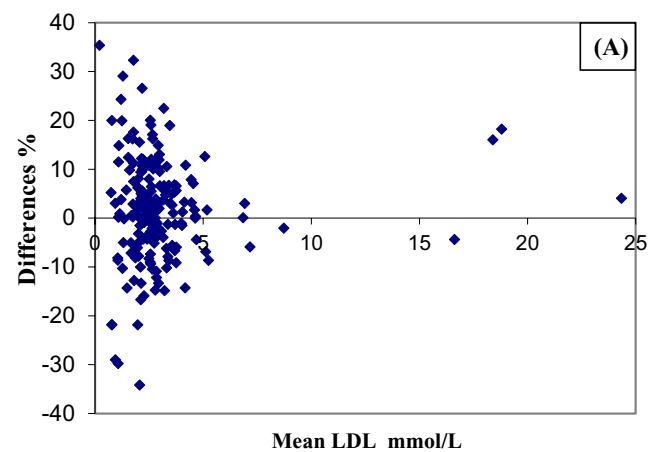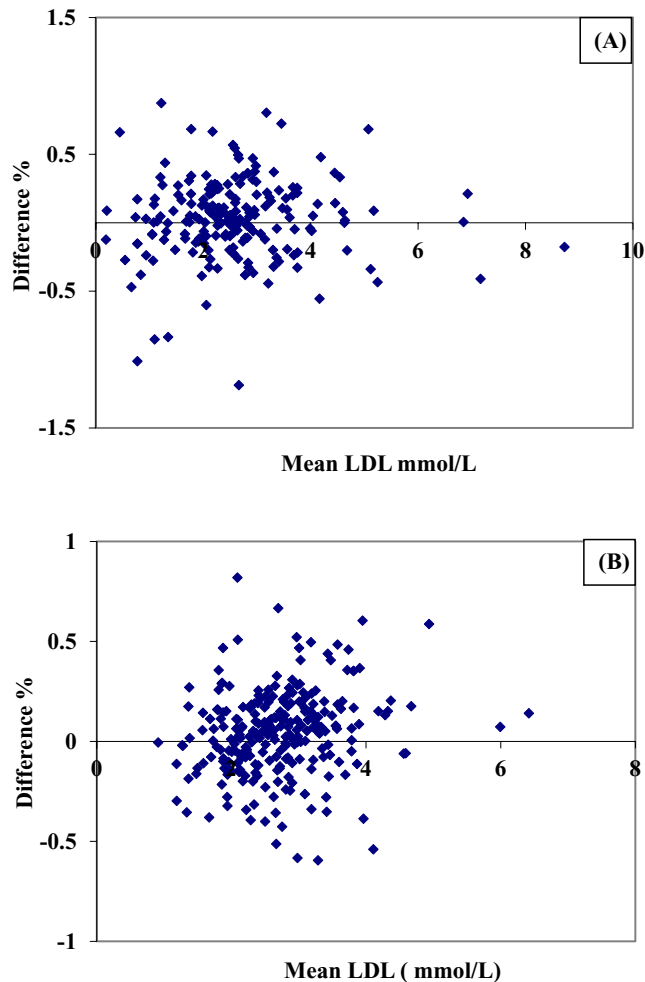
FIGURE 1 LDL-C direct vs LDL-C calculated in children (<12 y). Correlation of direct LDL-C and calculated LDL-C. Y = 0.9101x + 0.199, R^2 = 0.978, *P* = .148

and abnormal (≥1.13 mmol/L) TG levels (R = 0.978, 0.990, and 0.951, respectively). The rate of agreement was better at normal and borderline TG levels (80.5%) and (82.9%), respectively, than at abnormal levels (68.2%; Table 4).

Despite the good correlation (>0.95), a large positive bias (27%) between DLDL and CLDL at abnormally high TG levels was observed in children, indicating that around 27% of CLDL values were

underestimated. A positive bias was also observed in adolescents with a low rate of agreement (61.2%) in which 28% of CLDL values were underestimated (Table 5).

Similarly, there were good correlations between DLDL-C and CLDL-C in adolescents at normal (<1.02 mmol/L), borderline (1.02-1.46 mmol/L), and abnormal (≥1.47 mmol/L) TG levels (R = 0.927, 0.921, and 0.965, respectively). The two tests agreed well (93.5%) at normal TG levels below 1.02 mmol/L, but not above (75.5%; Table 5).

4 | DISCUSSION

This study investigated the accuracy of DLDL-C using direct color enzymatic assay on an Architect analyser system from Abbott. We compared DLDL-C to CLDL-C using the Friedewald formula on specimens obtained from children and adolescents visiting the pediatric and primary clinics in Riyadh, Saudi Arabia.

Our results are acceptable overall correlation values and rates of agreement between measured and calculated LDL-C concentrations in Saudi children and adolescents. Agreement in results was observed for 381 (82.1%) children and adolescents based on a 12% difference as the cutoff between DLDL-C and CLDL-C.

Comparison of the accuracy of the DLDL-C assay in the present study with that reported by Nauck and Rifai in evaluating the analytical and clinical performance of two homogeneous LDL-C assays from Roche and the Friedewald formula showed high correlation with the ultracentrifugation-dextran sulfate-Mg^{2+} method.[5,6] The study examined two homogeneous LDL-C assays, N-geneous (Genzyme Corp.) and Roche (Roche Diagnostics), to directly measure LDL-C. The authors concluded that these assays correlated highly with the ultracentrifugation-dextran sulfate-Mg^{2+} method. In contrast, some authors have demonstrated the limited utility of DLDL-C assays in children.[10,11] Nauck and Nader reported that the discrepancy in serum LDL-C levels might be due to differences in methods used to determine serum LDL-C levels in each study.[6] This explanation can also be applied to our method, as we used a color enzymatic method on a different analyzer from Abbott.

FIGURE 4 Correlation of DLDL-C with CLDL-C in childeren (A) and adolescent (B) using scatter plot

LDL mmol/L	R		Agreement
<1.0 n = 14	0.367	9 above – 12% 3 above + 12%	14.3%
<2.85 n = 133	0.844	20 above – 12% (overestimation) 18 above + 12% (underestimation)	71.4%
>2.85 n = 74	0.980	2 above – 12% 12 above + 12%	81.1%
<3.37			72.1%
>=3.37 n = 42	0.980	1 above – 12% 5 above + 12%	85.7%
All n = 207	0.978	22 above – 12% 31 above + 12%	74.4%

TABLE 1 Comparison of DLDL-C and CLDL-C at different LDL cutoff points using NCEP guidelines in children (<12 y, n = 207)

TABLE 2 Comparison of DLDL-C and CLDL-C using NCEP guidelines in adolescents (12-17 y, n = 257)

LDL mmol/L	R		Agreement
<2.0 n = 43	0.659	5 above − 12% 2 above + 12%	83.7%
<2.85 n = 151	0.806	15 above − 12% (overestimation) 9 above + 12% (underestimation)	84.1%
>2.85 n = 106	0.978	2 above − 12% 7 above + 12%	91.5%
<3.37			87.2%
>=3.37 n = 47	0.976	2 above − 12% 3 above + 12%	89.4%
All n = 257	0.972	20 above − 12% 14 above + 12%	86.8%

TABLE 3 Comparison of DLDL-C and CLDL-C value in children and adolescents groups

	n	DLDL-C (mean ± SD)	CLDL-C (mean ± SD)	P value
Children	206	2.97 ± 2.77	2.89 ± 2.56	.387
Adolescents	258	2.83 ± 2.79	2.79 ± 1.35	.385

TABLE 4 DLDL-C vs CLDL-C at different triglyceride cutoff points in children

At Triglycerides mmol/L	R		Agreement
<0.85 n = 87	0.978	14 above − 12% 3 above + 12%	80.5%
0.85-1.12 n = 35	0.990	4 above − 12% 2 above + 12%	82.9%
≥1.13 n = 85	0.951	4 above − 12% 23 above + 12%	68.2%
Overall n = 207	0.978	22 above − 12% 26 above + 12%	76.8%

TABLE 5 DLDL-C vs CLDL-C at different Triglycerides levels in adolescents

Triglycerides mmol/L	R		Agreement
<1.02 n = 153	0.927	9 above − 12% 1 above + 12%	93.5%
1.02-1.46 n = 53	0.921	4 above − 12% 2 above + 12%	88.7%
>1.02 n = 102	0.959	9 above − 12% 16 above + 12%	75.5
≥1.47 n = 49	0.965	5 above − 12% 14 above + 12%	61.2%
Overall n = 257	0.972	18 above − 12% 16 above + 12%	86.8%

The DLDL-C and Friedewald methods were comparable to the beta quantification ultracentrifugation-precipitation reference method for appropriately classifying patients into the treatment groups established by the NCEP at a cutoff of 3.37 mmol/L.[10,12] The reported rates of agreement below and above of this cutoff were 55% and 83%, respectively, for DLDL-C and 82% and 67%, respectively, for CLDL-C. However, these results did not differentiate between children and adolescents.[10,12] Our data showed rates of agreement between DLDL-C and CLDL-C of 72.1% and 85.7% for children below and above this cutoff (3.37 mmol/L), respectively, and 87.2% and 89.4% below and above the cutoff, respectively, for adolescents. Moreover, the rate of agreement between these two methods improved in the adolescent population below and above this cutoff.

This finding is in contrast to that in a study by Tighe et al, which reported significantly different CLDL-C and DLDL-C concentrations (4.26 ± 0.88 vs 4.83 ± 1.06 mmol/L, respectively, P < .0001), in which 93% of DLDL-C measurements exceeded those by CLDL-C.[13] However, the population in that study was ambulatory adults which differed from the population in the present study. In addition, the means of both DLDL-C and CLDL-C in their study were higher than those in the present study. This may be attributed to differences in subject selection. The subjects in the study by Tighe et al were part of the Worcester-Area Trial for Counselling in Hyperlipidaemia II (WATCH II), a randomized study designed to implement and evaluate the effects of a systems-based nutrition intervention program in primary care patients with LDL-C concentrations in the upper quartile of the LDL-C distribution.[13]

Li et al earlier showed significantly overestimated uncorrected serum LDL-C by a mean of 4.1%, 8.5%, and 21.4%, respectively, for serum lipoprotein[a] concentrations below or equal to 300, 301-600, or above 600 mg/L, respectively.[14] This finding led the authors to conclude that the Friedewald formula might be modified by subtracting lipoprotein[a] - cholesterol to obtain a "true" LDL-C estimation.[14]

Can et al reported a significant correlation between LDL-C estimated using the Friedewald formula and the direct assay but noted a negative bias. Therefore, they concluded that this assay should be used cautiously as a surrogate for the Friedewald formula as these assays have not been standardized in large populations.[15] Another explanation for this bias is the findings reported by Bayer et al, who concluded that the bias in many direct LDL-C methods was associated with the VLDL-C/TG ratio, indicating that cholesterol enrichment of VLDL was an important source of bias.[8]

When TG levels were taken into consideration using the NCEP guidelines and cutoff, Tanno et al found high correlations between DLDL and CLDL-C in both fasting (R = 0.971) and non-fasting (R = 0.955) samples. They also reported 84.8% and 80.1% in concordance with NCEP categories for fasting for non-fasting samples, respectively.[10] However, the bias between the two measurements increased in samples with TG concentrations above 1.69 mmol/L, especially in non-fasting samples.[10] These data are in agreement with our observations of overall fasting correlations of (R = 0.978) and (R = 0.971) in children and adolescents, with 74.4% and 86.8% agreement, respectively. We also observed decreased agreement and correlation with increasing TG concentrations in the fasting population. This finding was also noted by Teerakanchana et al, who observed a good correlation between DLDL-C and CLDL-C and concluded that the Friedewald formula did not perform homogeneously when estimating LDL-C levels in samples with different TG levels.[9,16]

Our findings correlate with those reported by Ahmadi et al,[17] in which CLDL-C was significantly overestimated for TG < 1.13 mmol/L and no significant difference was observed between DLDL-C and CLDL-C for TG between 1.69 and 3.95 mmol/L. We observed CLDL-C overestimation and underestimation in children with TG < 0.85 and >1.13 mmol/L, respectively. Similar findings were observed in adolescents.

The limitations of this study are as follows. The Friedewald equation did not consider differences in race and specific characteristics, which were not addressed in this population. Additionally, this study did not perform additional comparisons using beta quantification as a reference method.

In conclusion, we found good agreement between DLDL-C and CLDL-C in both children and adolescents. The LDL-C levels were better estimated in fasting patient serum samples using the Friedewald formula. The percent difference in f LDL-C can also be utilized as a quality indicator to check laboratory analyzer performance in healthy subjects.

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