Original Article

COMPARISON OF CALCULATED METHOD WITH MEASURED METHOD FOR LOW DENSITY LIPOPROTEIN CHOLESTEROL

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ABSTRACT:

OBJECTIVE: Most of the labs use calculated method for measurement of Low density lipoprotein cholesterol. We conducted this study to know the accuracy of calculated method versus measured method for determination of LDL cholesterol level.

MATERIAL AND METHODS: This Cross sectional hospital based study included 153 consecutive healthy individuals aged 17 to 75 years. The patients were selected from Pathology department of Punjab Institute of cardiology, Lahore over a period of 6 months from 1st June 2014 to 31st December 2014. Patients with alcohol consumption, history of smoking, Diabetes Mellitus, liver disease, renal disease or on any lipid lowering agent and without proper fasting were excluded from the study. Data was analyzed using SPSS version 20.0

RESULTS: Out of 153 patients, 90 (58.8 %) were male while 63 (41.3 %) were female patients. The mean age of the participants was 46.46± 12.58 years, Low density lipoprotein cholesterol measured by measured method and calculated by Friedewald formula showed uniformity in Triglyceride group ≤100 but there was significant difference in other groups ranging from 101 – 401 mg/dl of TG. It indicated that the Friedewald formula was not rightly predicting the accurate mean Low density lipoprotein cholesterol value as the TG level goes beyond the 100mg/dl.

CONCLUSION: Calculated Low density lipoprotein cholesterol is not reliable when serum triglyceride (TG) levels exceed 100mg/dl and measured method is more accurate than calculated method at different TG levels.

KEYWORDS: NCEP: National Cholesterol Education Program, LDL_C: Low density lipoprotein cholesterol, CAD: Coronary artery disease, TG: Triglyceride, Friedewald formula: FF, HDL-C: High density lipoprotein cholesterol

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INTRODUCTION:

Hyperlipidemia is playing an important role in the pathogenesis of coronary artery disease[1]. Cardiovascular disease risk assessment increases with an increase in hyperlipidemia levels as 2.2% in subjects with LDL cholesterol ≤3.0 mmol/L, 5.4% for LDL cholesterol between 3.01 to 4.0 mmol/L, 11.5% for LDL cholesterol between 4.01 to 5.0 mmol/L and 11.8% for LDL cholesterol >5.0 mmol/L.[2]

In men, the relative risk of CAD increased at an apoB/apoA-I ratio of 1.0, and the comparative figure for females was 0.86.[3] The diagnosis routine measurement in the evaluation and management of hyperlipidemia is largely based on LDL-C level. There are several competing methods for measurement of lipoprotein particle concentrations and size. Direct measurement is used in the evaluation and management of LDL-C, but this is too expensive for use in most laboratories.[4]

Friedewald in 1972 established a formula to estimate LDL-C as an alternative to tedious ultra centrifugation. Because VLDL carries most of the circulating TGs, VLDL-C can be estimated reasonably well from measured TGs divided by 5 for mg/dl units. LDL-C is then calculated as total Cholesterol minus HDL-C minus estimated VLDL-C.

The formula requires fasting plasma high density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), and TG, and is calculated as LDL-C = TC − HDL − (TG / 5) for mg/dl). This formula is less accurate at extremes of TG values or in patients with renal dysfunction and DM & other co-morbid conditions, but is widely used. Several other formulae have been developed, but these did not perform better than Friedewald's calculation or had varying results in different population groups and including those considering TG ratios.[5]

Method for direct measurement of LDL-C is not simple and accurate. Therefore, the calculation of LDL-C by Friedewald formula, which has been

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widely accepted as an accurate alternative and efficient in terms of cost and performance.[6] Because of the importance of LDL-C in CAD risk assessment, the measurement of LDL should be accurate. LDL measurement should be carried out directly.[7]

This study was conducted to check the validity and reliability of Friedewald formula to make the lipid profile cost effective with respect to direct method for LDL-C measurement in clinical labs. We compared measured LDL-C method with the widely used calculated (Friedewald's) method to estimate the differences and correlation of LDL-C and to assess whether any of the above mentioned method affects the classification of the patients for CAD risk according to the guidelines of NCEP.

MATERIALS AND METHODS:

This cross sectional hospital based study of 153 consecutive healthy individuals between the age group 17 to 75 years were selected from Pathology department of Punjab Institute of cardiology, Lahore over a period of 6 months from 1st June 2014 to 31st December 2014. Patients with alcohol consumption, history of smoking, Diabetes Mellitus, history of liver disease, renal disease or taking any lipid lowering agent and those without proper fasting were excluded from the study.

Before taking sample for lipid profile, informed consent was obtained from each patient.

Blood samples were obtained after a 12 to 14 hour fast, and centrifuged at 2,000 x g for 5 minutes. After specimen collection, serum was separated and the assays were performed within 02 hours of sample collection.

All biochemical lipid analysis was done on Hitachi 912 chemistry auto analyzer by using kit by (Roche Diagnostic). [8]

Serum total cholesterol (TC) was measured by enzymatic endpoint method with a coefficient of variation (CV) of 3.1%. Serum triglyceride (TG) was measured by enzymatic method with a CV of 3.6%. Serum high density lipoprotein cholesterol (HDL-C) was measured by direct homogeneous assay with a CV of 5.6%. Serum low density lipoprotein cholesterol (LDL-C) was measured by direct Enzymatic Assay with a CV of 4.9%.[9, 10]

In calculated LDL-C assay, cholestest- LDL contains two ready to use stable liquid reagents that directly measure the concentration of LDL-C by homogenous method based on detergent technology. The detergent 1 in Reagent 1disrupts the structure of HDL, VLDL and chylomicrons and causes release of cholesterol. The cholesterol esterase releases free cholesterol. Cholesterol oxidase releases hydrogen peroxide from free cholesterol, which reacts with 4- aminoantipyrine in the presence of peroxidase to give a colorless product. The second step starts with the addition of Reagent 2. Detergent 2 in Reagent 2. specifically acts on LDL releasing cholesterol. With the action of Cholesterol esterase and Cholesterol oxidase, hydrogen peroxide is liberated from free cholesterol of LDL. The coloring agent N, N- bis (4- Sulfobutyl) m- toluidine disodium salt (DSBmT) reacts with hydrogen peroxide in the presence of Peroxidase to give a bluish purple product measured at 546nm main and 660 subsidiary. The intensity of color is proportional to concentration of LDL-C. Cholestest calibrator was used for calibration of both HDL-C and LDL-C.

LDL-C levels were also calculated by Friedewald's formula (FF); LDL-C = TC - (HDL-C +TG/5).10

Data was analyzed by using SPSS (Statistical Package for Social Sciences) Version 20.0 for Windows. Difference between the quantitative variables with both methods was analyzed by using paired sample t test or univariate analysis. P-value ≤ 0.05 was considered as significant. All tests applied were two tailed.

RESULTS:

Out of total 153 patient, 90 (58.8 %) were male and 63 (41.3 %) were female. The overall mean age of the participants was 46.46±12.58 years and range was 17-75 years.

The mean of LDL-measured was lower than LDL-calculated (104.7±36.19 mg/dL vs. 114.3±36.33 mg/dL). The mean difference between the two methods was significant, which was 9.6 ± 14.3

Figure-1: Correlation between the LDL-C measured by direct method and calculated by friedewald's formula.

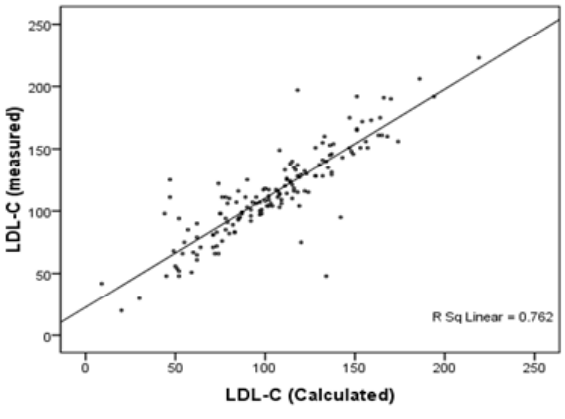

Table-1: Difference between mean of LDL-C measured by direct method and calculated by Friedewald's formula with respect to different grouped TG distribution.

Triglyceride Range	LDL-C (measured)	Friedewald's LDL-C (calculated)	Mean difference (error)	P-value
1-100	92.90 ± 35.38	92.94 ± 37.47	0.04	0.998
101-200	118.81 ± 33.86	108.60 ± 35.32	10.21	0.0103
201-300	120.41 ± 31.80	110.14 ±28.29	10.41	0.0031
301-400	127.27 ± 41.23	101.36 ± 41.10	25.91	0.001
>401	115.60 ± 54.62	93.60 ± 63.03	22.0	0.001

Table-2: Classification of the patients on the basis of LDL obtained by measured and calculated method.

LDL-C	LDL-calculated	LDL-measured
<130	115 (75.16%)	108(70.58%)
≥130	38 (24.83%)	45(29.41%)

mg/dl (P < 0.001). However, the correlation between measured and calculated LDL levels was high, as r= 0.873 and adjusted r2 = 76.2% with a P-value 0.001 (fig-1).

Measured LDL-C produced higher results as compared with calculated LDL-C. The mean and standard deviation (SD) of LDL-C measured and calculated showed insignificant uniformity in group TG (1 – 100) but there was significant difference in other groups ranging from 101 – 401 mg/dl of TG, indicated that the calculated LDL-C was not independently predicting the accurate mean LDL obtained by measured method as the TG level goes beyond the 100mg/dl. It has been delineated that if serum TG level exceeds 100-300 mg/dl, the formula cannot be accurate.

Patients were classified as low and high cardiac risk taking 130 mg/dl LDL-C as cut- off levels. By LDL-C calculated, 24.83% of patients had high risk and 29.41% of patients had high risk by LDL-C measured (table-2).

DISCUSSION:

Our results demonstrated a highly significant correlation between LDL-measured and LDL-calculated. However, the LDL-measured underestimated the LDL level compared to the LDL-calculated method. The mean level of LDL-calculated was approximately 0.096 mg/ dl more than that of LDL-measured. LDL-measured classified 29.41% of patients as high cardiac risk whereas there were 24.83% by LDL-calculated assay.

We confirmed previous findings that the LDL-calculated performance decreases with increasing TG levels. We found insignificant differences in the measured method and LDL-calculated (Friedewald formula) when TG level was < 100 mg/dl, but when TG level was 100 - 399 mg/dl the difference between both methods widened progressively.

Our results are comparable with the previous literature by Anwar M[10] et al reported highly significant correlation between LDL-measured and LDL-calculated r=0.93. However, the LDL-measured underestimated the LDL level compared to the LDL-calculated method (2.81±0.82 mmol/L vs. 2.93±0.81 mmol/L). The mean level of LDL-measured was approximately 0.12 mg/ dl less than that of LDL-calculated. LDL-calculated classified 2% of patients as high cardiac risk whereas there were 2.7% by LDL-measured assay, also reported that LDL-calculated homogenous assays have a uniform performance for LDL-C estimation at TG levels ≤ 150 mg/dl while at TG level > 150 LDL-calculated performance was decreases.

A further study by Bairaktari ET[11] et al reported that LDL-measured underestimated the LDL level compared to the LDL-calculated method as 102.7 mg/dl ± 32.9 mg/dl vs. 113.9 mg/dl ± 40.2 mg/dl. The correlation between the two methods was r = 0.86. LDL-calculated have a uniform performance for LDL-C estimation at TG levels ≤200 mg/dl while the performance of LDL-calculated was inconsistent as the TG level > 200 mg/dl.

Kamal[12] et al, and Chen[13] et al established that the LDL-calculated method have a uniform performance for LDL-C estimation at TG levels ≤100 mg/dl while the performance of LDL-calculated was poor as the TG level goes beyond the 200 mg/dl (p-value <0.05). The mean LDL-measured was lower than calculated as (117±42 mg/dL vs. 134±35 mg/dL;P-value = 0.001) 17 ± 26 mg/dL higher than measured LDL-C.

Another study Knopfholz J[14] et al scrutinized the patients with metabolic syndrome and he found that Friedewald formula is a reliable method to estimate serum LDL-c in patients with MS.

Martins J[15] et al found that the mean LDL obtained by calculated method was higher than by measured as (112 ± 45 mg/dl vs. 108 ± 48 mg/dl). Reported good correlation between LDL-calculated and LDL-measured method as r=0.963, also reported higher specificity 95.4% while lower sensitivity 84.9% for high cardiac risk taking 130 mg/dl LDL-C as cut- off levels showing an accuracy of 90.2% and measured LDL performance decreases with increasing TG levels.

Ahmadi[16] et al reported that the mean LDL-C estimated by calculated and measured method

showed significant difference at lower TG ranges of <100 mg/dl while at TG level 150- 300 mg/dl insignificant difference was found, although both methods showed insignificant correlation (r > 0.976; P-value= 0.867).

Another study by Suchanda[6] et al who established that LDL measured classified 23.5 % of patients as high cardiac risk whereas there were 17.58% by LDL-calculated assay, also showed a good correlation between LDL-C levels obtained by measured and calculated methods, r= 0.88. The mean LDL-C estimated by calculated and measured method showed a significant difference at lower TG ranges of <200 mg/dl respectively. Present study showed dissimilar results due to the different technique, regents and instrument use for the measurement of HDL- Cholesterol (HDL-C), Triglycerides (TGs) and total Cholesterol (TC) and population variation.

Present study has certain limitations. LDL calculated from Friedwald formula contains TG/5 ratio for the calculation of VLDL should be readjusted for large scale.

CONCLUSION:

LDL-measured method is more valid, reliable, accurate and precise for the estimation of LDL-C level as compared to measured by LDL-calculated at TG level > 100 mg/dl. Therefore LDL-measured method should be used for the LDL-C measurement in routine clinical laboratories.

REFERENCES

1.Carmena R, Duriez P, Fruchart JC. Atherogenic lipopro tein particles in atherosclerosis. Circulation. 2004;109[suppl III]:III-2–III-7.

2.Mohan V , Deepa R , Rani SS, Premalatha G. Prevalence of Coronary Artery Disease and Its Relationship to Lipids in a Selected Population in South India. J Am Coll Car diol.2001;38(3):682-687.

3.Mudd JO, Borlaug BA, Johnston PV, Kral BG, Rouf R, Blumenthal RS et al. Beyond Low-Density Lipoprotein Cholesterol: Defining the Role of Low-Density Lipoprotein Heterogeneity in Coronary Artery Disease. J Am Coll Car diol. 2007;50(18):1735-1741.

4.Berglund L, Brunzell J D, Goldberg A C, Goldberg I J, Sacks F, Murad M H, & Stalenhoef A F H . Evaluation and Treatment of Hypertriglyceridemia: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2012 Sep; 97(9): 2969–2989

5.Martins J, Olorunju S A, Murray L M, Pillay T S.Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients. Clin Chim Acta. 2015 Apr 15;444:137-42.

6.Sahu S, Chawla R, Uppa B. Comparison of two methods of estimation of low density lipoprotein cholesterol, the direct versus friedewald estimation. Indian. J Clin Biochem. 2005 Jul; 20(2): 54–61.

7.Boshtam M, Ramezani MA, Naderi G, Sarrafzadegan N. Is Friedewald formula a good estimation for low den sity lipoprotein level in Iranian population? J Res Med Sci. 2012;17(6):519-522.

8.Friedewald WT, Levi RI, Fredrickson DS. Estimation of the concentration of low density lipoproteins cholesterol in plasma without use of the ultracentrifuge. Clin Chem 1972;18:499–502.

9.Jabbar J, Siddiqui I, Raza S Q, Baig A. To Compare the Total Cholesterol and HDL-Cholesterol before and after Ultra- Centrifugation in Lipemic Samples. JPMA. 2005 Jun;55(6):239-42.

10.Anwar M, Khan D A and Khan F A. comparison of freide wald formula and modified Friedewald formula with direct homogenous assay for low density lipopro tein cholesterol estimation. J Coll Physicians Surg Pak. 2014 Jan;24(1):8-12.

11.Bairaktari ET, Tzallas C, Kalientzidoub M, Tselepis AD, Siamopoulosb KC, Seferiadis KI et al. Evaluation of al terna tive calculation methods for determining low-density lipoprotein cholesterol in hemodialysis patients. Clinical Biochemistry 2004;37 :937 – 940

12.Kamal AH, Hossain M, Chowdary S, Mahmud N. A com parison of calculated with direct measurement of low den sity lipoproteincholesterol level. J Chittagong Med Coll Teach Assoc 2009; 20:19-23.

13.Chen Y, Zhang X, Pan B, Jin X, Yao H, Chen B, et al. A modified formula for calculating low density lipoprotein cholesterol values. Lipids Health Dis. 2010 May 21;9:52

14.Knopfholz J, Disserol CCD, Pierin AJ, Schirr FL, Streisky L, Takito LL et al. Validation of the Friedewald For mula in Patients with Metabolic Syndrome. Cholesterol. 2014; 2014:1-5.

15.Martins J, Olorunju SAS, Murray LM, Pillay TS. Compari son of equations for the calculation of LDL-cholesterol in hospitalized patients. Clinica Chimica Acta 444 (2015) 137–142.

16.Ahmadi SA, Boroumand MA, Gohari-Moghaddam K, Tajik P, Dibaj SM. The impact of low serum triglyceride on LDLcholesterol estimation. Arch Iran Med 2008; 11 :318-21.