Comparative Study of Direct - Measured and Calculated LDL in Clinical Use[†]

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Objective: Compare DLDL-C and CLDL-C obtained during regular service in hospital.

Material and Method: The study at Bangkok Hospital included 9,285 lipid profiles of different individuals that contained total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C), and directly-measured (by homogeneous method) LDL cholesterol (DLDL-C). The population has mean age 45.92 ± 12.43 years, 48% were male. LDL-C values were also calculated by Friedewald equation (CLDL-C). However, it was known to have limitation when TG > 400 mg/dL.

Results: The DLDL-C is $13.4 \pm 8.8\%$ higher than CLDL-C. The authors could obtain CLDL-C closer to DLDL-C in wider TG range, including other explanatory variables in the equation to calculate LDL-C, by these two equations, $DLDL\text{-}C = 0.98\,TC - 0.84\,HDL - 0.12\,TG + 0.056\,age + 0.071\,BMI$, and $DLDL\text{-}C = 0.98\,(TC - HDL) - 0.12\,TG + 0.1\,age + 2.4\,sex + 0.2\,BMI$.

Conclusion: DM and using lipid-lowering medications had no effect on the correlation of CLCL-C and DLDL-C.

Keywords: Friedewald equation, LDL measurement, LDL, LDL equation

Elevated LDL cholesterol (LDL-C) is a major cause of coronary heart disease (CHD). LDL-lowering therapy reduces risk for CHD and becomes primary target of therapy. More intensive lowering is recommended in persons having a relatively high risk for CHD[1].

Previously, direct LDL-C (DLDL-C) measurement was conducted by beta-quantification after ultracentrifugation and precipitation, which was a complicated and time-consuming method, needed expensive instrumentation, and skillful technicians[2]. Friedewald offered a more convenient way to estimate LDL-C (CLDL-C) from easily measurable total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) by equation $C_{LDL} = C_{plasma} - C_{HDL} - TG/5$, assuming TG/VLDL ratio = 5, in mg/L unit[3]. The Friedewald's report has become frequently cited and calculation has become the benchmark for routine LDL-C quantification[4]. However, there are some limitations in using this equation. The well-known limitation includes the invalidity associated with samples in which plasma TG higher than 400 mg/dL, the cases of hyperchylomicronemia, and in the cases of dysbetalipoproteinemia. Other observed limitations are the need of apolipoprotein B measurement for more accuracy or the findings of TG/VLDL ratio variations for different ranges of TG level[5-9].

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Clinical studies of hyperlipidemia in cardio-vascular diseases have been done using the Friedewald equation to estimate LDL-C. At present, the direct measurement of LDL-C can be performed more easily using homogeneous assays system and the need for TG in fasting samples to be used in the Friedewald equation can be eliminated[4,10]. Homogeneous assays of LDL-C seem to be able to meet current the National Cholesterol Education Program (NCEP) requirement for LDL-C testing when samples collected from non-fasting individuals are used[4]. The objectives of this study are to compare DLDL-C and CLDL-C obtained during regular service in hospital.

Material and Method
We retrieved all the lipid profiles containing TC, TG, HDL-C, and DLDL-C in period of July-December 2004 at Bangkok Hospital. The quantification of LDL-C was carried in the hospital laboratory using homo-geneous assay of Kyowa Medex methods[4] on Roche automated clinical chemistry analyzers. The patients were informed to fast for 10-12 hours before blood specimens were collected. The specimens were brought to the hospital laboratory within 10 minutes after blood collection and centrifuged at 3,000 rpm for 10 minutes before further processed in automated analyzers and results reported. The results were obtained within one hour. The %CV for TC were 1.69/1.86 and TG were 1.71/1.71 using precinorm U/ precipath U as IQC materials; and HDL-C 4.8/6.94, LDL 3.1/4.03 using precinorm L / precipath HDL/LDL. All the patients' records were reviewed by full-time internists of the study group. The data related to diagnosis of diabetes mellitus (DM), lipid-lowering medications use and body mass index (BMI) values were reviewed. History related to lipid-lowering medications was classified as none, using statins, using fibrates or using combined drugs for < 6 weeks or $\geq$ 6 weeks.

Statistical analyses
Descriptive statistics were used to present characteristic data. Linear regression analysis was used to study correlation of CLDL-C and DLDL-C. Comparisons of various factors were performed by paired t-test. A p-value of less than 0.05 was considered statistically significant.

Results
Nine thousand two hundred eighty five cases were registered and 2.75% (255 cases) had TG values > 400 mg/dL, 2.36% (219 cases) had DLDL-C values less than CLDL-C values, and 23.42% (2,175 cases) had DLDL-C values within $\pm$ 10% 0f CLDL values. The general characteristics of analyzed population were demonstrated in Table 1 and Table 2.

The mean age of the population was 45.92 $\pm$ 12.43 (16-95) years with mean BMI of 24.21 $\pm$ 4.13 (11.89-49.70) Kg/m^2. The mean $\pm$ SD values of TC, TG, HDL, DLDL-C, and CLDL-C were 208.35 $\pm$ 39.41, 123.99 $\pm$ 83.41, 61.04 $\pm$ 16.57, 140.73 $\pm$ 37.10, and 122.51 $\pm$ 36.13 mg/dL, respectively. Forty-eight percent of the population was male. DM was diagnosed in 5.7% and 92.48% of the population were not using lipid-lowering medication while 3.68% were using statins for 6 weeks or longer.

The results showed that DLDL-C is 13.4 $\pm$ 8.8% higher than CLDL-C. The correlation between CLDL-C and DLDL-C levels is shown in Fig. 1. The correlation was high and statistically significant (p < 0.001). Using linear regression analysis, it could

Table 1. Descriptive statistics of numerical data (n = 9,285)

	Mean $\pm$ SD	Range
Age (years)	45.92 $\pm$ 12.43	16-95
Cholesterol (mg/dL)	208.35 $\pm$ 39.41	51-573
Triglyceride (mg/dL)	123.99 $\pm$ 83.41	11-1,151
HDL-C (mg/dL)	61.04 $\pm$ 16.57	8-146
DLDL-C (mg/dL)	140.73 $\pm$ 37.10	12-369
CLDL-C (mg/dL)	122.51 $\pm$ 36.13	1.2-352
BMI (kg/m^2)	24.21 $\pm$ 36.13	11.89-49.70

Table 2. Descriptive statistics of characteristic data (n = 9,285)

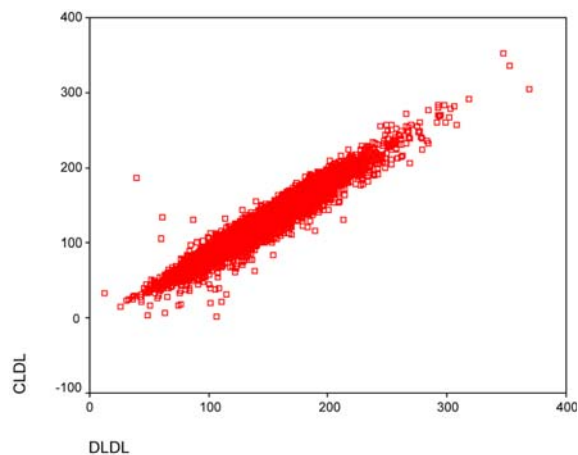|	Frequency	Percent
Sex		
Male	4,457	48.0
Female	4,828	52.0
Diabetes mellitus		
Yes	529	5.7
No	8,756	94.3
Lipid lowering medications		
Not using	8,587	92.48
Statins using < 6 weeks	12	0.13
Statins using $\geq$ 6 weeks	342	3.68
Fibrates using < 6 weeks	0	0.00
Fibrates using $\geq$ 6 weeks	51	0.55
Using combined statins		
and fibrates < 6 weeks	1	0.01
$\geq$ 6 weeks	7	0.08
Others	285	3.07

Fig. 1 The plot of calculated LDL (Friedewald Equation-derived) versus direct-measured LDL r = 0.96, p < 0.001

demonstrate better correlations of DLDL-C and calculated LDL-C by also using the age, sex, and BMI as explanatory variables in equations. The DM diagnosis and the use of lipid-lowering medications have no effects on the difference of DLDL-C and CLDL-C. There are two equations obtainable to calculate LDL-C that was better correlated to DLDL-C as follow.

Equation 1 incorporated "age" (regression coefficient = 0.056) and "BMI" (regression coefficient = 0.071) in the equation. Equation 2 included "age", "sex", and "BMI" (regression coefficient = 0.1, 2.4, and 0.2, respectively). (Values for sex are male = 1, female = 2). The equations yield better correlation between DLDL-

C and calculated LDL-C and are much less affected by TG level as demonstrated in Fig. 2 and Fig. 3.
Equation 1:
$$DLDL\text{-}C = 0.98\,TC - 0.84\,HDL - 0.12\,TG + 0.056\,age + 0.071\,BMI$$
Equation 2:
$$DLDL\text{-}C = 0.98\,(TC - HDL) - 0.12\,TG + 0.1\,age + 2.4\,sex + 0.2\,BMI$$

Discussion

In this study, the DLDL-C values that were directly measured by homogeneous method were averagely 13.4 + 8.8% higher than CLDL-C. The higher values of DLDL-C than CLCL-C were also reported by Puavilai[5], Wongtiraporn[11], Bairaktari[6], Legault[7], Akanji[12], Hirany[13] and Lindsey[14]. The findings of higher DLDL-C values than CLCL-C values were described as conditions lead to an overuse of lipid lowering medications by DLDL-C[11] and the other way, the underestimation of CLDL-C[13], and might result in a loss of goal attainment for half of the patients with CHD or a CHD risk equivalent [14].

The Friedewald equation is beneficial to the care of hyperlipidemia and in the prevention and treatment of cardiovascular diseases. However, there have been a number of critics on the limitations of this equation such as the suggestion to include apolipoprotein B measurement[6, 7, 15, 16] in the equation to assess LDL-C of type 2 DM as well as the incorporation of lipoprotein (a)[17] in the equation for more accuracy despite the limited availability of these measurements. In this study we have searched factors to modify the calculate
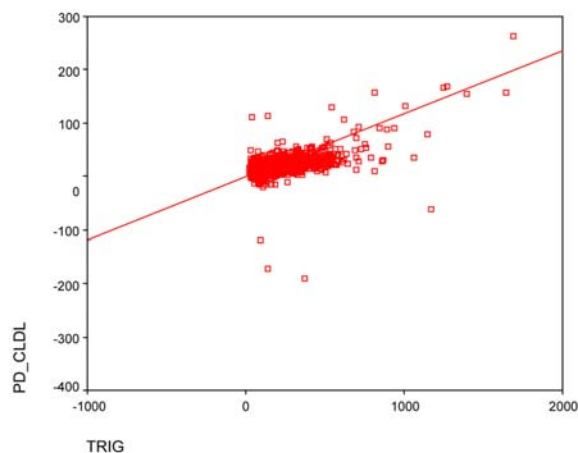

Fig. 2 Relation between triglyceride and the calculated LDL-C using Equation Friede wald (validation group)
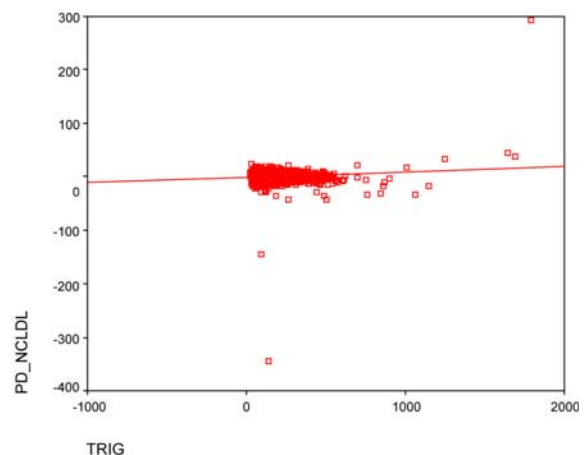

Fig 3. Relation between triglyceride and the calculated LDL-C by our methods (validation group)

equation and do not find the effect of DM diagnosis in the difference of DLDL-C and CLDL-C. The authors found no effect from using lipid-lowering medications, which was never been described before in relation to correlation of DLDL-C and CLDL-C.

In this study, the authors have proposed two equations:

Equation 1:

DLDL-C = 0.98 TC – 0.84 HDL -0.12 TG + 0.056 age + 0.071 BMI

and

Equation 2:

DLDL-C = 0.98 (TC – HDL) – 0.12 TG + 0.1 age + 2.4 sex + 0.2 BMI

These two equations could calculate LDL-C that has the values more closed to DLDL-C values and can be used in a wider range of triglyceride levels. The other explanatory variables in the equations, besides TC, TG and HDL-C level i.e. "age" (years), "sex" (male = 1, female = 2), and BMI (Kg/m^2) are easily obtainable and at a very minimal or no cost.

Other critics on the Friedewald equation are the variety of TG multiplier in the equation that is varied from 0.158 to 0.421 in mg/L unit of TG[5-9,18-22]. The proposed refinement of the equation such as the use of triglyceride multipliers give only marginally better LDL values and may not apply to all populations[7].

For all of its simplicity and limitations, the Friedewald formula probably remains the single best equation for estimating LDL-C in clinical laboratories. Although this study proposed the factor of age, sex, and BMI to correct the LDL-C estimation for wider range of triglyceride, the equations are not simple as in Friedewald's.

DLDL-C measurement that previously was recommended when there were limitations to the use of Friedewald equation, is now much more convenient to perform by homogeneous methods and may give more benefits than previously suggested. In DM, it is more reliable, rapid, and cost effective despite the need for triglyceride evaluation in some cases[10,13]. Non-fasting venous samples, which yield more convenience, may be used by USPSTF recommendation[23]. However, there were some disagreement. Miller found the post-prandial changes in homogeneous assays of DLDL-C that were similar to beta-quantification reference method and not recommend the use of non-fasting specimen due to postprandial variability among patients[24].

The finding of higher DLDL-C value than CLDL-C may reinforce intensive control by using DLDL-C as monitor. Control by diet and exercise are safe and useful. Using lipid-lowering medications need more cautions if we consider the findings that lipids were not found to be the risk of a five-year mortality by Tocharoenvanich in southern Thailand[25] or cardiovascular mortality in 12-17 year follow-up by Sritara in a cohort of 3,499 urban Thais[26].

Conclusion

Direct LDL-C measurement is convenient, usually yield higher value than calculated LDL -C from Fiedewald equation. The Friedewald equation can be modified to create better correlation between direct and calculated LDL-C by including sex, age, and BMI as variables.

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การศึกษาเปรียบเทียบ แอล ดี แอล ที่วัดโดยตรง กับ แอล ดี แอล ที่คำนวณจากสมการ

สุรชัย รุ่งธนาภิรมย์, บุญส่ง องค์พิพัฒน์กุล, ทนงศักดิ์ เกียรติบำรุงพันธ์, จงรักษ์ ภักดีกุล, สุเทพ อาชวนันทกุล, ชัยศิลป์ แตระกุล, จันทนา พงศ์สงวนสิน, วีรวิทย์ เจริญเลิศ, กฤษณพงศ์ ตันสงวน

จากการศึกษาที่โรงพยาบาลกรุงเทพ โดยเก็บข้อมูลจากคนไข้ที่ไม่ซ้ำคน 9,285 ราย ที่มีการตรวจไขมันใน เลือดครบทุกตัวในการตรวจครั้งเดียวกันได้แก่ คอเลสเตอรอล (TC) ไตรกลีเซอไรด์ (TG) เอช ดี แอล (HDL-C) และ แอล ดี แอล ที่วัดโดยตรงโดยวิธี homogeneous (DLDL-C) เทียบกับค่า แอล ดี แอล ที่ได้จากการคำนวณโดยใช้ สูตรของ Friedewald (CLDL-C) พบว่า DLDL-C มีค่าสูงกว่า CLDL-C ประมาณ 13 ± 8.8 % และอาจใช้ตัวแปรอื่น ได้แก่ อายุ เพศ และค่าดรรชนีมวลกาย เข้าร่วมในสมการที่คำนวณหาค่า CLDL-C ด้วย เพื่อให้ได้ค่าที่ใกล้เคียงกับ DLDL-C มากขึ้น และใช้ได้กับการมีระดับ TG แตกต่างกันมากขึ้น ดังสมการ 2 ข้อ ข้างล่างนี้ ส่วนการเป็นเบาหวาน และการใช้ยาลดไขมันไม่มีผลต่อความสัมพันธ์ของ LDL-C ที่วัดโดยตรง และที่คำนวณจากสมการ

1) DLDL-C = 0.98 TC − 0.84 HDL − 0.12 TG + 0.056 age + 0.071 BMI

2) DLDL-C = 0.98 (TC − HDL) − 0.12 TG + 0.1 age + 2.4 sex + 0.2 BMI