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Factors Causing Disagreement between Measured and Calculated Low Density Lipoprotein-Cholesterol (LDL-C) in Clinical Laboratory Services

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
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Literature Search F
Funds Collection G

ABCDEF **Veeravan Lekskulchai**

Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok, Thailand

Corresponding Author: Veeravan Lekskulchai, e-mail: veeravah@g.swu.ac.th
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Background: Since measured low density lipoprotein-cholesterol (LDL-C) has been available in clinical laboratories, there have been concern about the disagreement between measured and calculated LDL-C and the factors causing their disagreement.

Material/Methods: Serum lipid concentrations were collected from 1,339 medical records of patients admitted to hospital between 2013 and 2015. They were grouped by their total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C) concentrations and the agreement between measured and calculated LDL-C was statistically analyzed.

Results: A strong relationship was found between measured and calculated LDL-C. Significantly disagreements between measured and calculated LDL-C were found in all groups in 2013 and 2014 when lipids were analyzed by Cobas C501. Disagreements found in groups of low TG and low HDL-C concentrations in 2015 were when lipids were analyzed by Abbott Architect ci8200. In groups of calculated LDL-C <1.81 mmol/L, around 80% had the measured LDL-C >1.81 mmol/L. Among various atherogenic indices, non-HDL-C showed the strongest relationship with LDL-C, while TC to HDL-C ratio showed the strongest agreement with the LDL-C.

Conclusions: The disagreement between measured and calculated LDL-C in a clinical laboratory seemed to depend on the analytical system used, and was probably associated with individual laboratory variations.

MeSH Keywords: Cholesterol, LDL • Coronary Disease • Lipoprotein, HDL

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Background

The analysis of serum lipids in a clinical laboratory, as recommended by the Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program (2001) [1], consists of direct measurements of total cholesterol (TC), triglycerides (TG), and high density lipoprotein- cholesterol (HDL-C) along with calculated low-density lipoprotein-cholesterol (LDL-C). LDL-C has been recommended as an important risk factor of coronary heart disease (CHD) and its serum level is necessary for decision-making for prevention strategies for coronary heart disease (CHD) [2]. Due to the limitation in calculations and doubtful precision of calculated LDL-C [3,4], direct LDL-C assays have been developed and are currently performed in most clinical laboratories. There have been studies about the relationship and agreement between measured and calculated LDL-C to help ensure that measured LDL-C could eventually replace calculated LDL-C, however, the agreement between these two values has been found to be inconsistent [5] and the factors associated with these disagreements were found to be diverse [3,5–9].

CHD risk assessment and prevention, based on the ATP III criteria that emphasize LDL-C and other serum lipids are used mainly for calculating LDL-C [1]. However, if direct LDL-C measurement is applied instead, the importance of other serum lipids is reduced. To increase their clinical benefit, other atherogenic indices calculated from these lipid values have been proposed to be more accurate in CHD prevention than TC or TG alone and can be a supportive or even an alternative marker of LDL-C [10–18].

Therefore, the aims of this study were to investigate the relationship between measured and calculated LDL-C in clinical laboratory, to find possible factors causing the agreement between these two values, and to find a potential atherogenic index for supporting LDL-C in preventing coronary heart disease.

Material and Methods

This work was conducted at the HRH Princess Maha Chakri Sirindhorn Medical Center, Nakhon Nayok, Thailand. It was approved by the Srinakharinwirot University Ethics Committee for Human Research (license code: SWUEC-021/60X). Medical records of patients admitted between 2013 and 2015 were randomly accessed and serum lipid concentrations from these records were collected. Calculated LDL-C was estimated using Friedewald formula (cLDL-C). The collected cases were grouped by their TC, TG, and HDL-C concentrations based on the recommended serum lipid cutoff points [2]. Other atherogenic indices, i.e., TC: HDL-C, TG: HDL-C, LDL-C: HDL-C, and non-HDL-C were calculated and interpreted using previously suggested cutoff points [12,16-18]. The agreement between measured and calculated LDL-C was analyzed by paired *t*-test and the correlation was analyzed by linear regression analysis. Statistical analyses were performed using Microsoft Excel 2007 (file version 12.0.6665.5003). Statistical significance was defined when *p*-value was less than 0.05.

Results

Case information

A total of 1,339 records were included in this analyses. The number of records collected in 2013, 2014, and 2015 was 453, 418, and 468, respectively. The means of TC, TG, HDL-C, mLDL-C and cLDL-C are shown in Table 1. On average, mLDL-C was higher than cLDL-C in 2013 and 2014, whereas, mLDL-C was slightly lower than cLDL-C in 2015 (Table 1). High TC, high TG, and low HDL-C was detected in 36%, 30%, and 19% of the patients, respectively (Table 2).

Relationship and agreement between mLDL-C and cLDL-C

A strong positive correlation was found between the measured and calculated LDL-C (Table 3). Statistical analysis revealed mLDL-C was significantly higher than cLDL-C in all groups in

Table 1. Mean of serum lipid concentration of the study patients.

Year	Mean ±SD (mmol/L)				
	TC	TG	HDL-C	mLDL-C	cLDL-C
2013	4.95±1.06	1.85±1.10	1.45±0.41	3.08±0.96	2.77±0.93
2014	4.92±1.06	1.82±1.09	1.45±0.39	3.16±0.98	2.75±0.96
2015	4.92±1.14	1.92±1.34	1.24±0.34	2.90±0.95	2.93±0.93
Total	4.92±1.09	1.86 +1.20	1.37±0.39	3.06±0.96	2.82±0.96

TC – total cholesterol; TG – triglycerides; HDL-C – high density lipoprotein-cholesterol; mLDL-C – measured LDL-C; cLDL-C – calculated LDL-C.

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Table 2. Number of patients with high TC, high TG, and low HDL-C.

Year	Number (%) of patients		
	TC >5.18 mmol/L	TG >1.70 mmol/L	HDL-C <1.04 mmol/L
2013	172 (38%)	138 (30%)	65 (14%)
2014	135 (32%)	118 (28%)	54 (13%)
2015	173 (37%)	140 (30%)	131 (28%)
Total	480 (36%)	396 (30%)	250 (19%)

TC – total cholesterol; TG – triglycerides; HDL-C – high density lipoprotein-cholesterol.

2013 and 2014 (Table 3) when all lipids were analyzed by a Roche Cobas C501 analyzer (Roche diagnostics, Indianapolis, IN, USA). However, mLDL-C was significantly lower than cLDL-C only in groups with low TG and low HDL-C in 2015 when serum lipids were analyzed using an Abbott Architect ci8200 integrated system (Abbott, Abbott Park, IL, USA).

Agreement between mLDL-C and cLDL-C at low LDL-C

In the group with TG >1.70 mmol/L and cLDL-C ≤1.81 mmol/L, around 80% of the patients had mLDL-C >1.81 mmol/L (Table 4).

Relationship and agreement between LDL-C and other atherogenic index

Table 5 shows there was a strong positive correlation between non-HDL-C and mLDL-C. Among studied atherogenic indices, TC: HDL-C gave the highest percentages of agreement with mLDL-C (Table 6).

Discussion

LDL-C concentration is clinically evaluated for decision-making in strategies for prevention of CHD. The recommended cutoff point is based on co-existence of other CHD risk factors and treatment goals [1,2]. In this study, the cutoff points for LDL-C were selected at >3.37 mmol/L and >4.14 mmol/L because most cases had at least one co-existing CHD risk factor; including diabetes (24% of total cases) or hypertension (27% of total cases). Following standard practice, LDL-C cannot be calculated if TG levels are >4.52 mmol/L. In this study, TG >4.52 mmol/L were found in only 2% of the patients; which was similar to that observed in a previous report [19].

The strong positive correlation between measured and calculated LDL-C that was found in this study has been previously reported in other studies [6–9]. Nonetheless, the findings in this study showed that the agreement between these two values was inconsistent. Changing the analytical system from Cobas C501 analyzer to Abbott Architect ci8200 caused

Table 3. Agreement and relationship between mLDL-C and cLDL-C based on TC, TG, and HDL-C concentrations.

Year	mLDL-C *vs.* cLDL-C	
2013	***p***	**r**
TC ≤5.18 mmol/L	<0.05	0.9319
TC >5.18 mmol/L	<0.05	0.9310
TG ≤0.79 mmol/L	<0.05	0.9813
TG=0.80–1.70 mmol/L	<0.05	0.9816
TG=1.71–3.39 mmol/L	<0.05	0.9724
HDL-C ≤1.04 mmol/L	<0.05	0.9666
HDL-C >1.04 mmol/L	<0.05	0.9726
2014	***p***	**r**
TC ≤5.18 mmol/L	<0.05	0.9451
TC >5.18 mmol/L	<0.05	0.9682
TG ≤0.79 mmol/L	<0.05	0.9829
TG=0.80–1.70 mmol/L	<0.05	0.9884
TG=1.71–3.39 mmol/L	<0.05	0.9844
HDL-C ≤1.04 mmol/L	<0.05	0.9736
HDL-C >1.04 mmol/L	<0.05	0.9793
2015	***p***	**r**
TC ≤5.18 mmol/L	0.3863	0.8526
TC >5.18 mmol/L	0.0857	0.8960
TG ≤0.79 mmol/L	<0.05	0.9698
TG=0.80–1.70 mmol/L	<0.05	0.9539
TG=1.71–3.39 mmol/L	0.1460	0.9419
HDL-C ≤1.04 mmol/L	<0.05	0.9599
HDL-C >1.04 mmol/L	0.3507	0.9354

TC – total cholesterol; TG – triglycerides; HDL-C – high density lipoprotein-cholesterol; mLDL-C – measured LDL-C; cLDL-C – calculated LDL-C.

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Table 4. Number of patients with disagreement between mLDL-C and cLDL-C when TG >1.70 mmol/L and mLDL-C or cLDL-C ≤1.81 mmol/L.

Year		Total (cases)	Number of cases (%) TG >1.70 mmol/L and	
			mLDL-C >1.81 mmol/L	cLDL-C >1.81 mmol/L
2013	mLDL-C ≤1.81 mmol/L	6	0	0
	cLDL ≤1.81 mmol/L	20	14 (70%)	0
2014	mLDL-C ≤1.81 mmol/L	3	0	0
	cLDL ≤1.81 mmol/L	21	18 (86%)	0
2015	mLDL-C ≤1.81 mmol/L	8	0	0
	cLDL ≤1.81 mmol/L	44	35 (80%)	0

TG – triglycerides; mLDL-C – measured LDL-C; cLDL-C – calculated LDL-C.

Table 5. Relationship between LDL-C and other atherogenic indices.

Atherogenic index	r-Value				
	mLDL-C	cLDL-C	TC: HDL-C	LDL-C: HDL-C	TG: HDL-C
Non-HDL-C	0.8950	0.9308	0.7378	0.7784	0.3434
mLDL-C		0.9461	0.4951	0.7053	0.0000
cLDL-C			0.5157	0.6814	0.0000
TC: HDL-C				0.9106	0.7179
LDL-C: HDL-C					0.4362

TC – total cholesterol; TG – triglycerides; HDL-C – high density lipoprotein-cholesterol; mLDL-C – measured LDL-C; cLDL-C – calculated LDL-C.

Table 6. Number of cases with high atherogenic index in accordance with high mLDL-C.

mLDL-C >3.37 mmol/L in accordance with	Number (%) of cases		mLDL-C >4.14 mmol/L in accordance with	Number (%) of cases	
TC: HDL-C >3	350	(82.94%)	TC: HDL-C >3	153	(91.62%)
cLDL-C >3.37 mmol/L	313	(74.17%)	cLDL-C >4.14 mmol/L	110	(65.87%)
Non-HDL-C >4.14 mmol/L	291	(68.96%)	Non-HDL-C >4.92 mmol/L	109	(65.27%)
TC: HDL-C >4	184	(43.60%)	TC: HDL-C >4	103	(61.68%)
LDL-C: HDL-C >3	133	(31.52%)	LDL-C: HDL-C >3	84	(50.30%)
TG: HDL-C >3	102	(24.17%)	TG: HDL-C >3	45	(26.95%)
TG: HDL-C >4	59	(13.98%)	LDL-C: HDL-C >4	28	(16.77%)
LDL-C: HDL-C >4	36	(8.53%)	TG: HDL-C >4	27	(16.17%)

Total cases with mLDL-C >3.37 mmol/L and >4.14 mmol/L were 422 and 167, respectively.

some differences in agreement between mLDL-C and cLDL-C. Despite performing with the same system, Abbott Architect ci8200, Choi et al. [20] found that the measured LDL-C was significantly higher than the calculated LDL-C at high TG levels.

In this study, using a system by the same manufacturer, the results in 2013 and 2014 were consistent with a previous report by Anwar et al. [6]. They found that lipids measured by a Hitachi 912 chemistry analyzer from Roche Diagnostics had

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significant differences between measured and calculated LDL-C at all TG levels. However, Nanda et al. [5] found lipids and LDL-C analyzed by a Cobas Integra 400 Plus from Roche Diagnostics gave no significant differences between measured and calculated LDL-C at all TG ranges.

Centois et al. [21] suggested that the results from LDL-C measurements could vary significantly as a result of different methods from different manufacturers, specifically the way LDL fractions were extracted. Furthermore, the inaccuracy of the Friedewald equation used to calculate LDL-C could result in the accumulation of inaccuracies and imprecision of TC, HDL-C, and TG measurements [21].

As aforementioned, the analytical variations and inaccuracies of both measured and calculated LDL-C can occur even when using systems from the same manufacturer. Systemic errors derived from lot-to-lot differences, unique calibrations by distributors, different calibrations from country to country, and reformulations of reagents can affect accuracy in individual laboratories [22]. Since a homogenous LDL-C assay interacts unequally with the different components of the LDL subclasses [4], it is possible that genetic variations controlling the proportion of these LDL subclasses may affect the accuracy in routine LDL-C measurements.

To prevent CHD effectively, the new treatment goal has been set at LDL-C <1.81 mmol/L [23]. For this reason, a good agreement is crucial between the measured and calculated LDL-C at such a low LDL-C, especially with a high TG level. As shown in Table 4, in groups with TG >1.70 mmol/L, the Friedewald estimation tended to classify LDL-C as <1.81 mmol/L despite measured LDL-C being >1.81 mmol/L. This finding was in accordance with previous reports [20,24,25], therefore, the underestimation of calculated LDL-C should be kept in mind when applying this treatment goal.

Simple atherogenic indices have been recommended for supporting LDL-C or as potentially better markers than LDL-C in CHD prevention [10–18]. In this study, non-HDL-C showed a strong correlation with LDL-C (Table 5). On the other hand, there was a strong agreement between TC: HDL-C and LDL-C. Consequently, results suggest that non-HDL-C and TC: HDL-C can add risk prediction power to LDL-C. Individuals with a high TC: HDL-C and/or non-HDL-C may have greater cardiovascular risk owing to the imbalance between the cholesterol carried by atherogenic and protective lipoproteins [11].

Conclusions

The results from this study support a strong positive correlation between measured and calculated LDL-C. The factors contributing to disagreements calculation and measurement in clinical routine tests are generally inconclusive. Variations and inaccuracies of the analytical system in use at an individual laboratory may locally affect the agreement between measured and calculated LDL-C. Among various recommended atherogenic indices, the results of this study showed that non-HDL-C and TC: HDL-C could potentially be a supportive or an alternative atherogenic index of LDL-C for preventing CHD.

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