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Brief Report

The Impact of Low Serum Triglyceride on LDL-Cholesterol Estimation

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Most clinical laboratories directly measure serum triglyceride, total cholesterol, and high-density lipoprotein cholesterol. They indirectly calculate low-density lipoprotein cholesterol value using the Friedewald equation. Although high serum triglyceride (>400 mg/dL or 4.52 mmol/L) devaluates low- density lipoprotein cholesterol calculation by using this formula, effects of low serum triglyceride (<100 mg/dL or 1.13 mmol/L) on its accuracy is less defined.

Two hundred thirty serum samples were assayed during a one-year period. In 115 samples, the triglyceride level was below 100 mg/dL and in 115 samples from age- and sex-matched patients the triglyceride level was 150 – 350 mg/dL (1.69 – 3.95 mmol/L). In both groups total cholesterol was above 250 mg/dL (6.46 mmol/L). On each sample, total cholesterol, high-density lipoprotein cholesterol, and triglyceride were directly measured in duplicate and low-density lipoprotein cholesterol measured directly and calculated with Friedewald equation as well.

Statistical analysis showed that when triglyceride is <100 mg/dL, calculated low- density lipoprotein cholesterol is significantly overestimated (average :12.17 mg/dL or 0.31 mmol/L), where as when triglyceride is between 150 and 300 mg/dL no significant difference between calculated and measured low-density lipoprotein cholesterol is observed.

In patients with low serum triglyceride and undesirably high total cholesterol levels, Friedewald equation may overestimate low-density lipoprotein cholesterol concentration and it should be either directly assayed or be calculated by a modified Friedewald equation. Using linear regression modeling, we propose a modified equation.

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Introduction

Elevation of serum low-density lipoprotein cholesterol (LDL-c) constitutes a major risk factor for the development of atherosclerosis and coronary heart disease.[1] Based on the serum LDL levels the National Cholesterol Education Program (NCEP) suggests different criteria for decision-making in treatment of hypercholesterolemic patients who have coronary heart disease or other risk factors.[2, 3]

The reference procedure for lipoprotein separation and measurement is analytical ultracentrifuge[4]; however, this method is not readily available in the routine laboratory evaluation and its use is confined to research and specialized laboratories.[1] In routine practice, LDL concentration (mg/dL) is estimated indirectly from the measured levels of triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), and total cholesterol (TC) using the Friedewald equation:

$$\mathbf{LDL = TC - HDL - (TG / 5)}\ ^{2}$$

When concentrations are expressed in mmol/L, TG is divided by 2.17 instead of 5.[1,4] Although the accuracy of Friedewald equation has been

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extensively reviewed, and the equation sometimes leads to contradictory results[5–9]; it is still recommended for routine use.[1] Three essential limitations are known for the Friedewald equation; when chylomicrons are present, in patients with type III hyperlipidemia, and when plasma TG exceeds 400 mg/dL (4.52mmol/L). In these circumstances LDL should be directly measured.[1, 4]

There are a few reports indicating the misleading effects of low serum TG (<100 mg/dL or 1.13 mmol/L) on LDL estimation by the Friedewald equation.[10] Therefore, in this study we aim to determine whether low TG may significantly deviate the LDL calculation in hypercholesterolemic patients and if so, can it be corrected by a modified equation?

Materials and Methods

In this study, 230 specimens (two sets of 115) were selected during a one-year study period (October 2002 – 2003) from the fasting adult outpatients referred to our two hospitals' laboratories for lipid profile checking. The patients were hypercholesterolemic but free of underlying diseases such as liver, kidney, or familial lipoprotein disorders. Their serum samples were nonhemolytic, nonicteric, and nonlactescent. Their measured TC level was above 250 mg/dL (6.46 mmol/L).

One set of 115 specimens with TG<100mg/dL (1.13 mmol/L) was chosen to compare with the other set of 115 samples with TG levels between 150 – 350 mg/dL (1.69 – 3.95 mmol/L) from the sex- and age-matched patients. All samples were stored at -20°C until thawing and measured in less than two months. For each sample TC, TG, HDL, and LDL were directly measured in duplicate using Technicon® RA-XT autoanalyzer (Technicon RA-XT, USA). All assay kits were based on enzymatic methods. In these methods TG or cholesterol are cleaved by specific enzymes and final products are linked to a chromogenic substrate. The intensity of light absorbed by chromogen is proportional to the TG or cholesterol concentration. The kits were purchased from a local distributor. The coefficient of variation (CV%) for TG, TC, and HDL methods are 2 – 3%, 1.5 – 2.5%, and 4%, respectively. LDL assay was based on a two-step (homogeneous) selective degradation of non-LDL lipoproteins. In this method only LDL is protected from degradation and then is assayed as above. CV% for this method is <3%.[2]

LDL was also calculated using Friedewald equation. Statistical analyses were performed using SPSS software version 11.5 (SPSS Inc., Chicago, IL). Descriptive statistics for both methods of LDL measurement were expressed as the mean, standard deviation (SD), and CV%. The degree of correlation between the results of the two methods was evaluated by calculating Pearson's correlation coefficient based on the study group. Likewise, we fitted a linear regression model by enter method, specifying directly measured LDL as a function of the TC, TG, and HDL in patients who had TG <100 mg/dL.

Results

Among 61 males and 54 females in any of the two study groups, the mean age±SD was 61±12.4 years in patients with TG<100 mg/dL (group A) and 61±12.2 years in patients with TG level between 150 – 350 mg/dL (group B). The mean, standard deviation, and CV% of measured and calculated serum lipids in duplicated analyses for each group are depicted in Table 1.

Pearson's correlation coefficient between the measured and calculated LDL by Friedewald equation in groups A and B was 0.901 and 0.991, respectively, which are graphically presented in Figures 1A and 1B.

In group A, calculated LDL was on an average 12.17 mg/dL (0.31 mmol/L) higher than measured LDL (CI 95%=10.34 to 14 mg/dL), while in group B, calculated LDL was about 1.48 mg/dL (0.03 mmol/L) lower than measured LDL (CI 95%= -4.21 to 1.25 mg/dL). Comparing the calculated and measured LDL levels by paired *t*-test showed no significant difference (P=0.285) in group B, but the difference was statistically significant (P< 0.001) in group A. The linear regression analysis using data of TC, TG, and HDL of group A patients to estimate LDL (mg/dL) produced the following equation:

LDL (mg/dL) = TC/1.19 + TG/1.9 – HDL/1.1 – 38

Stating the concentrations as mmol/L the equation will be as follows:

Table 1. Mean, standard deviation (SD), and coefficient of variation (CV%) of measured and calculated parameters in each group.

		Serum concentrations					
		TG	**TC**	**HDL**	**LDL-calculated**	**LDL-measured**	**VLDL**
Group A	**Mean**						
	mg/dL	78.47	266.45	55.90	193.38	181.21	17.21
	(mmol/L)	(0.88)	(6.89)	(1.44)	(5.0)	(4.68)	
	SD						
	mg/dL	10.66	20.99	10.25	22.71	19.394	2.15
	(mmol/L)	(0.2)	(0.54)	(0.26)	(0.58)	(0.50)	
	CV%	13.5	7.87	18.33	11.74	10.7	12.49
Group B	**Mean**						
	mg/dL	227.82	291.97	52.17	194.81	196.29	45
	(mmol/L)	(2.57)	(7.55)	(1.34)	(5.03)	(5.07)	
	SD						
	mg/dL	60.03	39.90	11.04	34.865	34.425	11.975
	(mmol/L)	(0.67)	(1.03)	(0.28)	(0.90)	(0.89)	
	CV%	26.34	13.66	21.16	17.89	17.53	26.6

TG=triglyceride, TC=total cholesterol, HDL=high-density lipoprotein, LDL=low-density lipoprotein, VLDL=very low-density lipoprotein.

LDL (mmol/L) = TC/1.19 + TG/0.81 – HDL/1.1 – 0.98

Using this modified equation, the Pearson's correlation coefficient between the direct and indirect LDL measurement in group A approaches 0.976 (Figure 1C) and the average calculated LDL is only about 0.67 mg/dL (0.01 mmol/L) less than measured LDL (CI 95% = -0.72 to 0.85mg/dL) with no significant difference between the two methods (*P*=0.867).

Discussion

Although the normal range of serum TG is 10 – 190 mg/dL (0.11 – 2.14 mmol/L), many hypercholesterolemic patients also have high TG levels. At the present time only TG concentration higher than 400 mg/dL (4.52 mmol/L) is considered a limiting factor for the application of routine Friedewald equation. This study indicates that in TG levels between 150 – 350 mg/dL (1.69 – 3.95 mmol/L) current Friedewald equation has

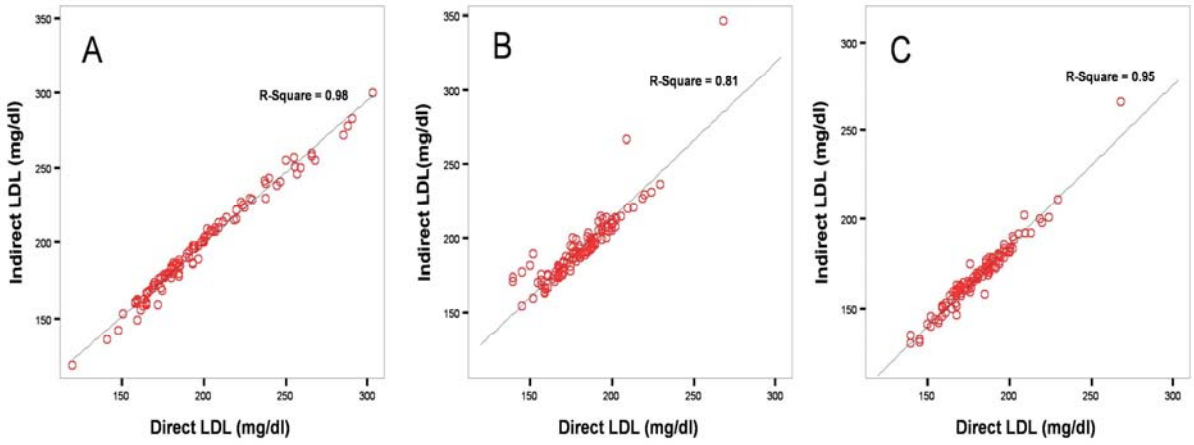

Figure 1. Correlation between directly measured and indirectly calculated LDL levels. A) In patients with TG>150 mg/dL (group B) using Friedewald equation, B) in patients with TG<100 mg/dL (group A) using Friedewald equation, and C) in patients of group A using the newly proposed equation. LDL=low- density lipoprotein, TG=triglyceride (dl>dLin the diagram).

only in an average 1.5 mg/dL (0.03 mmol/L) negative error in LDL estimation, which is not statistically significant and probably it is not important clinically. Therefore, the reliability of Friedewald equation in this group of Iranian population is confirmed. Similar to the reported case of Wang et al.,[10] this study indicates that with low concentrations of TG, LDL calculation by current method may overestimate the serum LDL to such a degree that may affect the clinical decision-making. As shown in Figure 1C, modified Friedewald equation compensates LDL over-estimation caused by low TG level. However, more studies using larger samples taken from different ethnic and geographic populations and preferably compared with reference method i.e., ultracentrifuge and precipitation would accomplish this work. Additionally, considering the different ranges of low TG (including levels between 100 – 150 mg/dL or 1.13 – 1.69 mmol/L) will certainly give more information about the degree of inaccuracy of Friedewald equation in these situations.

In conclusion, when using the unmodified Friedewald equation, low serum TG may positively affect the LDL calculation and to correct this error, the LDL level should either be directly measured or be adjusted by a modified formula. Such a modified equation may be practically applicable when associated with a laboratory reporting software.

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