Friedwald's Calculation; Is It Reliable in Estimating Risk of Coronary Heart Disease?

Shubho S Biswas*, Vaishali Jain, Prerna V Gokhale***, Vandana Agrawal******

* Professor, **Professor, *** Assistant Professor, Biochemistry, ****Professor, Pathology, Ln Medical College, Bhopal, MP,

Abstract: Background and Objectives: Accurate determination of LDL-C is important for the identification and management of patients at risk of CHD. The limitations of the Friedwald's equation led to the expensive direct homogenous assays and an interest in non-HDL-C as markers of risk of CHD, particularly in patients with elevated triglycerides. There are conflicting reports on whether the Friedwald's underestimates LDL-C in comparison to direct, so this study compared the two methods in 800 out patients. Also, comparison of CHD risk detection was done by LDL-C direct, LDL-C calculated and non-HDL-C. Methods and Results: LDL-C by direct method was significantly higher than calculated (mean difference 7.75 mg/dl) and the difference was progressively higher across all categories of TG and TC. The Friedwald's calculation underestimated 570 (71.25%) compared to direct method. Using NCEP risk categorization for LDL-C (<130mg/dl low risk) and non-HDL-C risk categorization of 30mg/dl above that of LDL-C, those at higher risk of CHD numbered 320 (40%) by the direct, 270(33.75%) by non-HDL-C and 220(27.5%) by the calculated method. Overall, 660 (82.5%) out of 800 were similarly classified by all three methods. Conclusion: Although the Friedwald's calculation performs reasonably well, it underestimates LDL-C compared to direct method. It misses identifying a significant number of patients at risk of CHD by direct LDL-C and by non-HDL-C. [Biswas S NJIRM 2017; 8(4):27-31]

Key words: Friedwald's calculation, direct method, non-HDL-C

Author for correspondence: Biswas Shubho Subrata, B-409, 5th Floor, Sagar Premium Towers-Phase 1,Shirdipuram, JK Hospital Road, Kolar, Bhopal-462042 T: 0755-4087001, M: 9302066808, E-Mail: drssbis@gmail.com

Introduction: Low density lipoprotein cholesterol (LDL-C) has strong association with atherosclerosis and coronary heart disease (CHD).[1, 2] Hence, accurate determination of LDL-C is important for the identification and management of patients at risk of CHD. The Report of the National Cholesterol Education Programme's Adult Treatment Panel III (NCEP-ATP III) found LDL-C appropriate to classify patients into three categories of risk for CHD, low risk <130 mg/dl, borderline high risk 130–159 mg/dl and high risk 160 mg/dl or more.[3] However, in the presence of two or more risk factors like diabetes, family history, hypertension, smoking, low high density lipoprotein cholesterol (HDL-C), it recommended that LDL-C should be kept below 100 mg/dl.

LDL-C measurement based on ultracentrifugation (beta-quantification method) is accurate but not feasible in routine use as it is costly and labour intensive.[4] The Friedwald's equation for calculated LDL-C is recommended by the NCEP as a reliable method of LDL-C measurement in routine practice.[5] However, it can be used only in the fasting state and cannot be used when triglycerides are above 400mg/dl.[6] These limitations led to the development of expensive direct homogenous assays which could be applied even in the non-fasting state or in presence of hypertriglyceridaemia.[7]

Further non-high density lipoprotein-cholesterol (non-HDL-C) is now regarded as a very effective marker of risk of CHD in patients with elevated triglycerides (> 200 mg/dl) as it represent cholesterol present in all atherogenic lipoproteins. The targeted goal for non-HDL-C has been established at 30 mg/dl above the patient's LDL-C for every risk category, based upon the observation that when triglyceride levels are ≤150 mg/dl, VLDL-C values are usually ≤30 mg/dl.[8]

Studies carried out in India comparing the performance of Friedwald's equation with the direct assay have reported conflicting results. Underestimation of LDL-C has been reported by both the Friedwald's calculation[9] and the direct method.[10] Since the Friedwald's calculation is in routine use, underestimation of LDL-C by it has significant implication in delaying identification of patients at risk of CHD.

We therefore, carried out this study to compare measurement of LDL-C by the direct and calculated methods. In addition, we compared the CHD risk detection by LDL-C direct, LDL-C calculated and non-HDL-C.

Methods: This study was carried out on 800 outpatients who came for lipid profile estimation. It had 540 males and 260 females of mean age 50.02 ± 12.84 in the age range of 20 to 85. Prior permission was obtained from the Institutional Ethical Committee and informed consent was taken from the patients.

Twelve hour overnight fasting venous blood samples were collected in plain vial and centrifuged.

Serum total cholesterol (TC) was measured by Accurex CHOD-PAP kit and serum triglyceride (TG) was measured by Erba GPO-Trinder kit, both based on enzymatic end point methods.[11,12] Samples with TG>400mg/dl were excluded from analysis. Direct HDL-C and Direct LDL-C were estimated by the AutoPure T HDL-C and AutoPure T LDL-C kit respectively, based on selective solubilization of HDL-C or LDL-C by a detergent enabling its measurement by a conventional enzymatic reaction with cholesterol esterase, cholesterol oxidase and peroxidase.[13] All the samples were processed on Biosystems A25 fully automated analyzer. Friedwald's formula was applied to obtain calculated LDL-C.[14] Non-HDL-C was obtained by subtraction of HDL-C from TC.

NCEP-ATP III categorization was used to classify patients into two categories of risk for CHD, low risk <130 mg/dl, else at higher risk, fusing the last two categories.[3] Risk of CHD categorization by non-HDL-C was made 30mg/dl above that of LDL-C i.e < 160 mg/dl was considered as low risk, else at higher risk.[8] Paired 't' test and Pearson correlation were used for statistical analysis, which were done by IBM SPSS-16.

Results: The study group had mean TC 185.20 ± 43.82, mean TG 179.80 ± 82.51, mean HDL-C 39.98 ± 08.45, mean non-HDL-C 145.23 ± 42.96, mean LDL-C by direct method 116.71 ± 40.24 and mean LDL-C by Friedwald's calculation 109.17 ± 36.72. Overall, 290 (36.2%) had TC more than 200 mg/dl and 440 (55%) had TG more than 150 mg/dl.

Correlation between LDL-C estimated by direct method and by Friedwald's calculation was significant (r = 0.954 and p<0.001). There was significantly higher LDL-C by direct method compared to Friedwald's calculation (mean difference 7.75 mg/dl, p< .001). This significant underestimation of LDL-C by Friedwald's calculation was present across all categories of TG and TC and the mean difference increased progressively in the higher categories of TG and TC (Table 1 and 2). Overall, compared to the direct, the Friedwald's calculation underestimated LDL-C of 570 (71.25%) and overestimated of 230 patients (28.75%) (Table 3). In only 15%, the mean difference between two methods was restricted to ± 5mg/dl. In different ranges of mean difference, the highest distribution of patients i.e. 22% was in the range between 5 to 10 mg/dl.

Those at at risk of CHD numbered 220 (27.5 %) by the Friedwald's calculation, 320 (40%) by the direct method and 270 (33.7% by non-HDL-C risk categorization. (Table 4).

Taking all three criteria combined together, 660 (82.5%) were similarly classified by the three methods. Of these, 210 (26.2%) were at risk of CHD by all three criteria, and 450 (56.2%) were not at risk by any of the three criteria. Those that were not similarly classified were 140 (17.5%) and these were at risk by at least one of the three criteria (Table 5)

Table 1: LDL-C by two methods at different ranges of serum TG

Serum TG	N	LDL-C Direct	LDL-C Calculated	Mean Difference	95% CI
1-100	190	103.95	99.78	4.17**	3.11 - 5.22
101-200	330	115.88	110.08	5.80***	4.37 - 7.23
201-300	210	119.86	110.40	9.46***	7.91 - 11.00
301-400	70	145.86	126.66	19.20***	16.39 - 22.01
Overall	800	116.71	109.17	7.75***	6.70 - 8.39

**p < .01, ***p < .001, n=number of patients

Table 2: LDL-C by two methods at different ranges of serum cholesterol

Serum TG	N	LDL-C Direct	LDL-C Calculated	Mean Difference	95% CI
101-105	190	74.53	70.81	3.72**	2.13-5.30
151-200	320	104.53	98.06	6.47***	5.14-7.80
201-250	200	145.50	135.29	10.21***	8.56-11.86
>250	90	185.11	171.58	13.53***	11.03-16.04
Overall	800	116.71	109.17	7.75***	6.70 - 8.39

**p < .01, ***p < .001, n= number of patients

Table 3: Distribution of mean difference of LDL-C

Differences in LDL-C by two methods (Range)	n (%)
< - 10 mg/dl	100 (12.5%)
-10mg/dl to -5mg/dl	50 (6.2%)
> -5mg/dl to 0 mg/dl	80 (10.0%)
> 0mg/dl to 5 mg/dl	40 (5.0%)
> 5mg/dl to 10 mg/dl	**180 (22.5%)**
> 10 mg/dl to 15 mg/dl	130 (16.2%)
> 15 mg/dl to 20 mg/dl	110 (13.7%)
> 20mg/dl	110 (13.7%)

n= number of patients

Table 4: CHD risk detection by the three criteria

Higher CHD risk criterion	Higher Risk n (%)	Low risk n (%)
Direct LDL-C > 130 mg/dl	320 (40%)	480 (60%)
Calculated LDL-C > 130 mg/dl	220 (27.5%)	580 (72.5%)
Non-HDL-C > 160 mg/dl	270 (33.7%)	530 (66.25%)

n= number of patients

Table 5: Combined CHD risk detection by three criteria

Criterion for assessment of CHD risk	n (%)
At higher risk by atleast one of the 3 criteria	350 (43.8%)
At higher risk by all 3 criteria	210 (26.2%)
At low risk by all 3 criteria	450 (56.2%)
Categorization agreement by all 3 criteria	660 (82.5%)
Categorization disagreement by any one of 3 criteria	140 (17.5%)

Three criteria: non-HDL-C, direct & calculated LDL-C, n=number

Discussion: The accuracy of LDL-C has relevant consequences in establishing a risk profile of CHD for appropriately adjusting the dietary and treatment strategies.[3] Friedwald had reported high correlation coefficients of calculated LDL-C with the reference ultra centrifugation method.[14] In routine practice, NCEP accepts the Friedwald calculation as a cheap, effective and reliable method of estimating LDL-C that can be used for classifying patients into at risk categories.[13]

On the other hand, the Direct homogenous assay for LDL-C has higher positive predictive value and negative predictive value and it is able to meet the more stringent NCEP requirements for precision (CV<4%) and accuracy (bias<4%).[15] It also shows very high correlation with the reference beta-quantification method and its clinical utility is largely unaffected by hypertriglyceridaemia.[13]

In our study, correlation coefficient of LDL-C estimation by the Friedwald calculation with the direct method was 0.95. Most studies have reported high correlation of LDL-C by direct and calculated methods.[10,16,17]

The underestimation of LDL-C by the calculated method found in our study progressively increased across all categories of TG and TC. Bansal et al reported similar underestimation by the calculation which was higher at higher levels of TC and TG. Evans et al reported that the calculated method underestimates and the direct method overestimates LDL-C compared to the reference ultracentrifuation method.[18] Baruch et al reported that when compared to the direct method, the calculated method underestimated LDL-C by more than 5 mg/dl in 60% and by more than 15mg/dl in one third.[19] In our study, the direct overestimation was more than 5mg/dl in 66% and more than 15mg/dl in 27%. In a large Korean study, Jun et al reported 96% had higher direct LDL-C with overall mean direct LDL-C being higher by 9mg/dl.[20] In our study, overall 71% had higher direct LDL-C and overall mean direct LDL-C was higher by 7.75 mg/dl.

Bansal et al and Anwar et al also reported 11% additional CHD risk detection by direct method compared to calculated. [9,16] In our study, there was additional 12.5% CHD risk detection by the direct method compared to the calculated method (40% vs 27.5%). Friedwald's calculation missed 100 subjects that were detected as high risk by the direct method (detected only 220 compared to 320 by direct method).

Friedwald's calculation of of LDL-C is prone to error as it involves three independent lipid estimations, TC, TG and HDL-C; and assumes a fixed relationship among them. VLDL carries most of the circulating TG in the fasting state, hence the Friedwald calculated VLDL-C as TG/5 and then calculated LDL-C as TC-(HDL-C +

VLDL-C). However, it does not take into account the presence of chylomicrons or intermediate density lipoproteins (IDL), which also carry TG in it. The values calculated by it can therefore be erroneous if there are alterations in IDL, as reported in diabetes mellitus, chronic renal failure, CHD etc. [6] Likewise, in the presence of chylomicrons, it results in overestimation of VLDL and underestimation of LDL-C.[15]

In general, reliability of the Friedwald's calculation decreases with hypertriglyceridaemia. In comparison to the reference ultracentrifugation method, Nauck et al reported that the percent of subjects with reasonable accuracy (defined as deviation < 10%) by calculated method decreased from 86-92% at TG < 200mg/dl to 41% at TG between 400-500 mg/dl.[15]

However, antagonistic results of LDL-C underestimation by the direct method have also been reported by some. Mora et al reported that mean fasting direct LDL-C was lower by 5.6 mg/dl.[21] Sahu et al reported 6% lower risk categorization by direct method.[10] Some authors tried to correct this underestimation of LDL-C by the direct method with new formulas. [22,23]

The risk detection by the non-HDL-C cut point was intermediate (270, in between 220 by calculated and 320 by direct). Baruch et al reported that non-HDL-C corrected similarly by 30mg/dl had intermediate values between direct and calculated LDL, but the clinical discordance (defined as placement in different ATP III goal cut point ranges or difference $\geq$ 12% or $\geq$ 10mg/dl) was greater between non-HDL-C and direct LDL-C rather than between non-HDL-C and calculated LDL-C.[24] Non-HDL-C is now regarded as a cheap alternative calculation of atherogenic risk that does not assume normal lipoprotein composition, has no requirement for a fasting sample, and reflects the entire serum cholesterol carried by all of the potentially atherogenic lipoproteins-LDL, VLDL, IDL and remnant lipoproteins.[25] Cui et al reported that non-HDL-C was in fact a somewhat better predictor of cardiovascular disease mortality than LDL-C.[26] However, non-HDL-C is affected by age, race and gender.[25] Also, attainment of non-HDL-C goal still remains poor in patients due to deficiency of provider's awareness and poor patient tolerance or compliance to higher doses of statins.[8] Prospective study with CHD as outcome and all three as markers of CHD is required to verify the relative predictive values of these markers.

In conclusion, our study found that although the Friedwald's calculation performs reasonably well, it underestimates LDL-C compared to the direct method and misses identifying a significant number of patients at risk of CHD by both the direct method and by non-HDL-C.

References:

1. Newman WP, Freedman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL, et al. Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. The Bogalusa Heart Study. N Engl J Med. 1986;314:138–44.
2. Anderson KM, Castelli WP, Levy D. Cholesterol and Mortality. 30 Years of Follow-up From the Framingham Study. J Am Med Assoc. 1987;257:2176–80.
3. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). J Am Med Assoc. 2001;285:2486–97.
4. Miller WG, Waymack PP, Anderson FP, Ethridge SF, Jayne EC. Performance of four homogeneous direct methods for LDL-cholesterol. Clin Chem. 2002;48:489–98.
5. Bachorik PS, Ross JW. For the National Cholesterol Education Program Working Group on Lipoprotein Measurements. The National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. Clin Chem. 1995;41:1414–20.
6. McNamara JR, Cohn JS, Wilson PW, Schaefer EJ. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. Clin Chem. 1990;36:36–42.
7. Rifai N, Iannotti E, DeAngelis K, Law T. Analytical and clinical performance of a homogeneous enzymatic LDL-cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg2+ method. Clin Chem. 1998;44:1242–50.
8. Virani SS. Non-HDL Cholesterol as a Metric of Good Quality of Care. Tex Heart Inst J. 2011;38:160–2.

9. Bansal E, Kaur N. Does friedewald formula underestimate the risk of ischemic heart disease? Indian J Clin Biochem. 2014;29:496–500.
10. Sahu S, Chawla R, Uppal B. Comparison of two methods of estimation of low density lipoprotein cholesterol, the direct versus friedewald estimation. Indian J Clin Biochem. 2005;20:54–61.
11. Meiattini F, Prencipe L, Bardelli F, Giannini G, Tarli P. The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clin Chem. 1978;24:2161–2165.
12. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28:2077–80.
13. Nauck M, Graziani MS, Bruton D, Cobbaert C, Cole TG, Lefevre F, et al. Analytical and clinical performance of a detergent-based homogeneous LDL-cholesterol assay: a multicenter evaluation. Clin Chem. 2000;46:506–14.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
15. Nauck M, Warnick GR, Rifai N. Methods for Measurement of LDL-Cholesterol: A Critical Assessment of Direct Measurement by Homogeneous Assays versus Calculation. Clin Chem. 2002;48:236–54.
16. Anwar M, Khan DA, Khan FA. Comparison of friedewald formula and modified friedewald formula with direct homogeneous assay for low density lipoprotein cholesterol estimation. J Coll Physicians Surg Pak. 2014;24:8–12.
17. Tighe DA, Ockene IS, Reed G, Nicolosi R. Calculated low density lipoprotein cholesterol levels frequently underestimate directly measured low density lipoprotein cholesterol determinations in patients with serum triglyceride levels < or =4.52 mmol/l: an analysis comparing the LipiDirect magnetic LDL assay with the Friedewald calculation. Clin Chim Acta. 2006;365:236–42.
18. Evans SR, Fichtenbaum CJ, Aberg JA. Comparison of Direct and Indirect Measurement of LDL-C in HIV-Infected Individuals: ACTG 5087. HIV Clin Trials. 2007;8:45–52.
19. Baruch L, Agarwal S, Gupta B, Haynos A, Johnson S, Kelly-Johnson K, et al. Is directly measured low-density lipoprotein clinically equivalent to calculated low-density lipoprotein? J Clin Lipidol. 2010;4:259–64.
20. Jun KR, Park HI, Chun S, Park H, Min WK. Effects of total cholesterol and triglyceride on the percentage difference between the low-density lipoprotein cholesterol concentration measured directly and calculated using the Friedewald formula. Clin Chem Lab Med. 2008;46:371-5
21. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL Cholesterol Concentrations by Friedewald Calculation and Direct Measurement in Relation to Cardiovascular Events in 27 331 Women. Clin Chem. 2009;55:888–94.
22. Anandaraja S, Narang R, Godeswar R, Laksmy R, Talwar KK. Low-density lipoprotein cholesterol estimation by a new formula in Indian population. Int J Cardiol. 2005;102:117–20.
23. Boshtam M, Ramezani MA, Naderi G, Sarrafzadegan N. Is Friedewald formula a good estimation for low density lipoprotein level in Iranian population? J Res Med Sci. 2012;17:519–22.
24. Baruch L, Chiong VJ, Agarwal S, Gupta B. Discordance of Non-HDL and Directly Measured LDL Cholesterol: Which Lipid Measure is Preferred When Calculated LDL Is Inaccurate? Cholesterol. 2013;2013:502948.doi:10.1155/2013/502948
25. Bittner V. Non-HDL Cholesterol- measurement, interpretation, and significance. Adv Stud Med. 2007;7:8–11.
26. Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, et al. Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. Arch Intern Med. 2001;161:1413–9.

Conflict of interest: None
Funding: None
Cite this Article as: Shubho B, Vaishali J, Prerna G, Vandana A. Friedwald's Calculation; Is It Reliable in Estimating Risk of Coronary Heart Disease? Natl J Integr Res Med 2017; 8(4):27-31