The Accuracy in Using Modified Friedewald Equation to Calculate LDL from Non-Fast Triglyceride: A Pilot Study

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Background: *Total cholesterol, HDL (high-density lipoprotein) and LDL (low-density lipoprotein) are important risk factors of coronary heart disease. It is costly to perform the LDL test for follow-up cardiovascular diseases (CVD) especially for Gold Card Holders (Thirty Bahts Universal Coverage). Hypertriglyceridemia is also important as it is associated with uncontrolled type 2 Diabetes mellitus, low HDL, and metabolic syndrome. Because the serum triglyceride level changes with time after meal consumption, blood test for triglyceride level should be taken after fasting 12 hours. However, this causes hunger and inconvenience in many patients.*
Objective: *To find out the optimal time to take blood for triglyceride measurement and using it for calculation of LDL with the original Friedewald Formula and the new Modified Friedewald Formula.*
Material and Method: *Patients were asked the approximate time of last meal/eating, drinking soft drink, milk. Additionally, the time of blood drawn from the patients was recorded. The blood samples were drawn as usual amounts and the tests were done as the physicians ordered. If enough sera were left, it would be analyzed for lipid profiles. LDL was also calculated by using standard Friedewald equation (sfLDL) and Modified Friedewald equation (mfLDL = total cholesterol - HDL - 1/6 triglyceride). Comparison between direct measured LDL (dmLDL), sfLDL, and mfLDL with time interval of last food, drink intake was done.*
Results: *There were 999 serum tubes left to be analyzed for lipid profiles and 919 sera (92.0%) left having triglyceride less than 300mg/dl. Of those, 381, 84, and454 samples came after fasting (nothing per oral = NPO) approximately less than 8 hours (h), 8-11.9 h, and 12 h or more respectively with sfLDL to dmLDL $\pm$ 10 mg, comparison of 64.0%, 65.5% and 68.3% respectively. In contrast, comparing mfLDL to dmLDL $\pm$ 10 mg being of 82.7%, 83.3% and 84.8% from the same samples and time intervals respectively thus, statistical significant (p-value < 0.001, odd ratios (OR) 2.59- 2.68). If blood drawn regardless of time from last food intake with triglyceride less than 300 mg/dl and with the above condition mfLDL, it gave 83.8% related to dmLDL while sfLDL gave only 66.3% p < 0.0001 and OR = 2.63.*
Conclusion: *The present pilot study showed 919 of 999 sera (92.0%) with serum triglyceride less than 300 mg/dl, regardless of the time of the last food intake. The authors used the new Modified Friedewald equation to calculate that the LDL had 83.8% accuracy when compared to direct measured LDL $\pm$ 10 mg. This equation is more accurate than the standard (original) Friedewald equation with OR of 2.63. The authors offer that to save the cost, the new Modified Friedewald equation should be used to calculate LDL. Then, direct LDL measurement could be reserved for patients with hypertriglyceridemia, in the treatment of LDL in high-risk CVD.*

Keywords: *Blood, Cholesterol, LDL, Triglycerides, Predictive value of tests, Risk factors*

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High level of total cholesterol, high LDL (low density lipoprotein), and low HDL (high density lipoprotein) are important risk factors for cardiovascular disease but high triglyceride remains controversial because high triglyceride tends to associate with low HDL[1]. Total cholesterol, HDL, and LDL levels can be measured directly from the non-fast serum but serum triglyceride level is changed with a meal. The patients have to fast conventionally 12 hours (h) before blood is drawn for triglyceride level. Hypertriglyceridemia is found with diabetes mellitus, low HDL, metabolic syndrome (MS), and if serum triglyceride is 150 mg/dl or more, being one of the criteria of MS[2,3]. Triglyceride (TG) consists of a three-carbon glycerol backbone, linked to three fatty acids, which these fatty acids have varied in chain length and presence of bonds. Human plasma concentrations of lipids are varied greatly according to the nutritional status and genetic. In general following dietary intake of fat, the triglyceride (TG) level increases dramatically, then it declines about 4 hours later[4]. Dietary fat, which is mainly in the form of TG with a small amount of cholesterol, undergoes emulsification with bile acids and phospholipids to form micelles in the small intestinal lumen, subsequently hydrolyzed by pancreatic lipase to form monoglyceride, free fatty acids, and cholesterol. These products are absorbed into the enterocytes of the small intestine and re-esterified to form triglycerides, then they are synthesized with small amounts of free cholesterol, phospholipid, and proteins to form chylomicrons. Apolipoprotein B-48 is genesis from chylomicrons in the intestine (in humans)[5-8]. These particles are heterogeneous in composition and size. The cholesterol is also esterified to form cholesteryl esters in the enterocytes.

These lipids are secreted into the intestinal lymphatics and they entered to the thoracic duct then to the venous circulation. TG is the main component of chylomicrons and very low density lipoprotein (VLDL) which these particles are also called triglyceride-rich lipoproteins. There are five TG molecules in VLDL, so Friedewald formula using serum triglycerides divided by 5 to represent for VLDL. It is very inconvenient to fast 12 h for blood drawn for serum triglyceride measurement especially in old age, diabetes patients because of hunger. In general screening for lipid, total cholesterol is measured and if the level is 200 mg/dl or less; being considered normal[2]. It is costly for serum LDL test from direct measurement, especially if it has to be tested several times in a year and for the Gold Card Holders (Thirty Baht Universal Coverage), so LDL level is usually calculated by using original Friedewald equation. However, it is shown that the level of LDL is less accurate if serum triglyceride level is higher than 200 mg/dl thus, for more accuracy the modified Friedewald equation is used[9]. It will be convenient for patients to have blood drawn for lipid profiles [total cholesterol, HDL, (LDL), and triglyceride] including of fasting blood sugar (FBS) after NPO (nothing per oral) only 8 h, and even more convenient if NPO only 2 h. The authors performed a pilot study by analysis of lipid profiles of the sera left from any tests (no preservative). The present pilot study was approved by local ethic committee of Rajavithi Hospital and granted by Rajavithi Research Fund.

Objective

To compare the levels of LDL from calculation by using the standard (original) Friedewald equation (sf LDL) and new Modified Friedewald equation (mf LDL) with direct measurement of LDL (dm LDL).

Material and Method

The patients were asked the approximate time of last meal, milk, and soft drink before the blood drawn and recorded. If there were enough sera (no preservative) left from tested orders, these sera would be analyzed directly by using Hitachi 717 analyzer for total cholesterol, HDL, triglyceride levels, and direct measured LDL assay by Clinical Chemistry Laboratory, Department of Clinical Pathology, Rajavithi Hospital. Calculation of LDL level by using standard (original) Friedewald equation (sf LDL) was done as follows:

LDL = total cholesterol - HDL - 1/5 triglyceride

In addition, the new Modified Friedewald equation (formula) was used to calculate LDL level (mf LDL) as follows:

mf LDL = total cholesterol - HDL - 1/6 triglyceride

The study compared directly the levels of sf LDL and mf LDL to direct measured LDL (dm LDL). The sample was labeled as satisfied if the difference was dm LDL within $\pm$ 10 milligrams (mgs), labeled as overestimated if more than 10 mgs (> +10), and labeled underestimated result if the difference was less than 10 mgs (> -10). Using Chi-square test to find out the significance if p-value being less than 0.05. Serum triglyceride was divided due to the approximate NPO of less than 8 hours (h), 8 to 11.9 h, and 12 h or more. Chi-square was used for statistic significance.

Results

There were 999 enough left sera for analysis, and 919 of them (92.0%) having serum triglyceride level less than 300 mg/dl regardless the time of last meal, milk and/or soft drink. The result of LDL from calculation from standard Friedewald and Modified Friedewald equations compared to direct measured LDL as followings. Overall mean sfLDL seemed to be correlated to dmLDL the same as mfLDL with the correlation of 0.880 and 0.884 respectively, but these values included the over and under estimated beside the satisfied values (Table 1, Fig. 1).

There were 919 (92.0%) sera with triglyceride levels being less than 300 mg/dl. The result of mfLDL was compared with dmLDL, being better than sfLDL with dmLDL significantly.

When serum triglyceride was less than 300 mg/dl, mfLDL value was more correlated to direct measured LDL than sfLDL with statistical significance by p-values being of < 0.0001 (95% CI = 1.91-3.75, OR = 2.68); = 0.008 (95% CI = 1.27- 5.46, OR= 2.63); < 0.0001 (95% CI = 1.87- 3.58, OR = 2.59); and < 0.0001 (95% CI = 2.10-3.28, OR = 2.63) from blood drawn < 8 h, 8-11.9 h, ≥ 12 h and at any time of last meal, respectively (Table 2, Fig. 2).

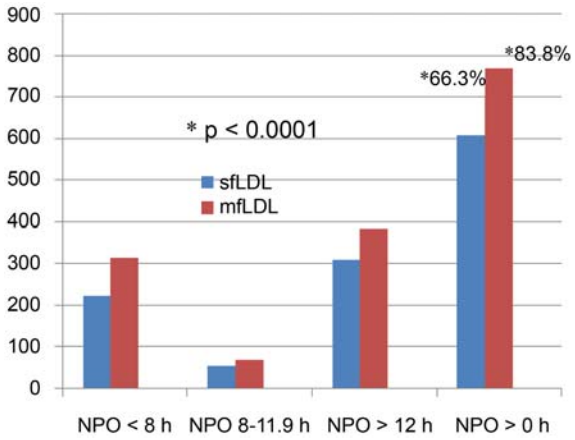
Discussion

The authors demonstrate the usefulness of new modified Friedewald equation (formula), which is LDL = total cholesterol - HDL - 1/6 triglyceride[9], to calculate serum LDL level from non-fast triglyceride, regardless of the time of last meal if serum triglyceride level is less than 300 mg/dL. This study shows that the LDL will be in the range of 82.7 - 84.8% of direct measured LDL within ± 10 mg (Table 2) although, the correlation of mean sfLDL and mfLDL to mean dmLDL were almost the same, 0.880 and 0.884 respectively (Table 1). The new Modified Friedewald Formula is more accurate than the original one with p-values being less than 0.001 (0.008 - < 0.0001) and odd ratios are in the range of 2.59 - 2.68 with mean OR of 2.63 regardless to the time of blood drawn when triglyceride is less than 300 mg/dL (Table 2, Fig. 1). After meal, fat is emulsified then hydrolized partly to monoglyceride and absorbed into enterocytes that re-esterified to form triglyceride then synthesized to chylomicrons and finally entered to venous circulation via the thoracic duct. Chylomicrons are rapidly lipolized by lipoprotein lipase (LPL) that resides on the surface of capillary endothelium of arterial system in peripheral tissues to give free fatty acids and made chylomicrons smaller,

Table 1. Correlation of mean sfLDL, mfLDL to dmLDL when Triglyceride level was less than 300 mg/dL regardless the time of last meal

LDL of 919 sera	Mean	Std. deviation	mean diff. to dmLDL	Correlation to dmLDL
dmLDL	131.3643	± 42.2224		
sfLDL	123.1110	± 45.0364	8.2524	0.880
mfLDL	127.3706	± 45.3427	3.9929	0.884

n = number; Std. deviation = standard deviation; mean diff. = mean difference, dmLDL= direct measured LDL, sfLDL = standard Friedewald calculated LDL, mfLDL= modified Friedwald calculated LDL



sfLDL= standard Friedewald calculation for LDL;
mfLDL = modified Friedewald calculation for LDL

Fig. 1 Illustration of numbers of sera (in percents) which sfLDL, mfLDL levels compared to dmLDL within ± 10 mg at different time of last meal when triglyceride less than 300 mg/dl

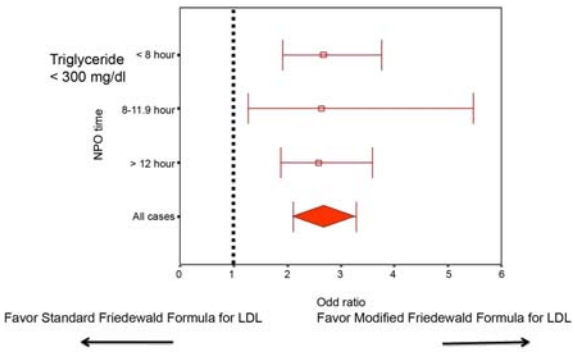


Fig. 2 Illustration of odd ratios of mfLDL and sfLDL in different time interval when triglyceride was less than 300 mg.dl

Table 2. The differences of LDL level between calculations and direct measurement in serum triglyceride, which was less than 300 mg/dl, and different time in NPO

NPO (h)	Calculated LDL differences from dmLDL (mgs)						
	≥ +11		0 ± 10		≤ -11		p-value
	sfLDL (%)	mfLDL (%)	sfLDL (%)	mfLDL (%)	sfLDL (%)	mfLDL (%)	
< 8	123 (32.3)	44 (11.6)	244* (64.0)	315* (82.7)	14 (3.7)	22 (5.7)	<0.0001
8-11.9	27 (32.1)	12 (14.3)	55[#] (65.5)	70[#] (83.3)	2 (2.4)	2 (2.4)	0.008
≥ 12	136 (29.9)	56 (12.3)	310[@] (68.3)	385[@] (84.8)	8 (1.8)	13 (2.9)	<0.0001
0- ≥12	286 (31.1)	112 (12.2)	609[$] (66.3)	770[$] (83.8)	24 (2.6)	37 (4.0)	<0.0001

* numbers of sera were significantly different at p-value < 0.0001
[#] numbers of sera were significantly different at p-value = 0.008
[@] numbers of sera were significantly different at p-value < 0.0001
[$] numbers of sera were significantly different at p-value < 0.0001

shrunken, and relatively richer in cholesterol, which are called chylomicron remnants and are removed from plasma by chylomicron remnant receptors in hepatocytes[10,11]. Chylomicron remnants have a reduced triglyceride content and are enriched in cholesterol and apolipoproteins B and E[12], which have already been associated with the presence and progression of cardiovascular disease since Apo-B100 is the sole protein constituent of LDL[1,7,13]. Free fatty acids are either oxidized by muscle cells for energy, stored by adipose tissues, or returned to the liver for oxidation, re-esterification to triglyceride used in VLDL synthesis. Chylomicrons are created from dietary fat or from fatty acids derived by catabolism of sugars, certain amino acids and other fatty acids[14]. Therefore, partly chylomicrons could be synthesized not related to meal, and are lipolized by LPL to chylomicron remnants, circulating in the blood, which are caught by the receptors in the liver. Chylomicron, and VLDL composed about 90% and 60% of triglycerides-rich lipoproteins (triacylglycerol-rich lipoproteins), respectively[15]. Anyhow, chylomicron remnants have more TG molecules than VLDL. It is more proper to use TG/6 for representation of VLDL, chylomicron remnants, few chylomicrons and other venous circulating triglyceride molecules. The new modified Friedewald formula (equation) can be used even fast TG less than 200 mg/dl, which LDL value being 91.3% and from standard (original) Friedewald formula, LDL being 94.1% with ± 10 mg of direct measured LDL, and no statistical significance[9]. As mentioned before, TG level declined 4 hours after meal[5]. From this report, there is less than 10% of sera containing of TG being of 300 mg/dl or more from the blood samples drawn at any time of last meal. Therefore, blood can be drawn at 4 hours or more after meals for total cholesterol, HDL, and TG in persons eating a normal diet (not heavy in fat). Then LDL can be calculated by new modified Friedewald formula and giving the value for 82-84% reliable compared to direct measured LDL ± 10 mg, if TG < 300 mg/dl (Table 2, Fig. 1). Patients who have a tendency to have fast TG 300 mg/dl or more such as uncontrolled type 2 diabetes, obese, familial hypertriglyceridemia, and genetic disorders, should have fast TG for the baseline then non-fast TG may be used. If non-fast TG is very high, fast TG has to be used and if it is higher than 400 mg/dl, direct measured LDL should be performed. However, this report is a pilot study, it needs a prospective study to know the exact time of blood drawn after the regular diet and re-test the new Modified Friedewald formula. With this new formula, maybe a two hours after meals period for diabetic patients could be used.

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ความแม่นยำของ สูตร Modified Friedewald Equation ในการคำนวณค่า LDL จากระดับ serum triglyceride ที่ไม่ได้อดอาหาร: การวิจัยนำร่อง

วิไล พัววิไล, ดอนพิชิต เหล่ารักพงศ์, ชัยชาญ ดีโรจนวงศ์, น้ำทิพย์ มัธพงษ์ถาวร, ประเทือง ศรีเลิศ

ภูมิหลัง: *คอเลสเตอรอล, HDL และ LDL เป็นปัจจัยเสี่ยงสำคัญของโรคหัวใจขาดเลือด การตรวจระดับ LDL มีราคาแพง แพทย์ส่วนหนึ่งนำค่าLDL มาจากการคำนวณจากสูตรของ Friedewald ซึ่งต้องใช้ค่า fast-triglyceride โดยผู้ป่วยต้องอดอาหาร 12 ชั่วโมง ซึ่งไม่สะดวกและหิว*

วัตถุประสงค์: *หาเวลาที่เหมาะสมในการเจาะเลือดตรวจระดับ triglyceride ใช้ในการคำนวณค่า LDL จากสูตรของ Friedewald หรือ new modified Friedewald แล้วนำมาเปรียบเทียบกับค่าตรวจโดยตรงของ LDL*

วัสดุและวิธีการ: *ถามผู้ป่วยถึงเวลาที่รับประทานอาหาร นม เครื่องดื่มที่ผสมน้ำตาล ครั้งสุดท้ายก่อนถูกเจาะเลือด โดยกะประมาณเวลาและบันทึกไว้ นำซีรัมที่เหลือจากการตรวจตามแพทย์สั่งมาตรวจคอเลสเตอรอล, HDL, LDL และ triglyceride นำผลมาคำนวณตามสูตร*

ผลการศึกษา: *มีซีรัมเหลือได้ตรวจ 999 หลอดทดลอง พบว่ามีซีรัมจำนวน 919 หลอดทดลอง (92.0%) มีระดับ triglyceride น้อยกว่า 300 มก./ดล. และพบว่าซีรัมจำนวน 381, 84 และ 454 หลอดทดลอง จากผู้ป่วยอดอาหารน้อยกว่า 8 ชั่วโมง, 8-11.9 และ 12 ชั่วโมง หรือ มากกว่าตามลำดับ เปรียบเทียบระดับ sfLDL (Friedewald) กับ dmLDL (วัดโดยตรง) $\pm$ 10 ม.ก. และนำ mfLDL (new modified Friedewald) เปรียบเทียบระดับ dmLDL เหมือนของ sfLDL พบว่าค่าของ mfLDL ดีกว่า sfLDL อย่างมีนัยสำคัญโดยค่า p-value < 0.001 และถ้า triglyceride น้อยกว่า 300 มก./ดล. โดยไม่เกี่ยงว่าเลือดถูกเจาะเมื่อใด พบว่าค่าของ mfLDL เกี่ยวพันกับ dmLDL ถึง 83.8% ขณะที่ค่าของ sfLDL เกี่ยวพันกับ dmLDL เพียง 66.3%, p-value < 0.0001, OR = 2.63*

สรุป: *จากการศึกษานำร่องพบว่า triglyceride น้อยกว่า 300 มก./ดล. โดยไม่เกี่ยงว่าเลือดถูกเจาะเมื่อใด มีจำนวน 919 จาก 999 หลอดทดลอง (92.0%) ค่า mfLDL เกี่ยวพันกับ dmLDL $\pm$ 10 ม.ก. ถึง 83.8%. คณะผู้นิพนธ์เสนอสูตรในการคำนวณค่า LDL โดย LDL = total cholesterol - HDL - 1/6 triglyceride ถ้าค่า triglyceride น้อยกว่า 300 มก./ดล.*