Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/15087
DOI URL: http://dx.doi.org/10.21474/IJAR01/15087

RESEARCH ARTICLE

LOW-DENSITY LIPOPROTEIN CHOLESTEROL ESTIMATION BY FRIEDEWALD'S FORMULA AND BY DIRECT MEASUREMENT - A COMPARISON

Dr. Nimi Bharathan, Sunitha S. Nair and Dr. Rini Raveendran

Manuscript Info

Manuscript History
Received: 29 May 2022
Final Accepted: 30 June 2022
Published: July 2022

Key words:-
LDL-C, Friedewald's formula, direct homogenous assay

Abstract

Introduction: Low-density lipoprotein cholesterol (LDL-C) levels in serum is the basis of diagnosis, classification and management of hypercholesterolemia as per the current recommendations of the Adult Treatment Panel III of National Cholesterol Education Program. Friedewald's formula is used in most laboratories to measure LDL-C levels indirectly. But, Friedewald's formula is not reliable always. Now-a-days, various homogenous assay kits for direct estimation of LDL-C based on precipitation and solubilisation using specific detergents have been evolved. This makes estimation of LDL-C levels easier but costlier. So we conducted a study to compare the results obtained by estimating LDL-C level by direct assay kit and also indirectly by using Friedewald's formula in order to assess the cost effectiveness of both the methods.

Aim: To compare LDL-C levels obtained by direct homogenous assay (D-LDL-C) to that obtained by Friedewald's formula (F-LDL-C) in fasting serum samples.

Materials And Methods: Serum separated from 404 blood samples was analysed for Total cholesterol (TC), Triglycerides (TG), High-density lipoprotein (HDL) and LDL using specific assay kits. Simultaneously, LDL-C was also estimated in the same samples indirectly by using Friedewald's formula.

Results: Statistical analysis by Pearson correlation showed high correlation between D-LDL-C and indirect F-LDL-C. But in all ranges of TG, F-LDL-C showed underestimation of LDL-C when compared to D-LDL-C.

Conclusion: Use of Friedewald formula for estimation of LDL-C is not suitable in all cases. In samples showing hypertriglyceridemia, direct homogenous assay was found to be better for CHD risk assessment and management.

Copy Right, IJAR, 2022,. All rights reserved.

Introduction:-

LDL-C level in serum is considered as a strong risk factor and an accepted predictor with highest predictive value for Coronary Artery Disease (CAD) among all lipoproteins (1) . Many studies show high correlation between increased levels of LDL-C level and CAD (2,3) .Increase in LDL-C level is seen to correlate with the severity of atherosclerosis in CAD (4). As with CAD patients, in patients diagnosed with hypercholesterolemia also, LDL-C level is the corner-stone in diagnosis andmanagement.

Corresponding Author:- Dr. Nimi Bharathan

Ultra centrifugation followed by polyanion precipitation, also known as Beta quantification is the gold standard method for estimation of LDL-C as well as other lipoproteins (5,6). In this method, Sodium or Potassium bromide is used to separate lipoprotein particles according to their density. When plasma is ultracentrifuged at 105,000 x g for 18 hours at 10°C, VLDL, chylomicrons, and β-VLDL accumulate in a floating layer. Chylomicrons and very low-density lipoprotein cholesterol (VLDL) are triglyceride-rich and have density lower than LDL. The supernatant floating layer is removed by tube slicing or by using a syringe or pipette.The infranatant layer with a density greater than 1.006 g/mL will contain mostly LDL and HDL(7). Repeated spinning and slicing are employed until LDL is separated. As this method was time consuming and required high expertise and expensive machinery, it was soon replaced by other indirect methods. Since the publication of a landmark report by Friedewald in 1972, Friedewald's equation which used three independant parameters namely Total cholesterol (TC) level, Triacylglycerol (TG) level and High density lipoprotein (HDL) level is used widely for estimating LDL-C level indirectly. According to Friedewald's equation, LDL- C = TC- HDL- (TG/5)(8). TC represents the combination of LDL, VLDL, and HDL. Friedewald's equation was put forwardbased on the average ratio of triglyceride (TG) to cholesterol in VLDL. Amount of cholesterol in VLDL is assumed to be equal to 1/5th of the total TG concentration as majority of TG in serum. Friedewald assumed that, when TC and HDL are directly measured, taking the value for VLDL level as TG/5, would provide a fairly accurate method for deriving LDL level, theoretically (9). Friedewald and colleagues noted that simply dividing triglyceride values by 5 does not give an accurate estimate of VLDL-C (7). The TG/5 term, which serves as an estimate of cholesterol in VLDL is highly variable and unsuitable in (1) samples that have triglyceride concentrations above 400 mg/dL, (2) samples that contain significant amounts of chylomicrons (nonfasting specimen), or (3) patients with dysbetalipoproteinemia. In such cases, the factor TG/5 does not provide an accurate estimate of VLDL and can lead to large errors in calculated LDL cholesterol(8,9). Friedewald's equation will overestimate VLDL-C and underestimate LDL-C, if TG rich chylomicrons and chylomicron remnants are present in the serum sample, necessitating a fasting sample. The use of this formula is not recommended for type 2 diabetes, nephrotic syndrome and chronic alcoholic patients because accompanying abnormalities in lipoprotein composition render the underlying assumptions invalid for assessment of cardiovascular risk in these patients. The NCEP working group on lipoprotein measurements has recommended that the LDL-C analysis methods should have a total analytical error not exceeding 12% (≤4% imprecision (CV) and ≤4% inaccuracy) to guarantee correct patient classification into NCEP risk categories(10). It is difficult to obtain this analytical quality with Friedewald's formula (FF) because each component's analytical error is added (7).

Later, several methods were developed to measure LDL-C level in serum directly in order to overcome the shortcomings of both these methods (11). These homogenous assays that used a combination of solubilisation and precipitation using different surfactants and binding molecules, selectively measured cholesterol from LDL fraction(12). Many studies have shown that calculation of LDL-C level using Friedewald's formula (FF) correlates well with directly measured LDL-C level. But in order to replace FF by direct homogenous assay for LDL-C estimation, its cost-effectiveness and advantages have to be established by comparative studies as direct assay kits are more expensive and increase the cost of lipid profile estimation. The aim of this study is to compare the LDL-C levels obtained by calculation using Friedewald's formula and by direct assay.

Materials And Methods:-

Study was conducted after obtaining approval from Institutional ethics committee. Data obtained from lipid profile analysis of 410 aseptically drawn venous blood samples were studied. The specimens were allowed to clot for 30 minutes at room temperature. Serum was separated bycentrifugation at 3,000 rpm for 15 minutes. In all the samples, Total Cholesterol (TC), Triglycerides (TG), HDL-Cholesterol (HDL-C) and LDL-Cholesterol were estimated using CE certified kits manufactured by Erba Mannheim ltd. OnEM 360 Clinical Chemistry Analyzer, (TransAsia Bio-Medicals Ltd, Mumbai, Maharashtra, India). Simultaneously, in all samples, LDL-Cholesterol was calculated by using Friedewald's formula according to which, LDL-C = TC – HDL – (TG/5) mg/dL.

LDL-Cholesterol estimated by Homogenous Enzymatic Direct Assay was designated as D-LDL-C and LDL-Cholesterol obtained by Friedewald equation was designated as F-LDL-C. TC was estimated by Enzymatic endpoint CHOD- PAP method. TG was estimated by Enzymatic Glycerol Phosphate Oxidase/ Peroxidase method. HDL-Cholesterol (HDL-C) was estimated by Homogenous Enzymatic Direct Assay. Means and standard deviations were compared by paired t test. Correlation between D-LDL-C and F-LDL-C was assessed by Pearson's correlation.

Comparison of F-LDL-C and D-LDL-C were done at different Triglyceride ranges of < 100, 101-200, 201-300 and 301-400 mg/dL. Similarly, comparison was done at Total cholesterol ranges of 50-99, 100-149, 150-199, 200-249 and >250 mg/dL ranges. These ranges were selected based on the NCEP ATP III guidelines for management of patients with hypercholesterolemia (10). Mean percentage difference between F-LDL-C and D-LDL-C was calculated as %ΔLDL.

Results:-

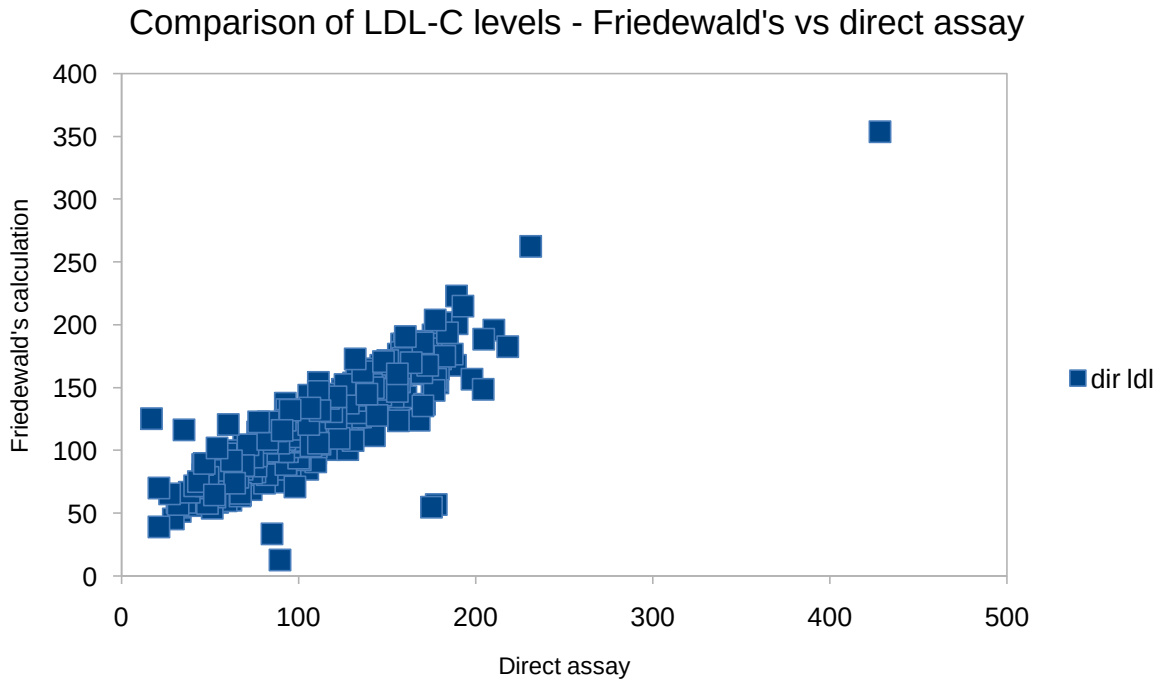
410 samples were analysed initially, out of which 4 samples having TG > 400mg/dL and 3 incomletelyanalysed samples were excluded from the study, making the number of total analysed samples 403.

Pearson's correlation between F-LDL-C and D-LDL-C showed significant correlation (correlation coefficient = 0.81,at 0.01 level, 2-tailed) as shown in Table 1 and Figure 1.

Table 1:- Correlation between LDL-C levels estimated by Friedewald's formula (F-LDL-C) and by direct assay (D-LDL-C).

		F-LDL-C	D-LDL-C
F-LDL-C	Pearson Correlation	1	.871**
	Sig. (2-tailed)		.000
	N	403	403
D-LDL-C	Pearson Correlation	.871**	1
	Sig. (2-tailed)	.000	
	N	403	403
**. Correlation is significant at the 0.01 level (2-tailed).			

Fig 1:- Scatter plot showing LDL-C levels Friedewald's vs Direct assay.

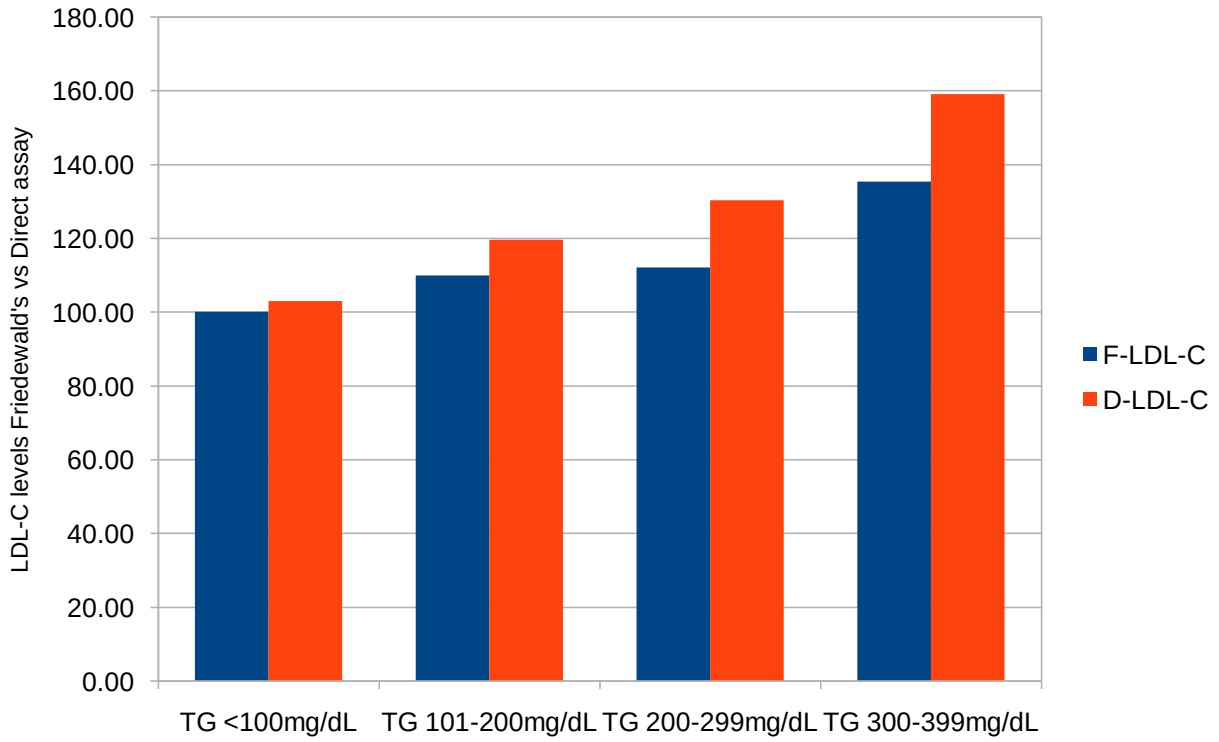


At the same time, mean and standard deviation of LDL-C estimated by Direct assay and by Friedewald formula showed statistically significant difference at all ranges of Triglyceride level as shown in Table 2. Calculation by Friedewald formula showed lower level than direct assay in 75% of samples. There was underestimation of LDL-C level when calculated by FF at all levels of TG which is evident from Figure 2. Underestimation was maximum at TG level >200mg/dL.

Table 2:- Comparison of LDL-C levels – Friedewald's vs Direct assay in different ranges of Triglyceride levels.

TG level (mg/dL)	n	F-LDL-C Mean +/- SD (mg/dL)	D-LDL-C Mean +/- SD (mg/dL)	p value
1-100	95	97.0 +/- 43.37	103.08 +/- 30.04	0.045
101-200	242	108.43 +/- 43.41	119.42 +/- 33.84	0.000
201-300	54	106.53 +/- 79.53	130.31 +/-52.29	0.000
301-400	11	135.46 +/- 50.42	159.15 +/- 32.50	0.012

Figure 2:- Bar chart showing F-LDL-C and D-LDL-C levels at different categories of Triglycerides.



The mean difference between LDL-C levelestimated by FF and directly measured LDL-C was6.08 +/- 22.64 mg/dL in TGlevel<100mg/dL,10.99 +/- 23.43 mg/dL in TG level 101-200mg/dL, 23.77 +/- 38.38 mg/dL in TG level 201-300mg/dL and23.68 +/- 25.77 mg/dL in TG level301-400mg/dL level. The mean % difference between the two values (%ΔLDL) ranged from 5.9% to 14.88% . Highest %ΔLDL was seen in TG range 201-300mg/dL group. (Table 3)

Table 3:- Mean % Difference of LDL-C between F-LDL-C and D-LDL-C.

TG level (mg/dL)	n	F-LDL-C Mean +/- SD (mg/dL)	D-LDL-C Mean +/- SD (mg/dL)	Difference (dir – cal f) Mean +/- SD	%ΔLDL
1-100	95	97.0 +/- 43.37	103.08 +/- 30.04	6.08 +/- 22.64	5.90

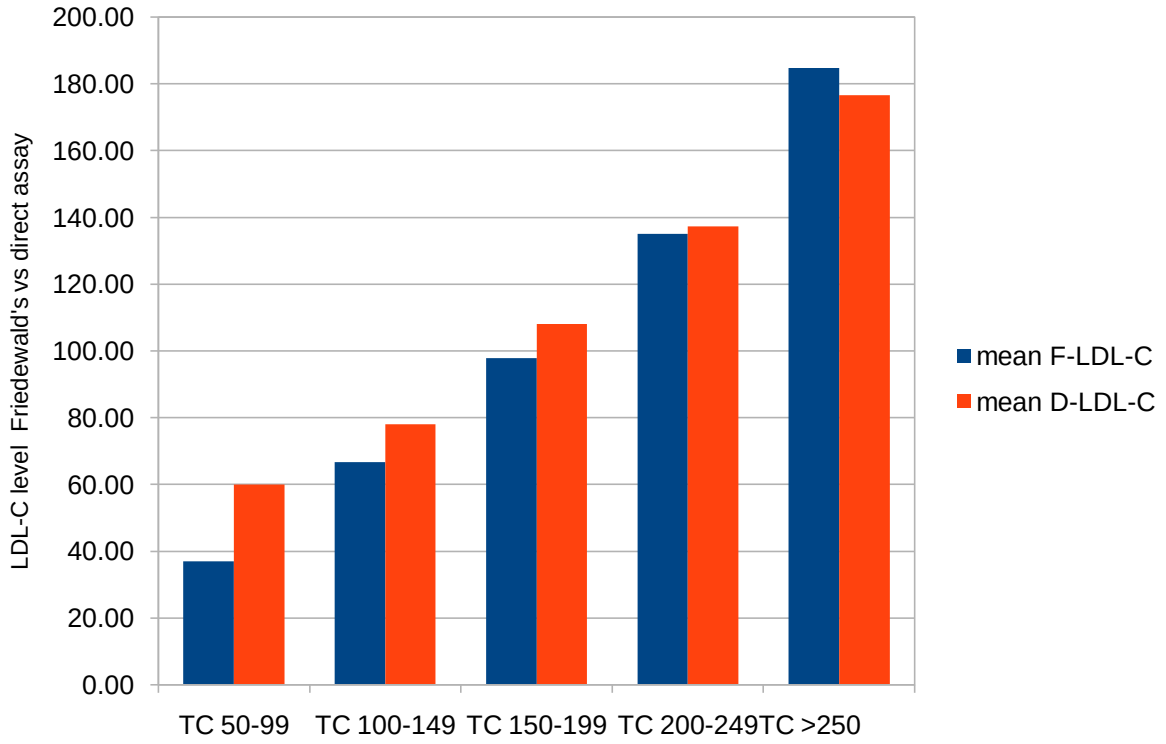
101-200	242	108.43 +/- 43.41	119.42 +/- 33.84	10.99 +/- 23.43	9.20
201-300	54	106.53 +/- 79.53	130.31 +/-52.29	23.77 +/- 38.38	18.24
301-400	11	135.46 +/- 50.42	159.15 +/- 32.50	23.68 +/- 25.77	14.88

On comparing D-LDL-C and F-LDL-C levels between different categories of TC, the difference between the two values was found to decrease with increase in TC level. Statistically significant difference was seen in all categories except when TC > 250mg%. (Table 4, Figure 3)

Table 4:- Comparison of LDL-C levels – Friedewald's vs Direct assay in different ranges of Total Cholesterol levels.

TC level (mg/dL)	n	F-LDL-C Mean +/- SD mg/dL)	D-LDL-C Mean +/- SD mg/dL)	Difference (dir – cal f)	P value
50-99	8	36.49 +/- 9.51	59.91 +/- 23.88	23.42	0.028
100-149	75	61.82 +/- 16.60	78.05 +/- 14.50	16.23	0.000
150-199	155	95.48+ - 18.41	108.03 +/-16.55	12.55	0.000
200-249	118	131.56 +/- 18.62	137.32 +/- 23.67	5.75	0.003
=/>250	47	181.11+/- 41.69	176.61 +/- 39.30	-4.49	0.314

Figure 3:- Bar chart showing F-LDL-C and D-LDL-C levels at different categories of Total Cholesterol.



When patients were categorised based on D-LDL-C level, as per NCEP ATP III guidelines (9), 127 patients (31.44%) showed optimal LDL-C levels, 130 patients (32.18%) showed Near optimal/above optimal levels, 94 patients (23.27%) showed Borderline high levels, 37 (9.16%) patients showed High levels and 12 (2.97%) patients showed Very high LDL-C levels. At the same time, when categorisation of patients was done based on F- LDL-C level, 183 patients (45.30%) showed optimal LDL-C levels, 103 patients (25.50%) showed Near optimal/above optimal levels, 74 patients (18.32%) showed Borderline high levels, 35 (8.66%) patients showed High levels and 7 patients (1.73%) showed Very high LDL-C levels. (Table 5)

Table 5:- Categorisation of patients according to F-LDL-C and D-LDL-C levels.

LDL Cholesterol level mg/dL	Categorisation as per NCEP ATP III guidelines	No. of patients by F-LDL-C level	No. of patients by D-LDL-C level
<100	Optimal	177 (43.81%)	127 (31.44%)
100-129	Near optimal/above optimal	104 (25.74%)	131 (32.43%)
130-159	Borderline high	73 (18.07%)	95(23.51%)
160-189	High	36 (8.91%)	38 (9.41%)
>190	Very high	13 (3.22%)	12 (2.97%)

Discussion:-

There are several studies that compare LDL-C levels estimated by direct homogenous assay and calculated by Friedewald formula (12,13,14,15,16). Correlation between F-LDL-C and D-LDL-C was significant in our study (correlation coefficient =0.81). Similar results were seen in other studies also (12,13,17).

Friedewald equation is based on the assumptions that, in fasting state, 1) chylomicrons are not present in circulation and total plasma cholesterol concentration is carried in VLDL, LDL, and HDL forms 2) almost all plasma TG are carried by VLDL 3) TG /cholesterol ratio in VLDL is constant and 4) Cholesterol concentration of VLDL (VLDL-C) is one fifth of TG concentration (18).

Various studies have demonstrated that the assumptions of Friedewald in deriving the formula are not always correct. Variation in TG/cholesterol ratio can be seen in different situations like type 2 diabetes, nephrotic syndrome and chronic alcoholic patients in whom there will be accompanying abnormalities in lipoprotein composition. These abnormalities in lipoprotein composition can make the basic assumptions of Friedewald's formula invalid for assessment of cardiovascular risk in these patients(19). In such cases, as Friedewald's formula becomes unreliable and calculation of LDL-C by Friedewald's formula can produce erroneous results even when TG levels are between 200 and 400 mg/dl(20). Comparison of LDL-C results at different levels of TGs by Waradeetal showed statistically significant difference ($P<0.001$) between measured values and those calculated by Friedewald's formula (18). In their study, Gupta et al reported underestimation of LDL by FF at all levels of TG (19), similar to the findings in our study. Lindsey etal found that comparison of these two methods demonstrated an underestimation of C-LDL of 19.5 ± 11.8 mg/dl. In their study, the degree of underestimation increased as the triglyceride level increased ($p<0.05$) (9). Kannan S etal also have noted that LDL-C estimation by Friedewald formula can underestimate LDL-C values especially in those having high TG levels (21). Agrawal also has stated in his study that there is substantial lack of agreement between D-LDL-C and C-LDL-C when he compared LDL-C values calculated by Friedewald formula with that obtained by using three different direct homogenous assay kits (22). But in a study by Sahuet al.,(12) they have noted that the mean LDL calculated by FF was significantly higher than the direct LDL measurement at TG between 1 and 300 mg/dl. In his study, Reignier also noted overestimation of calculated LDL-cholesterol level with respect to measured LDL-cholesterol (16). Such discrepancies in calculating LDL-C level by using Friedewald's formula must be owing to errors that can happen during measurement of TG, TC or HDL levels separately which can add up during the calculation (20).

In our study, when TC was >250 mg%, LDL-C level calculated by Friedewald formula was found to be higher than direct estimation unlike when TC was <250mg%. But this overestimation by Friedewald formula cannot be considered significant as the number of samples belonging to this category was low (n=47).

It was in an effort to overcome these discrepancies that several other formulas were derived by different study groups like the Modified Friedewald's formula (23), Anantharaja formula (24), Puavilai formula (25), Hata formula

(26), Martin formula (27) etc. Hata and Puavilai have used different ratios for calculation of VLDL cholesterol level, in an attempt to overcome the (25,26). Modified Friedewald's formula, Hata formula and Martin formula used different ratios between TG and cholesterol in different categories of TG level after finding that the same factor is not applicable to all levels of TG (23,26,27). Hata has found that triglyceride/cholesterol ratio in VLDL in Japanese population ranges from 3 to 5 depending on Trigleride level (26). Anantharaja obtained a new formula by multiple regression analysis which excluded HDL from the calculation, which they claimed to be more accurate than Friedewald formula in Indian population (24).In a study comparing 4 different formulas for calculating LDL-C values from TG, TC and HDL, Chowdary suggests that use of Martin formula is seen to correlate best with Directly measured LDL-C levels in Indian patients (28). These studies have shown that using different factors for estimating VLDL-C levels and deriving at LDL-C estimation accordingly provided more accurate estimation of LDL-C and better classification of subjects.

Even if the difference between D-LDL-C level and F-LDL-C level is not statistically significant, it can have varied effects on patient's management and outcomeas can be interpreted from our study.According to our study, estimation of LDL-C by Friedewald's formula will place more patients in the "optimal" LDL-C category, thus leading to a delay in initiation of therapeutic life style changes (TLC) which can be benefitial if started early, in hyperlipidemia. At the same time, falsely placing patients into "near/above optimal" category by Direct homogenous assay of LDL-C will only lead to earlier introduction of TLC which cannot be considered harmful. Naucketal also give similar opinion in their study (29). Thus, replacing indirect estimation of LDL-C by direct assay using third generation homogenous assay kits can be considered in clinical laboratories, especially in patients having hypertriglyceridemia, where calculation by Friedewald's formula can be inaccurate. Mora etal also opine that underestimation of LDL-C can lead to delay in initiation of adequate lipid-lowering therapy in high-risk patients. Similarly, overestimation of LDL-C can result in unnecessary pharmacological therapy as the patient is mistakenly placed in a higher risk strata (21, 30).

Thus, considering all the above factors, use of homogenous direct assay kit for lipid profile studies can be considered only after conducting more studies involving more samples in each category of TG and TC. Non-HDLC which can be derived from routine lipid profile panel itself should be considered in clinical biochemistry laboratories instead of using formula derived LDL-C values for CHD work up of patients in risk categories. As non-HDL can be calculated easily from routine lipid profile parameters, it does not involve additional expenditure. Non HDL can thus be considered for patients in whom F-LDL-C is expected to be inaccurate, as stated by Baruch (31). NCEP ATP III also recommends using Non-HDLC as a secondary target of lipid lowering, after adequate control of LDL-C is achieved and if TGs are elevated (200 mg/dl) (10).

Conclusion:-

Use of Friedewald formula for estimation of LDL-C is not suitable in all cases. In samples showing hypertriglyceridemia, direct homogenous assay was found to be better for CHD risk assessment and management.

Limitations

The number of patients included in higher ranges of Total cholesterol and Triglyceride were less when compared to lower range as patients were included before serum analysis. This made statistical correlation in higher range categories less significant. Clinical details, treatment details or outcomes of our patients were not available to us during the study. Grouping of patients and their potential implications were based on lipid profile only.

Reference:-

1. W P Castelli, R J Garrison, P W Wilson, R D Abbott, S Kalousdian, W B Kannel Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study JAMA 1986 Nov 28;256(20):2835-8.
2. Anderson, K.M., Castelli, W.P. and Levy, D.(1987) Cholesterol and Mortality: 30 years of follow-up from the Framingham Study. JAMA 257, 2176-2180.

3. Gordon, T., Kennel, W.D., Castelli, W.P. and Dauber, T.R. (1981) Lipoproteins, Cardiovascular Disease and Death: The Framingham Study. Arch. Intern. Med. 141, 1128-1131.
4. Newman, W.P., Freedman, D.S., Voors, A.W. etal. (1986) Relation of serum lipoprotein levels and systolic blood pressure to early arterioscleroses: The Bogalusa Heart Study. N Engl. J. Med. 314, 138- 144.
5. Marniemi, J., Maki, J., Maatela, J., Jarvisalo, J. and Impivaara, O. (1995) Poor applicability of Friedewald formula in the assessment of serum LDL- Cholesterol for Clinical purposes. Clin. Biochem. 28, 285-289.
6. Miller, W.G., Waymack, P.P., Anderson, F.P., Ethridge, S.F. and Jayne, E.C.
(2002) Performance of four homogenous Direct Methods for LDL- Cholesterol. Clin. Chem. 48 (3), 489-498.
7. Rifai, N., Warnick G.R., Remaley A.T., Lipids, Lipoproteins, Apolipoproteins,
and Other Cardiovascular Risk Factors, pg 424, chapter 23, Tietz fundamentals of clinical chemistry, 6 th Ed,2008.
8. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without the use of preparative centrifuge. Clin. Chem. 18, 499-502.
9.Lindsey C C, Graham E R, Johnston T P, Kiroff C G, Freshley A A clinical comparison of calculated versus direct measurement of low-density lipoprotein cholesterol level Pharmacotherapy 2004 Feb;24(2):167-72.
doi: 10.1592/phco.24.2.167.33142.
10.NCEP. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 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) final report. **Circulation**2002;106:3143-421.
11. Vesper H W, Danilenko U, Cholesterol Reference Method Laboratory Network (CRMLN) LDL Cholesterol Certification Protocol for Manufacturers updated: June 2018, Division of Laboratory Sciences, Centers for Disease Control and Prevention.
12. Kamal AHM, Hossain M, Chowdhury S, Mahmud NU. A comparison of calculated with direct measurement of low density lipoprotein cholesterol level. JCMCTA. 2009;20:19–23. [Google Scholar]
13. Atabi F, Mohammadi R Clinical Validation of Eleven Formulas for calculating LDL-C in Iran, Iran J Pathol 2020 Fall; 15(4): 261–267.
14. 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. [PMC free article]PubMed] [Google Scholar]
15. Choi S Y, Park H E, Kim M, Shin C S, Cho S, Oh B Difference between calculated and direct-measured low-density lipoprotein cholesterol in subjects with diabetes mellitus or taking lipid-lowering medications Mar-Apr 2012;6(2):114-20. doi: 0.1016/j.jacl.2011.12.007. Epub 2011 Dec 23.
16. Reignier A1, Sacchetto E1, Hardouin JB2, Orsonneau JL1, Le Carrer D1, Delaroche O1, Bigot-Corbel EComparison of calculated LDL cholesterol (LDL-C) versus measured ldl cholesterol (LDL-M) and potential impact in terms of therapeutic management.Ann Biol Clin (Paris). 2014 Oct 1;72(5):593-598.
17. Agrawal M, Spencer H J, Faas F H Method of LDL cholesterol measurement influences classification of LDL cholesterol treatment goals: clinical research study J Investig Med 2010 Dec;58(8):945-9. doi: 10.231/JIM.0b013e3181fb7ca7
18. WARADE JP, DAHAKE H, KAVITHA R. COMPARISON BETWEEN DIRECT ESTIMATION OF LDL AND FRIEDEWALD'S FORMULA. IAIM, 2016;3(2): 10-17.
19. Gupta S, Verma M, Singh K. Does LDL-C Estimation using anandaraja's formula give a better agreement with direct LDL-C estimation than the friedewald's formula? Indian J Clin Biochem. 2012;27:127–33. [PMC free article] [PubMed] [Google Scholar]
20. Rubies-Prat J, Revere RJ, Senti M, et al. Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. Diabetes Care. 1993;16:1081–1086. doi: 10.2337/diacare.16.8.1081. [PubMed] [CrossRef] [Google Scholar]
21.Kannan S, Mahadevan S,[1] Ramj Bi,[2] Jayapaul M,[3] and Kumaravel V[4]LDL-cholesterol: Friedewald calculated versus direct measurement-study from a large Indian laboratory database. Indian J Endocrinol Metab2014 Jul-Aug; 18(4): 502–504.
22. Mayank Agrawal, MD, MPH,[1]Horace J Spencer, MS,[2] and Fred H Faas, MD The Method of LDL Cholesterol Measurement influences Classification of LDL Cholesterol to Treatment Goals J Investig Med. 2010 Dec; 58(8):945-949

23. Ghasemi A, Asgari S, Hadaegh F, Kheirandish M, Azimzadeh I, Azizi F, Tohidi M, New modified Friedewald formulae for estimating low-density lipoprotein cholesterol according to triglyceride levels: extraction and validation 2018 Nov;62(2):404-411. doi: 10.1007/s12020-018-1685-2. Epub 2018 Jul 24.
24. 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.
25. Puavilai W, Laorugpongse D, Deerochanawong C, Muthapongthavorn N, Srilert P. The accuracy in using modified Friedewald equation to calculate LDL from non-fast triglyceride: a pilot study. J Med Assoc Thai 2009;92(02):182–187.
26. Hata Y, Nakajima K. Application of Friedewald's LDL-cholesterol
estimation formula to serum lipids in the Japanese population.Jpn Circ J 1986;50(12):1191–1200
27. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. Comparison of a novel method vs the Friedewald equation for estimating Low-Density Lipoprotein cholesterol levels from the standard lipid profile. JAMA 2013;310: 2061–8. doi: 10.1001/jama.2013.280532 [PMC free article] [PubMed]
28. Chowdary M. V. P. Comparison of Formula-Based Methods with Diverse TGL: VLDL-C Ratio for Calculating LDL-C in a Tertiary Care Hospital J Lab Physicians 2022;14:65–73.
29. Nauckk M, Warnick G R, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation Clin Chem 2002 Feb;48(2):236-54.
30. 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. [PMC free article] [PubMed] [Google Scholar
31. Baruch L, Chiong V.J, Agarwal S, Gupta B.,Discordance of Non-HDL and Directly Measured LDL Cholesterol: Which Lipid Measure is Preferred When Calculated LDL Is Inaccurate? Cholesterol, Volume 2013 (2013), Article ID 502948, 6 pages.