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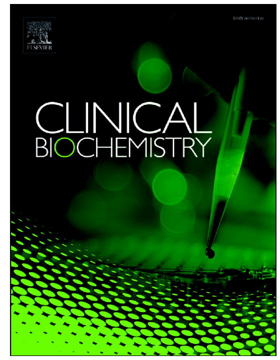

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Validation of the Friedewald formula for the estimation of low density lipoprotein cholesterol in a sub-Saharan African population

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Abstract

Background

Low density lipoprotein cholesterol (LDL-C) levels are used to estimate cardiovascular disease (CVD) risk and to guide prescriptions. To circumvent the challenges of direct LDL-C measurement, guidelines recommend the use of Friedewald formula derived LDL-C levels. Despite reported limitations of this formula, its validity in sub-Saharan Africans has not been adequately investigated

Objective

To assess the validity of the Friedewald formula derived against directly (homogeneous) measured LDL-C in adult Cameroonians.

Methods

We reviewed the fasting lipid profiles of 2500 patients, performed between March 2012 and January 2016 using enzymatic colorimetric method (reference), at the Douala General Hospital laboratory. The Friedewald formula was used to calculate LDL-C from total cholesterol, high density lipoprotein cholesterol and triglyceride levels. Calculated LDL-C values were compared to the reference values, and clinical significance of differences between the two methods was assessed using total error allowable (TEa).

Results

The difference between means of calculated and the reference LDL-C values was neither statistically nor clinically significant (3.33±1.51 vs. 3.33±1.25 mmol/l; p=0.704). The calculated LDL-C correlated positively with the measured LDL-C value (r=0.749) and both methods showed a good agreement on Bland-Altman plot. Conversely, there was only moderate agreement (kappa=0.478, 95% CI: 0.455-0.502) between the two values in the stratification of cardiovascular risk according to the National Cholesterol Education Program/Adult Treatment Panel III. Consequently, 40.6% of the participants were misclassified.

Conclusion

Friedewald formula is technically accurate but has a modest clinical accuracy which can translate into a substantial misclassification of patients' cardiovascular risk and subsequent inappropriate therapeutic decisions.

Keywords: Low density lipoprotein cholesterol; Friedewald formula; direct homogenous assay; agreement; cardiovascular risk; sub-Saharan Africa; Cameroon

Introduction

Cardiovascular diseases (CVD) continue to be a serious problem worldwide [1]. Cameroon like many other African countries is experiencing the epidemiological transition characterized by increasing CVD-related mortality [2]. Observational and interventional studies have established a causal relationship between low density lipoprotein cholesterol (LDL-C) level and atherosclerotic CVD [3]. LDL-C level, as calculated by the Friedewald formula (FF) in routine patient care, has a pivotal role in CVD risk estimation and reduction across clinical practice guidelines worldwide [4–6].

According to the FF, LDL-C level can be estimated from the difference between total cholesterol (TC) and the cholesterol content of other lipoprotein particles, namely high density lipoprotein (HDL-C) and very low density lipoprotein (VLDL-C), through the equation LDL-C (mmol/l) = TC - HDL-C –[Triglycerides (TG)/2.17], where TG/2.17 is an estimate of serum VLDL-C concentration [7]. This formula was introduced into clinical practice over four decades ago; because ultracentrifugation to directly measure LDL-C was time consuming, costly, and unavailable for routine clinical practice. Friedewald and colleagues however, recognized that the term TG/2.17 could not accurately estimate VLDL-C especially at triglycerides values> 4.52 mmol/l. Such inaccuracy could be tolerated because serum VLDL-C concentration is small relative to LDL-C concentration, but with the epidemics of other cardiovascular risk factors [8], such an assumption could jeopardize the standard of care offered to patients. Attempting to redress these problems, the expert panel of National Cholesterol Education Program in 1995 recommended the development of direct homogenous assay for precise and accurate measurement of LDL-C [9]. However, the direct homogenous assay method remains unavailable and expensive for patients, especially in low income countries [6]. Furthermore, studies have shown that FF can underestimate LDL-C values when compared to the ultracentrifugation [10] or to the direct homogeneous assay [11], or to overestimate it [12]. All these may lead either to failure to give medical attention to a deserving patient, or to needless and expensive polypharmacy, respectively. In the sub-Saharan African population where CVD is now a major public health concern [13] the formula has remained in routine use with little scrutiny. Besides, studies have found differences in metabolism of lipids between Caucasians and Africans [14,15]. Despite the above, only studies with small sample size have attempted to validate the FF (which was established based on fasting lipid profiles of 448 Caucasians) [7] in Africans.

In this study, we have used a larger sample to assess the validity of the Friedewald-calculated against the measured (by direct homogeneous assay) LDL-C in adult Cameroonians, by comparing the absolute mean values, assessing the continuous association, determining the level of agreement between estimated and measured LDL-C, and finally assessing the clinical significance of differences between estimated and measured LDL-C in clinical decision making.

Methods

Study design and setting

In this study, we reviewed the records of fasting lipid profiles performed at the laboratory of Douala General Hospital (DGH). DGH is a reference healthcare teaching hospital located in the Littoral Region of Cameroon. The laboratory undergoes annual external and internal quality control and was accredited in 2012 (accreditation N^{o} ISO 15189-2012). Since March 2012, the DGH laboratory has been systematically measuring LDL-C directly on a Roche-Hitachi Cobas C311® analyzer (Roche Diagnostics GmbH, Mannheim, Germany; Hitachi

High-Technology Corporation, Tokyo, Japan) using a colorimetric autoanalyzer kit which is Centers for Disease Control and Prevention (CDC)-certified, accurate and precise for lipid analysis. The same enzymatic colorimetric method were being used for total cholesterol, HDL cholesterol and triglycerides.

Data collection

We studied the fasting lipid profile records of patients managed at the Douala General Hospital from March 2012 to January 2016. All consecutive lipid profiles of patients aged 18 years and above performed during the study period were included. Each patient's record contained measured serum concentrations of each parameter of the lipid profile. Records were excluded if demographic data (age and gender) were missing, the lipid profile was incomplete, or the TG level was > 4.52 mmol/l. Individual LDL-C levels were then calculated using the Friedewald formula. Hypercholesterolemia was defined as serum total cholesterol > 5.0 mmol/L, and hypertriglyceridemia as serum triglycerides level > 1.70mmol/l.

Statistical analysis

Data were analyzed using the R statistical software version 3.2.2 (The R Foundation for Statistical Computing, Vienna, Austria). Variables were summarized as mean and standard deviation, median and 25th-75th percentiles, and count and percentages. The Shapiro-Wilk W test was used to determine whether the LDL-C values were normally distributed based on probability threshold of $p > 0.1$. Skewness was assessed with the D'agostino test [16] and Kurtosis with the Anscombe-Glynn test [17].

Analysis of the variance, Kruskal-Wallis test, and chi square test were used to compare characteristics across gender. Measured LDL-C served as the reference for all comparisons.

Paired-sample t-test and Wilcoxon test were used to compare differences in means of measured and calculated LDL-C concentrations overall and within subgroups.

The continuous association between measured and calculated LDL-C was assessed using the Pearson and Spearman correlation tests. Linear regression models were used to derive the regression coefficients, which helped us to predict the reference (measured) values from the calculated LDL-C values. Adjusted coefficient of determination (adjusted R-squared) was calculated to assess the performance of models. Assessment of systematic bias was judged using Bland and Altman plots [18] implemented with the use of 'Research Methods' package of R. Agreement in stratifying cardiovascular risk was assessed using Kappa statistics [19] with 95% confidence interval (95%CI) derived from bootstrap percentile methods, based on 2000 replications. We used the NCEP/ATP III, 2002, cut off points for cardiovascular risk stratification to compare the level of agreement between the two methods in categorizing participants in various risk groups.

To gauge the clinical importance of statistically significant differences between measured and estimated LDL-C, we used the total allowable error (TEa) [20] which was based on within- and between-subject variations. The mean of the calculated LDL-C was then compared with the mean of measured LDL-c. The former had to fall within clinical range of reference mean ± TEa. To get the TEa, we calculated the percentage difference as: 100*[(Calculated–Measured)/Measured LDL-C] and multiplied it by the mean of the calculated LDL-C. If the mean of the calculated was out of the range (reference mean ± TEa), the difference was considered clinically significant, which means that it could cause potentially harmful clinical decisions.

Ethical approval

This study was approved by the Institutional Ethics Committee for Research on Human Health of the University of Douala (N°IEC-UD/447/02/2016/T). Administrative clearance was obtained from the authorities of the DGH. Confidentiality, anonymity and privacy of all records were guaranteed at all levels of this study by using only specific codes.

Results

Characteristics of participants and lipid profiles

Of the 2500 records included, 1254 (50.2%) were from men. The mean age of the participants was 54.1 years. Mean values of lipid profile parameters and comparison between men and women is shown in Table 1. In all 58.2% of the sample had hypercholesterolemia while 16.5% had hypertriglyceridemia, with prevalence higher in women than in men for hypercholesterolemia ($p<0.001$), but the opposite for hypertriglyceridemia ($p<0.001$), Table 1.

Table 1: General characteristics of participants overall and by gender

Characteristics	Overall	Men	Women	p-value*
N (%)	2501 (100)	1254 (50.2)	1246 (49.8)	
Age, years	54.1 (12.6)	53.7 (12.3)	54.5 (12.9)	0.104
Total cholesterol, mmol/l	5.40 (1.49)	5.21 (1.57)	5.58 (1.38)	<0.001
HDL-C, mmol/l	1.45 (0.90)	1.37 (0.94)	1.61 (0.86)	<0.001
Triglycerides, mmol/l	1.24 (0.81)	1.33 (0.91)	1.15 (0.68)	<0.001
Total cholesterol >5.0 mmol/l, %	58.2	52.5	64.0	<0.001
Triglycerides >1.70 mmol/l, %	16.5	19.4	13.6	<0.001

*P-value for comparison between men and women.

Data are presented as mean (standard deviation), unless stated otherwise; HDL-C: high density lipoprotein-cholesterol.

Comparison of measured versus calculated LDL-C

Figure 1 shows a leptokurtic distribution of measured and calculated LDL-C. Measured LDL-C curve overlapped with estimated LDL-C curve, suggesting similar variability of LDL-C values from the mean for both methods. This was similar within genders. The non normal distribution was confirmed by the Shapiro Wilk test p-values < 0.0001 overall and within genders (Table 2). The difference between means of calculated and measured LDL-C values was not statistically significant (Table 2).

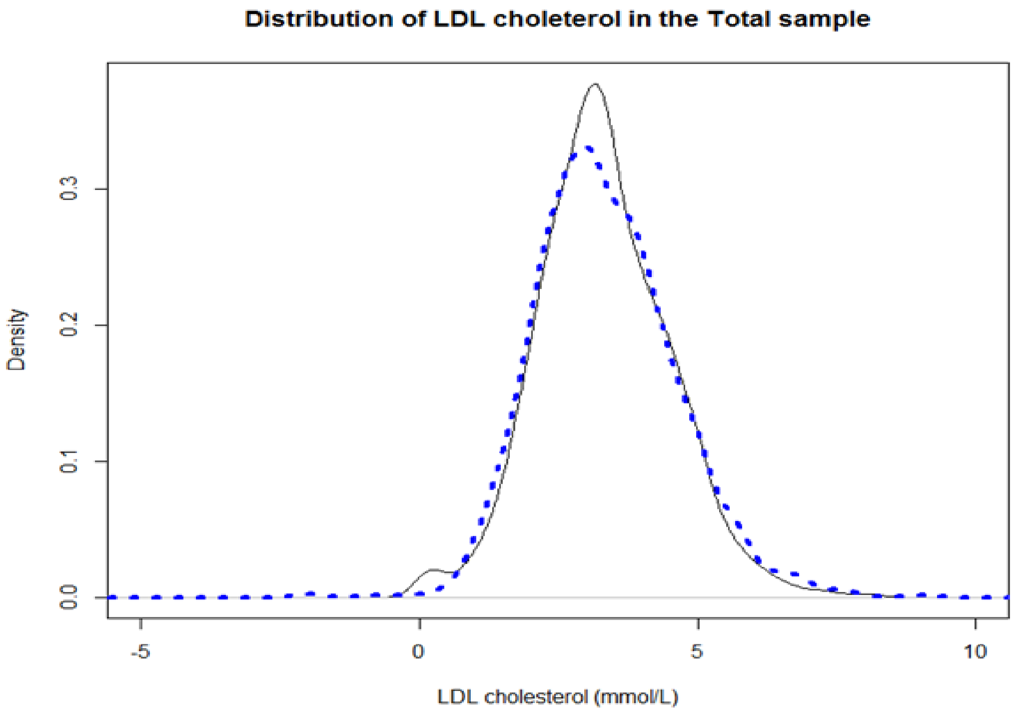

Figure 1: **Distribution curves for Measured LDL-C and calculated LDL-C for the whole study population.** Measured LDL-C is represented by solid black line, and calculated-LDL-C is represented by broken blue line.

Table 2: Mean difference and correlation between measured and estimated LDL-C

Characteristics	Overall	Men	Women	p-value*
Measured LDL-C (mmol/L)				
Mean (SD)	3.33 (1.25)	3.25 (1.35)	3.41 (1.15)	0.001
Shapiro p	<0.0001	<0.0001	<0.0001	
Coefficient of variation (%)	37.7	41.5	33.6	
Calculated LDL-C (mmol/L)				
Mean (SD)	3.33 (1.51)	3.23 (1.60)	3.44 (1.40)	
Shapiro p	<0.0001	<0.0001	<0.0001	
Coefficient of variation (%)	45.3	49.5	40.9	
Measured – Calculated LDL-C (mmol/L)				
Mean (95%CI)	-0.008 [-0.047-0.032]	0.014 [-0.044-0.072]	-0.029 (-0.083-0.025)	
Paired t-test**	0.704	0.646	0.288	
Correlation coefficient				
Pearson (95% CI)	0.749 (0.731-0.765)	0.760 (0.735-0.782)	0.731 (0.704-0.756)	
Spearman	0.848	0.846	0.845	

LDL-C: Low density lipoprotein cholesterol, CI: Confidence interval, *p-value men *vs.* women; **p-value measured *vs.* estimated LDL-C

Assessment of the association between measured and estimated LDL-C

We found a positive correlation between estimated and measured LDL-C values in the overall sample and within genders (Table 2 and Figure 2).The linear regression equation linking the calculated to the measured LDL-C values in the overall sample, men and women were respectively: calculated LDL-C = 0.901*measured LDL-C + 0.337, calculated LDL-C = 0.901*measured LDL-C + 0.307, and calculated LDL-C= 0.897*measured LDL-C + 0.379 with respective adjusted R^2 of: 0.560, 0.577, and 0.534.

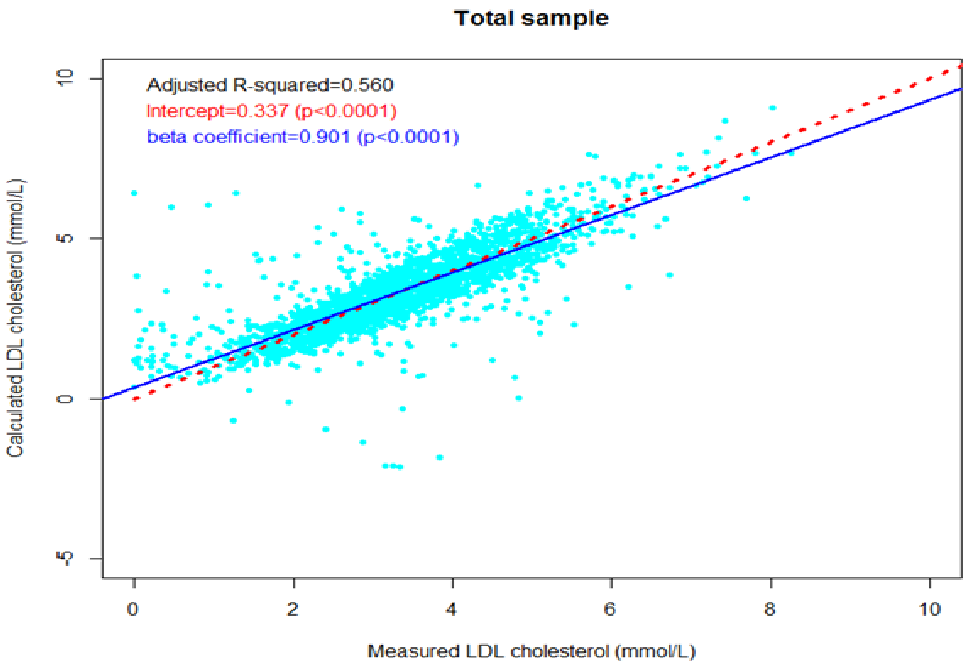

Figure 2: **Linear regression curves showing the continuous association of measured with calculated LDL-C for the whole study population.**

The dotted diagonal line is the line of perfect agreement, and the blue line is the regression line between calculated and measured LDL-C in our study popualtion. Adjusted R-squared is the adjusted coefficient of determination.

Bland and Altman plots of differences between measured and estimated LDL-C values plotted on the y-axis and the mean of these values on the x-axis were used to assess systematic bias in the overall sample. Most of the plotted points lied around the line of perfect agreement (light dotted blue line through zero). The solid green line which is the difference between the two methods (mean bias), overlaps with the line of perfect agreement. This was also true in the two subgroups, suggesting a good technical agreement between the two methods. Nonetheless, there were multiple outliers in negative and positive regions of the graph signifying probable discordance between the two methods at extreme LDL-C values (Figure 3).

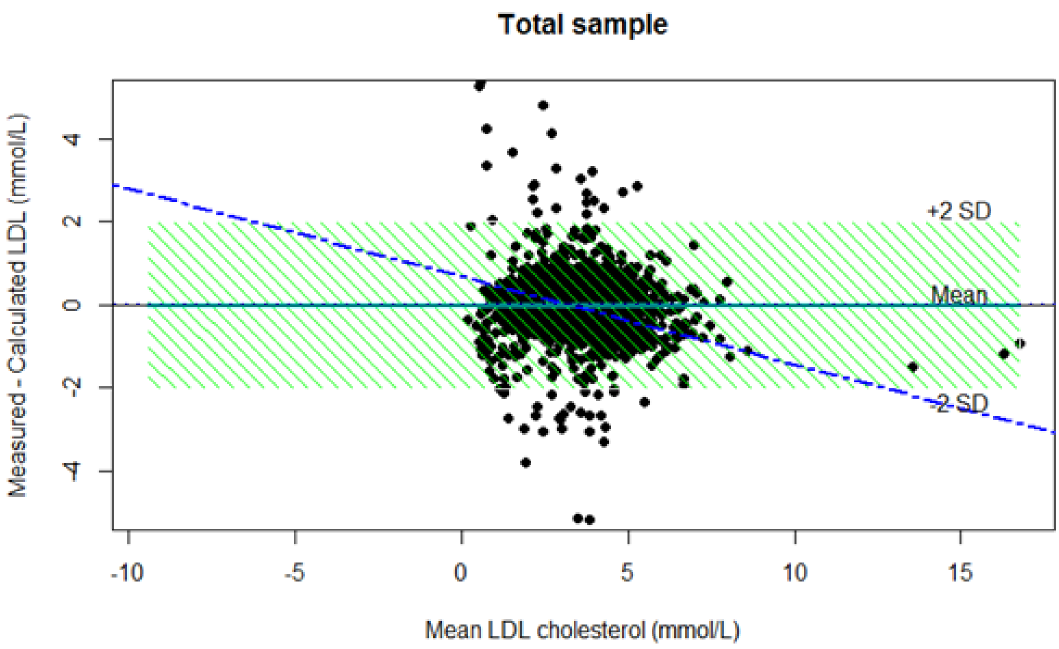

Figure 3: **Bland-Altman plot of agreement between estimated and measured LDL-C for the overall sample.**

SD: standard deviation. +2SD and -2SD are the upper and lower limits of agreement. The solid green line is the difference between the 2 methods (mean bias); the lighter dotted blue line through zero is the line used to assess the discrepancy of the observed mean difference (it is a line of perfect agreement between the two measurements), and the shaded zone represent limits of agreement (within 2 SD). The linear curve of best fit is also shown (broken superimposed curve).

Table 3: Agreement between estimated and measured LDL-C in classifying patient's cardiovascular risk categories (NCEP/ATPIII), overall and by gender

	Categories of measured LDL					Kappa
Categories of Calculated LDL-C	**<2.58**	**]2.58 to 3.35]**	**]3.35 to 4.11]**	**]4.11 to 4.88]**	**>4.88**	
Overall population						0.478 (95%CI: 0.455-0.502)
<2.58	528 (79.6)					
]2.58 to 3.35]		377 (54.4)				
]3.35 to 4.11]			254 (47.2)			
]4.11 to 4.88]				159 (44.2)		
>4.88					167 (67.9)	
Men						0.478 (95%CI: 0.443-0.511)
<2.58	304 (80.4)					
]2.58 to 3.35]		168 (51.1)				
]3.35 to 4.11]			126 (47.9)			
]4.11 to 4.88]				75 (44.6)		
>4.88					78 (67.2)	
Women						0.475 (95%CI: 0.443 to 0.509)
<2.58	224 (78.6)					
]2.58 to 3.35]		209 (57.4)				
]3.35 to 4.11]			128 (46.5)			
]4.11 to 4.88]				84 (43.7)		
>4.88					89 (68.5)	
Normotriglyceridemia						0.472 (95%CI: 0.444 to 0.500)
<2.58	433 (78.4)					
]2.58 to 3.35]		344 (56.0)				
]3.35 to 4.11]			219 (47.6)			
]4.11 to 4.88]				121 (42.2)		
>4.88					119 (68.4)	
Hypertriglyceridemia						0.497 (95%CI: 0.438 to 0.555)
<2.58	95 (85.6)					
]2.58 to 3.35]		33 (41.8)				
]3.35 to 4.11]			35 (44.9)			
]4.11 to 4.88]				38 (52.0)		
>4.88					48 (66.7)	

Data are presented as counts (percentage)

Overall the level of agreement between the two measurements in cardiovascular risk stratification was only moderate; kappa (95% CI) was 0.478 (0.455-0.502) and similar in men and women. As a consequence, up to 1015 participants (40.6%) were misclassified by calculated LDL-C, with about half of them (20.9%) misclassified into higher risk group and 19.7 % into lower risk group compared to measured LDL-C. The observed agreement between estimated and measured LDL-C was high at extreme LDL-C values. 79.6% for LDL-C <2.58 mmol/l and 67.9 % for LDL-C >4.88 mmol/l (Table 3). Between these levels, the level of agreement decreased as the LDL-C level increases (Table 3). By status for hypertriglyceridemia, the agreement statistic was kappa 0.472 (95%CI 0.444-0.500) for participants with normotriglyceridemia, and 0.497 (0.438-0.555) among those with hypertriglyceridemia. When participants were grouped by quarters of total cholesterol, the agreement was 0.392 (0.309 to 0.471) in the bottom quarter (TC<4.44 mmol/l) and 0.282 (0.226 to 0.340) in the top quarter (TC≥6.28 mmol/l).

Using the TEa, the difference between the measured and the calculated LDL-C values was not clinically significant, either in the overall population or in the two genders (Table 4).

Table 4: Clinical significance based on total error allowable

Subgroup	**measurement**	**N**	**Mean (SD)**	**SD**	**% difference**	**Statistical significance**	**TE %**	**Mean*TE%**	**Allowable range**		**Clinical significance**
									min	max	
Overall	Measured	2501	3.33	1.25	0	0.704	11.9	0.40	2.90	3.70	Not significant
	Calculated	2501	3.33	1.51	0.2						
Men	Measured	1254	3.25	1.35	0	0.646	11.9	0.39	2.90	3.60	Not significant
	Calculated	1254	3.23	1.60	-0.4						
Women	Measured	1246	3.41	1.15	0	0.288	11.9	0.41	3.00	3.80	Not significant
	Calculated	1246	3.44	1.41	0.9						

TE: Total error

Discussion

Worldwide guidelines recommend Friedewald-estimated LDL-C for cardiovascular risk assessment and therapeutic target [4–6]. In this study, we found that the mean difference between Friedewald-estimated and measured LDL-C was neither statistically nor clinically significant. There was a positive association between the two methods and they also displayed good agreement on Bland-Altman plot. Nonetheless, the two methods showed only moderate agreement in cardiovascular risk stratification according to the NCEP-ATPIII.

Many similar studies have been carried out on this subject, mostly in developed countries. In the current study, we showed that the mean difference between estimated and measured LDL-C was not significant, regardless of the gender. Our finding are congruent with those of few other studies [21,22]. Nevertheless, many studies have shown significant differences in the mean values of Friedewald-estimated and measured LDL-C [11,23–26]. The accuracy of the result obtained by the FF is dependent on a number of factors, namely 9-12 hours fasting prerequisite, analysis of TC, HDL-C and TG as well as the disease status of an individual. Thus, due respect of these prerequisite may explain the differences observed.

We found a strong correlation of 0.749 between the two methods. Many studies have also shown a strong correlation between estimated and measured LDL-C [27]. Even with the strong positive correlation, the actual test of technical accuracy applicable was the Bland-Altman plots which showed a good agreement between the two methods. On the contrary, most of the above mentioned studies have shown that Friedewald-estimated LDL-C underestimates or overestimates cardiovascular risk, which was displayed by positive or negative mean bias on Bland-Altman plot respectively. The difference between those studies and ours can be explained by differences in socio-demographic background, study setting, study design and even sample size. Whether the type of food eaten by our participants could have been the reason behind our differences as noted by Fukuyama *et al.* [27] in Japan could not be ascertained in this study.

While it is generally unlikely that different methods will exactly agree, the question should be whether the magnitude of any bias affects clinical judgment. Correctly estimating patients' LDL-C is invaluable as reporting a wrong value can convey a wrong message about cardiovascular risk leading to inappropriate treatment. The NCEP/ATP III cut-off concentrations are important parameters in therapeutic decisions. When we used these cut-off points to stratify participants' cardiovascular disease risk, we found that overall, the level of agreement between the two methods was only moderate (kappa=0.478), with a consequent misclassification in 40.6% patients by estimated LDL-C. This implies that estimated LDL-C in our population may overestimate or underestimate about two out of every five patient's cardiovascular risk. It should however be noted that with the advent of the 2013 American College of Cardiology/American Heart Association guidelines that are more focused on risk groups rather than multiple LDL-C categories [28], the problem of misclassification is currently of very limited interest.

Our findings are however likely to be of greater relevance because patients managed in a reference hospital usually have other cardiovascular risk factors, hence overestimating their risk of CVD may leads to polypharmacy which may further complicate their pre-existing condition. On the contrary, underestimating their risk may undermine, and sometime would deny medical attention to the deserving patients in our population. This is especially important in our population where the rising trend of other cardiovascular comorbidities such as hypertension and diabetes is already established [29].

We acknowledge the following limitations that should be considered when generalizing the results. Firstly, FF was proposed to be used for epidemiological studies and not for diagnosis or following-up of CVD patients as in our study. However, worldwide recommendations have prescribed FF to be used for such purposes [4–6]. Secondly, our study examined a single measurement of LDL-C, which is the common practice in clinical decision-making, however guidelines also advocate serial measurements to establish greater accuracy or assess changes in serum LDL-C levels following intervention [4,6]. Other potential limitations pertain to confounders that may influence the calculated or the directly-measured LDL-C. For instance, calculated LDL-C may be influenced by HDL-C measurement errors or by elevated Lipoprotein (a), whereas direct homogeneous LDL-C measurement may have been influenced by errors in samples from dyslipidemic patients or from diseased patients in our study population.

Conclusions

Compared to the direct homogeneous measurement of LDL-C, the Friedewald formula is technically accurate but its clinical accuracy is modest; as a consequence, Friedewald-estimated LDL-C may misclassify cardiovascular risk of two out of every five patients. This conveys a potential wrong clinical and epidemiological decisions, in terms of individual CVD risk stratification and therapeutic decisions. Thus, with the current trend of cardiovascular disease in our setting, there is need to use Friedewald-estimated LDL-C with caution especially when accuracy matters most.

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Authors' contributions

SPC: study conception and design, data collection and interpretation, draft and review of the manuscript

TM: study conception and design, data collection and interpretation, draft of the manuscript

JPD: data collection and review of the manuscript

CAD: data analysis and review of manuscript

YMD: data analysis and drafting of manuscript

ES: study conception, review of the manuscript

APK: study design, data analysis and interpretation, review of the manuscript

All authors made significant intellectual contributions and have read, reviewed, and approved the final manuscript.

Competing interest

The authors declare no competing interest relevant to this article.

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Highlights

- Friedewald formula accurately estimates LDL cholesterol in Cameroonians
- Friedewald-calculated LDL cholesterol correlates well with measured LDL cholesterol
- Using Friedewald formula may however misclassify the CV risk of 40% of patients