

Comparative study of determination methods for low-density lipoprotein by direct assay and modified Friedewald's formula calculation*

Penprapa Jaiwang**

Viroj Wiwanitkit** Nara Paritpooke**

Jaiwang P, Wiwanitkit V, Paritpooke N. Comparative study of determination methods for low - density lipoprotein by direct assay and modified Friedewald's formula calculation. Chula Med J 2000 Mar; 44(3): 189 - 94

Objective : *To compare determination methods for low-density lipoprotein by direct assay and modified Friedewald's formula calculation*

Design : *Descriptive study*

Materials : *Fasting serum from 100 individual cases sent to the Division of Laboratory Medicine, King Chulalongkorn Memorial Hospital*

Methods : *Specimens were examined for total cholesterol, high-density lipoproteins (HDL) and triglycerides using automated clinical chemistry for basic information in calculation of LDL levels. Each specimen was also processed by direct LDL assay (Boehringer Mannheim) by the same automated process. Results from each determination were collected and then analyzed. Precision analysis for the direct LDL assay was performed. Linear regression was performed to assess significant differences in determination of LDL by direct assay and the calculation method.*

Results : *Precision analysis of direct LDL assay (n = 10) gave with-in run precision = 4.25 % and between run precision = 1.89 %. The least - squares equation from comparing direct LDL assay to the calculation method gave $Y = 0.890 X + 20.42$ ($r = 0.787, p < 0.05$)*

* Presented in the 41th Annual Scientific Meeting , Faculty of Medicine, Chulalongkorn University

**Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University

Conclusions : *The direct non-precipitating LDL assay is a new technology for the clinical chemistry laboratory. It can be a useful tool in treatment and follow - up of the hypercholesterolemic patients such as non-compliance groups. It should routinely be provided in the laboratory for assessment and management of patients.*

Key words : *Low - density lipoprotein, Determination.*

Reprint request: Jaiwang P, Department of Laboratory Medicine, Faculty of Medicine,
Chulalongkorn University, Bangkok 10330, Thailand.

Received for publication. November 5, 1999.

เพ็ญประภา ใจหวัง, วิโรจน์ ไชวานิชกิจ, นารา ผริตโกติ. การศึกษาเปรียบเทียบการตรวจวัดระดับของไลโปโปรตีนชนิดความหนาแน่นต่ำด้วยวิธีวัดโดยตรง และการคำนวณโดยใช้สูตร Friedewald แบบดัดแปลง. จุฬาลงกรณ์เวชสาร 2543 มี.ค; 44(3): 189 - 94

- วัตถุประสงค์** : เพื่อศึกษาเปรียบเทียบการตรวจวัดระดับไลโปโปรตีนชนิดความหนาแน่นต่ำด้วยวิธีวัดโดยตรงและการคำนวณโดยใช้สูตร Friedewald แบบดัดแปลง
- รูปแบบการศึกษา** : การศึกษาเชิงพรรณนา
- วัสดุ** : ตัวอย่างเลือดจำนวน 100 ตัวอย่าง ณ ห้องปฏิบัติการเวชศาสตร์ชั้นสูตรโรงพยาบาลจุฬาลงกรณ์
- วิธีการ** : ทำการตรวจตัวอย่างเลือดทั้งหมดเพื่อหาระดับของคลอเรสเตอรอล, ไลโปโปรตีนชนิดความหนาแน่นสูง และไตรกลีเซอไรด์ด้วยเครื่องอัตโนมัติทางเคมีคลินิกเพื่อใช้เป็นค่าพื้นฐานในการคำนวณหาระดับไลโปโปรตีนชนิดความหนาแน่นต่ำ และได้ใช้เครื่องอัตโนมัติเดียวกันตรวจหาระดับไลโปโปรตีนชนิดความหนาแน่นต่ำด้วยวิธีวัดโดยตรง ได้ทำการวิเคราะห์ความแม่นยำของการวัดระดับดังกล่าวได้ทำการหาสหสัมพันธ์เชิงเส้นระหว่างการหาระดับของไลโปโปรตีนชนิดความหนาแน่นต่ำของทั้งสองวิธี
- ผลการศึกษา** : จากการวิเคราะห์ความแม่นยำของการตรวจวัดระดับไลโปโปรตีนชนิดความหนาแน่นต่ำด้วยวิธีวัดโดยตรงพบความแม่นยำในกลุ่มเท่ากับร้อยละ 4.25 และระหว่างกลุ่มเท่ากับร้อยละ 1.89 สมการความสัมพันธ์ระหว่างการหาระดับไลโปโปรตีนชนิดความหนาแน่นต่ำด้วยวิธีวัดโดยตรง เทียบกับวิธีการคำนวณเป็นดังนี้ $Y = 0.890X + 20.42$ ($r = 0.787, p < 0.05$)
- บทสรุป** : การตรวจวัดระดับระดับไลโปโปรตีนชนิดความหนาแน่นต่ำด้วยวิธีวัดโดยตรงเป็นเทคโนโลยีชนิดใหม่ทางห้องปฏิบัติการเคมีคลินิก จัดเป็นการตรวจที่มีประโยชน์ในการรักษาและติดตามผู้ป่วย โดยเฉพาะในรายที่ผู้ป่วยไม่มีความพร้อมสำหรับการตรวจ จึงเป็นการตรวจที่ควรใช้ในการตรวจพื้นฐานสำหรับการประเมินและดูแลรักษาผู้ป่วย
- คำสำคัญ** : ไลโปโปรตีนชนิดความหนาแน่นต่ำ, การตรวจวัดระดับ

The association between levels of lipoprotein cholesterol and risk of coronary heart disease (CHD) is well documented.⁽¹⁾ Low-density lipoprotein (LDL) is an important lipoprotein cholesterol fraction that plays an important role in the pathogenesis of coronary heart disease. Therefore, a number of methods have been developed in order to determine LDL levels⁽²⁻³⁾ such as ultracentrifugation, electrophoresis, precipitation and the calculation method. In general practice these days, the calculation method is the most favored method, and the most acceptable and reliable calculation method is DeLong's modified Friedewald's formula.⁽⁴⁾

In this study, a new direct LDL determination method by chemical reaction was tested for its properties and compared to the calculation method. Results from this study can be useful in selection of the most proper method for lipoprotein cholesterol determination.

Materials and Methods

Fasting sera from 100 individual cases with wide range of cholesterol and triglyceride concentrations were included in this study. Specimens were determined for total cholesterol, high-density lipoproteins (HDL) and triglycerides using a Hitachi automated clinical chemistry analyzer for basic information in calculation of the LDL levels. Each specimen was also processed by direct LDL assay (Boehringer Mannheim) by the same automation process. Results from each determination were collected and then analyzed. Precision analysis for the direct LDL assay was performed. Linear regression were performed to assess significant differences in determination of LDL by the direct assay and the

calculation method. $P = 0.05$ was accepted as a significant level in the comparisons.

Procedure

1. Calculation method

In this study, calculation method of LDL level was calculated using DeLong's modified Friedewald's formula, a modification from study in large population.⁽⁴⁾ The formula was "Calculated LDL = Total cholesterol - (HDL + 0.16 x Triglyceride)"

2. Direct LDL assay

Direct assay mentioned in this study was performed using chemical reagents of Boehringer Mannheim, Thailand. There was two major reagents in the assay. Reagent 1 contains 40 mmol/L BIS-TRIS-buffer, 24 mmol/L magnesium chloride, preservative and detergent. Reagent 2 is composed of 40 mmol/L BIS-Tris-buffer, tentacle polyanion PAMPS 0.15 mg/mL, preservative and detergent. 5 microliter of sample combined with 250 microlitre of reagent 1 was incubated at 37 Degree Celcius for 5 minutes. Then 50 microlitre of reagent 2 was added. Complex of LDL in sample, detergent, magnesium ion and PAMPS was formed. Then Photometry for turbidity of comple at wavelength 700 and 505 nanometer was used to determine LDL level.

Results

Data from the lipid determination by automated clinical chemistry is presented in Table 1. The average of level of LDL by direct assay was 139.44 ± 46.19 milligram/deciliter and the average of level of LDL by calculation method was 133.78 ± 46.13 milligram/deciliter. Precision analysis of direct LDL assay ($n = 10$) gave with-in run precision = 4.25 %

Table 1. Characteristics of samples used in this study.

Determination	Mean (mg/dL)	Standard deviation (mg/dL)	Maximum (mg/dL)	Minimum (mg/dL)
Total cholesterol	203.91	50.44	347	44
Triglyceride	136.04	63.20	376	28
HDL	48.36	19.51	160	3
Direct LDL	139.44	46.19	316	44
Calculated LDL*	133.78	46.13	279	18

* DeLong's modified Friedewald's formula was used in calculation

$$\text{Calculated LDL} = \text{Total cholesterol} - (\text{HDL} + 0.16 \times \text{Triglyceride})$$

and between run precision = 1.89 %. The least - squares equation from comparing direct LDL assay to the calculation method gave $Y = 0.890X + 20.42$ ($r = 0.787$, $p < 0.05$) (Figure 1).

Discussion

Low - density lipoprotein cholesterol is the cholesterol fraction which affects the pathogenesis of coronary heart disease. Numerous methods have been developed to determine its levels. The direct LDL

assay⁽⁵⁾ is the new method based on the chemical reaction method using photometry of the LDL complex. This method is rather good comparing to the reference ultracentrifugation method (betaquat) and can overcome the problem of calculation method in cases of hypertriglyceridemia and hyperlipoproteinemia type III.⁽⁶⁾

In this study, the average level of direct LDL was slightly higher than calculated LDL. Considering the plot of linear regression in Figure 1, many lower values of calculated LDL can be observed. This can be explained by the fact that major limitation of such calculation method is abnormal low result in case if high triglyceride and abnormal lipoproteins.⁽³⁾

It revealed that the precision of the assay (within run and between-run precision) was quite good. And the results of direct LDL assay determination was well correlative to calculation method using modified Friedewald's formula ($r = 0.787$). Furthermore, use of the direct LDL assay can overcome the problem about fasting preparation of the subjects. Therefore, in case of non-compliance group as diabetic,⁽⁷⁾ geriatric, and pediatric patient,⁽⁸⁾ this assay should be considered

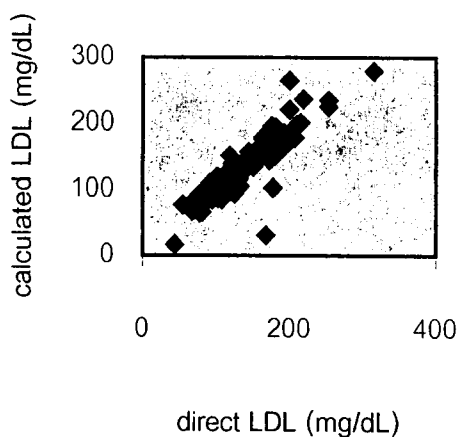


Figure 1. Comparison of direct LDL assay and calculation method.

to be the LDL determination method.

Direct LDL assay also provides a single step chemical reaction and can be performed by using automated clinical chemistry analyzers. This is preferable over other methods such as ultracentrifugation and precipitation methods which required multi-steps. Certainly it can provide better and faster results as compared to the modified Friedewald's formula due to the fact that it is direct determination and it is a one - step technique while the calculation method is based on three substance determinations before the calculation. Therefore, this technique can be the preferred solution for the determination of LDL. It can also be a useful tool in treatment and follow-up of hypercholesterolemic patients such as the non-compliance groups.

In conclusion, the direct non-precipitating LDL assay is a new technology for the clinical chemistry laboratory. It should routinely be provided in laboratories for assessment and management of patients.

References

1. Gordon T, Kennel WB, Catelli WP, Dawber TR. Lipoproteins, cardiovascular disease, and death: the Framingham study. *Arch Intern Med* 1981 Aug; 141(9): 1128 - 31
2. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr. Measurement of low-density-lipoprotein cholesterol in serum: a status report. *Clin Chem* 1992 Jan; 38(1): 156-60
3. Belcher JD, McNamara JR, Grinstead GF, Rifai N, Warnick GR, Bachorik P, Frantz Jr I. Measurement of low-density lipoprotein cholesterol concentration. In: Rifai N, Warnick GR, eds. *Methods for Clinical Laboratory Measurement of Lipid and Lipoprotein Risk Factors*. Washington D.C.: AACCPress, 1991: 75-86
4. DeLong DM, DeLong ER, Wood PD, Lippel K, Rifkind BM. A comparison of methods for the estimation of plasma low-density and very low-density lipoprotein cholesterol. *The Lipid Research Clinics Prevalence Study. JAMA* 1986 Nov 7; 256(17): 2372 - 7
5. Okada M, Matsui H, Ito Y, Fujiwara A, Inano K. Low-density lipoprotein cholesterol can be chemically measured: a new superior method. *J Lab Clin Med* 1998 Sep; 132(3): 195 - 201
6. Okubo S, Hashimoto Y, Futamura A, Morita K, Mashige H, Nakarai H, Nakahara K. Comparison of LDL-C measure with the automated method and the ultracentrifugation method in severe hypertriglyceridemia, and the prevalence and the life-style of patients with hypertriglyceridemia. *Rinsho Byori* 1999 Mar; 47(3): 275 - 80
7. Hirany S, Li D, Jialal I. A more valid measurement of low-density lipoprotein cholesterol in diabetic patient. *Am J Med* 1997 Jan; 102(1): 48-53
8. Ticho BS, Neufeld EJ, Newburger JW, Harris N, Baker A, Rifai N. Utility of direct measurement of low-density lipoprotein cholesterol in dyslipidemic pediatric patients. *Arch Pediatr Adolesc Med* 1998 Aug; 152(8): 781 - 91