A COMPARISON OF CALCULATED WITH DIRECT MEASUREMENT OF LOW DENSITY LIPOPROTEIN CHOLESTEROL LEVEL

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Abstract

The management of dyslipidemia are largely based on the concentration of low-density lipoprotein cholesterol (LDL-C). Though most clinical laboratories estimate the concentration of LDL-C by using calculation formula, direct methods for an accurate quantification of LDL-C are needed. Aim of the study was to determine if, and to what extent, LDL-C level is underestimated when it is estimated by using calculation formulas compared with the LDL-C level measured by a direct method and compare the percentages of patients meeting LDL-C goal using calculation formulas & direct method. Total cholesterol, HDL-C and TG were measured by using an Abbott VP Auto analyzer and Sigma Reagents, LDL-C was measured by using homogeneous assay (Wako method) and the estimation of the LDL-C was calculated by using the Friedwald formula, modified Friedwald formula, Anandaraja formula. From 820 lipid profiles 755 were included (TG <400 mg/dl) in the analysis. Study showed significant statistical difference (p <.001) between measured and calculated LDL-C level.

Though strong correlation was found between measured and calculated LDL-C level the calculated LDL-C methodology underestimated LDL-C levels by 11 to 23 mg/dl in different calculation formulas compared with measured LDL-C. The degree of underestimation increased as the triglyceride level increased ($p<0.05$) and resulting in a loss of LDL-C goal attainment for one third of the patients when LDL-C level was measured versus calculated ($p<0.0001$).The results of our studies showed that the direct LDL-C cholesterol assay is a more reliable and accurate method than the calculation formula for LDL-C cholesterol determination.

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Introduction

Elevated plasma low density lipoprotein cholesterol (LDL-C) concentration is a well-known atherogenic risk factor with highest predictive value for coronary heart disease (CHD) among all lipoproteins [1]. Separation of lipoproteins by combining ultracentrifugation with precipitation-'β quantification' is considered the gold standard for measuring LDL-C level [2]. Although β quantification is the method of choice, this process is not readily suited for routine use, as it is labor intensive, time consuming, and requires expensive instruments [3, 4]. More than 90% of laboratories in the United States estimate LDL-C levels using the Friedewald formula (FF): LDL-C = TC – HDL-C - (TG/5) [2, 5]. LDL-C level cannot be accurately estimated if the triglyceride value exceeds 400 mg/dl, as the triglyceride: total cholesterol ratio of VLDL-C will differ [5]. Caution should be taken against using this formula for patients with chylomicrons or dysbetalipoproteinemia [5]. Some other formulas, like modified Friedewald formula (mFF): LDL-C = TC-HDL-C - (TG/6) [6], Anandaraja formula (AF): LDL-C = (0.9 TC-(0.9 TG/5)-28 [7] etc also used in some countries to calculate LDL-C value. These formulas also underestimate calculated LDL-C value than measured value.

Homogeneous assays, developed in 1998 in an effort to overcome the limitations existing with both β quantification and the Friedewald formula, represent the third generation of LDL-C direct measurements. The assays contain different detergents to achieve specificity for LDL-C. Expected improvement in precision over earlier methods, including the Friedewald calculation, has been confirmed with the homogenous assays, and each of the five commercially available assays has been certified by the Cholesterol Reference Method Laboratory Network of the Centers for Disease Control and Prevention[2]. One of this homogeneous assay by using the Olympus AU640 analyzer (Olympus America, Inc., Melville, NY) is a two-reagent system using the Wako method of LDL-C quantification [2]. The method was compared with β quantification and shown to be accurate and precise[9]. After implementation of this assay, the

KCVAMC lipid clinic staff suspected that the LDL-C levels formerly calculated using the Friedewald formula may have underestimated the LDL-C value. In addition, patients previously meeting NCEP-defined goals were no longer below target.

We designed a study to determine if, and to what extent, the LDL-C value was underestimated when calculated by the Friedewald, modified friedwald & Anandaraja formula when compared with the Olympus AU640 homogeneous assay. Another objective was to determine and compare the percentages of patients achieving LDL-C goal using each of these two methods.

Methods

Data were collected including patient of at least 18 years of age and those who came to the laboratory for doing a complete lipid profile (total cholesterol, HDL, D-LDL-C, and triglycerides) from September 2009-January 2010. Patients were excluded if the lipid profile was incomplete. Lipid profiles containing a triglyceride level above 400 mg/dl also were excluded.

Lipoprotein analysis

Prior to obtaining blood from an antecubital vein, patients assumed a sitting position for 5 min, since postural changes can alter serum cholesterol concentrations. The blood samples were collected into tubes without anticoagulant and centrifuged to harvest serum after separation from the clot within 2 h. Samples were analyzed in same day. Total cholesterol, HDL-C and TG were quantitated by using an Abbott VP Auto analyzer and Sigma Reagents. LDL-C was determined indirectly by using the FF [5] as follows: LDL-C = TC_HDL-C_TG/5, mFF: LDL-C = TC- HDL-C - (TG/6) [6] and AF: LDL-C = (0.9 TC-(0.9 TG/5)-28 [6]. The direct LDL-C measurement of LDL-C was performed by using the homogeneous LDL-C assay from Wako Chemicals, which is distributed by Sigma Diagnostics, contains two ready-to-use reagents. Reagent 1 consists of Good's buffer [pH 6.8; N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline, sodium salt], cholesterol esterase, cholesterol oxidase, catalase, polyanions, and amphoteric surfactants, which selectively protect LDL-C from enzyme reaction. The non-LDL-C cholesterol reacts with cholesterol esterase and cholesterol oxidase, producing hydrogen peroxide, which is consumed by catalase. Reagent 2 contains Good's buffer (pH 7.0), 4-aminoantipyrene, peroxidase, sodium azide, and deprotecting reagent. The nonionic surfactants remove the protecting agent from LDL-C, enabling the specific reaction of cholesterol esterase and cholesterol oxidase with LDL-C. The resulting hydrogen peroxide yields color with Trinder's reagent and 4-aminoantipyrene in the presence of peroxidase. Serum (3μL) is added to 270 μL of reagent 1 and incubated at 37 °C for 5 min; 90 μL of reagent 2 is then added and incubated for another 5 min. The blue color complex produced has an absorbance peak at 600 nm and is measured at 600 nm (primary) and 700 nm (secondary) [9, 10].

Statistical analysis

Data are reported as mean ± SD. Paired t-test and Pearson correlation analyses were performed to assess significant differences and correlation in LDL-C concentrations obtained by calculation and direct measurement. A 'p' value of less than 0.05 was considered statistically significant. Microsoft Excel and SPSS, version 11.5 (SPSS Inc., Chicago, IL), statistical programs were used for analysis of the study data.

Results

A total of 820 lipid profiles were assessed. Of these, 65 (7.9%) were excluded because they contained a triglyceride level greater than 400 mg/dl. Most of the profiles were from men in their fifth decade of life. Paired't' test was done and it showed significant statistical difference ($p < .001$) between measured and calculated LDL-C level (Table-I). NCEP LDL-C goal is below 130 mg/dl.[5] From total of 755 lipid sample 481 (63.7%) were at goal when the LDL-C level was calculated using the FF, 444 (58.8%) by using modified FF, 513 (67.9%) by using Anandaraja formula compared with 367 (48.6%) when LDL-C was measured directly ($p<0.001$). A strong correlation was found between measured-LDL-C and all calculated LDL-C (m-LDL-C vs FF-LDL-C: $r = 0.786$ (Fig-1); m-LDL-C vs mFF-LDL-C: $r = 0.796$ (Fig-2); m-LDL-C vs AF-LDL-C: $r = 0.81$ (Fig-3)). Based on the regression equation, the C-LDL-C methodology underestimated LDL-C levels by 16.7 mg/dl in FF-LDL-C, 10.5 mg/dl in mFF-LDL-C, 22.35 mg/dl in AF-LDL-C compared with m-LDL-C. As triglyceride levels increased, the absolute difference between the two methods also increased; statistically significant differences ($p<0.01$) existed between each of the cohorts (Table II).

Table I : Paired samples statistics & correlations

	Mean Value	Mean Difference	Correlation (r)	t	'p' Value
mLDL-C vs fLDL-C	134 ± 35.9 117 ± 42.5	16.7	0.786	17.4	<.001
mLDL-C vs mfLDL-C	134 ± 35.9 123.5 ± 42.7	10.5	0.796	11.2	<.001
mLDL-C vs Anandaraja	134 ± 35.9 111.7 ± 41	22.35	0.810	25.37	<.001

Table II: Summary of measurement of total cholesterol, HDL-C, LDL-C (Direct), LDL-C (Friedewald formula), LDL-C (modified Friedewald) & LDL-C (Anandaraja formula) according to triglyceride levels; presented as mean ± standard deviation.

Triglycerides	Total CHolesterol	HDL-C	LDL-C (Direct)	LDL-C Friedewald formula	LDL-C modified Friedewald	LDL-C Anandaraja formula
≤ 100mg/dl	159.45±39.37	40.1±110.26	118.12±28.73	101.35±34.76	104.34±34.87	98.2±34.82
101-200 mg/dl	187.5±44.9	38.63±10.1	132.4±35.5	119.46±41.7	124.3±41.7	114.6±40.2
201-300 mg/dl	204.6±48.8	35.9±9	141.45±35.8	119.77±45.3	127.93±45.3	111.8±43.8
301-400 mg/dl	223.05±47.4	36.2±8.3	148.97±33	118.53±43	129.9±43.1	111.26±42

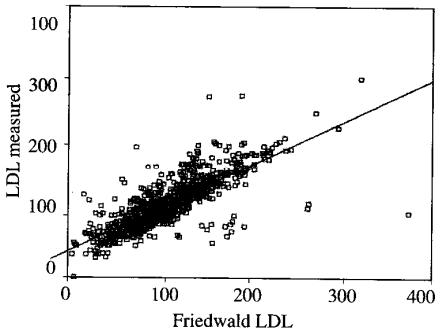

Fig 1: Correlation of measured value of LDL-C with Friedwald formula (r = .786, p<.001)

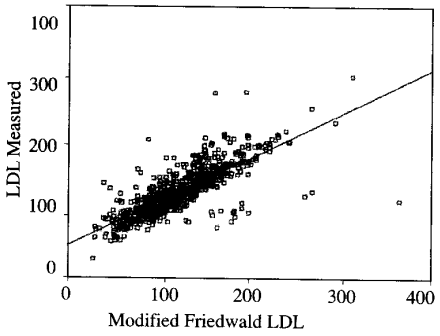

Fig 3: Correlation of measured value of LDL-C with modified Friedwald formula (r = .796, p<.001)

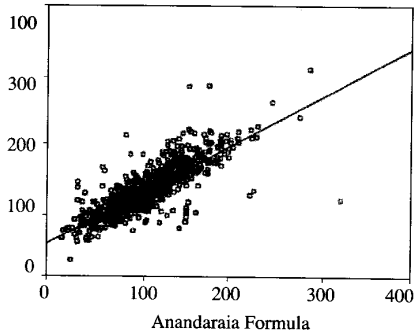

Fig 2: Correlation of measured value of LDL-C with Anandaraja formula (r = .810, p<.001)

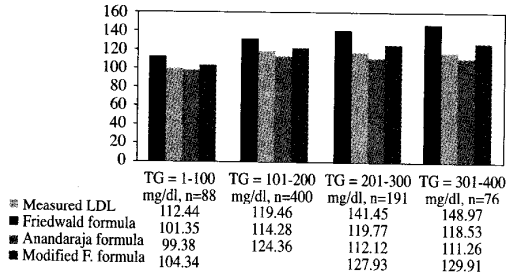

Fig 4: Comparison of measured vs calculated values of LDL-C

Discussion

Although a correlation exists between the measured and calculated LDL-C values of study subjects, use of the calculated LDL-C determined by the Friedewald, modified Friedwald & Anandaraja formula underestimated the LDL-C level when compared with the Olympus AU640 homogeneous assay. This difference broadens with increased triglyceride levels. In addition, almost one third of patients at or below their NCEP-defined LDL-C goal were no longer at target when D-LDL-C was employed in the assessment process.

Most studies of compliance with NCEP goals and CHD risk reduction have used the Friedewald formula rather than direct measurement of LDL-C [11-14]. An exception is the Heart Protection Study, which directly measured LDL-C level in more than 20,000 adults aged 40-80 years with coronary disease, other occlusive arterial disease, or diabetes [8, 15]. Primary outcomes of this randomized study were mortality and fatal or nonfatal vascular events. Results demonstrated a 25% reduction in vascular disease risk when lowering D-LDL-C from 116 mg/dl to less than 77 mg/dl, implying the need for more aggressive treatment than currently recommended.

An additional method of assessing CHD risk nuclear magnetic resonance may be available in the near future and shows promise for routine measurement of lipoprotein levels. This method quantifies lipoproteins by subclasses based on size. It is not influenced by variability in cholesterol composition. Currently, outcome data are not available for this method of measure. However, frozen plasma samples from ongoing or complete clinical trials will be analyzed by this approach to determine if it improves prediction of coronary artery disease outcomes [16].

One other study has compared a third-generation homogenous assay to calculated LDL-C levels [17]. A strong correlation (r = 0.86) existed between direct (EZ LDL-C Cholesterol; Sigma Diagnostics, St. Louis, MO) and calculated LDL-C. In addition, this study demonstrated an overestimation of the measured LDL-C level of 18 mg/dl compared with the calculated LDL-C level determined by the Friedewald formula. This study's end point was similar to that of our study, but it used a different homogenous assay, had a small sample size, and lipid samples were collected over a single week.

For our analysis, data were collected retrospectively. However, all data extractions from the computerized patient record system were conducted by one analyzer to limit the chance of variability between raters. Lipid profiles were collected over 3 months to minimize the effect of confounding variables. In addition, our laboratory used only one direct method for obtaining LDL-C; therefore, studies that use a different method may obtain different results. We assumed that lipid profiles were collected in a fasting state, without food for a minimum of 12 hours. It is important to note that a nonfasting state can increase the triglyceride level and potentially underestimate the value of C-LDL-C, whereas the direct method is not limited by timing of food ingestion. All patients at the KCVAMC are sent a letter before the date of their laboratory appointment reminding them of the necessity to fast. Various patient populations and lipid abnormalities have the potential to influence either method [5, 18-20]. This study was not designed to evaluate all potential subgroups. In addition, this study might have benefited from an additional comparison using quantification. Although the study had a sufficiently large sample size to determine the difference between measured vs calculated methods, it was not of sufficient duration to evaluate CHD-related outcomes.

Conclusion

An underestimation of approximately 17 mg/dl, 11 mg/dl, and 22 mg/dl was found when LDL-C levels resulting from application of the Friedewald formula, modified Friedewald formula & in anandaraja formula were compared with the Wako method-derived direct LDL-C level measurement. These resulted in loss of LDL-C goal attainment for one third of the patients. In applying the NCEP ATP III guidelines in patient management, clinicians as well as laboratorians should be aware of the circulatory heterogeneity of LDL-C particles and the potential limitations of the calculation formula. They should avoid application of the calculated LDL-C. It should be kept in mind That the standardization of the LDL-C assays is obvious. Adjusting the LDL-C measurements would be expected to invalidate to some extent the NCEP ATP III risk-based cutoff values for LDL-C.

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