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LDL CHOLESTEROL ESTIMATION – A COMPARISON OF FRIEDEWALD'S FORMULA AND ANANDARAJA'S FORMULA WITH DIRECT HOMOGENOUS ASSAY.

Biochemistry

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ABSTRACT

Friedewald's formula is commonly used to calculate LDL cholesterol level from Total cholesterol, HDL and Triglyceride values. A newer formula proposed by Anandaraja and colleagues need to be analysed to know if it can be used in place of Friedewald's formula. In present study, we assessed the effectiveness of Anandaraja's formula and compared it with Friedewald's formula and direct homogenous assay method. LDL cholesterol levels were assessed in 403 serum samples by Direct homogenous assay (D-LDL), Friedewald's formula (F-LDL) and by Anandaraja's formula (A-LDL) and analysed statistically. Mean % difference (%ΔLDL) of both methods was found out. Both calculated methods underestimated LDL cholesterol level measured by direct homogenous assay. Mean % difference was higher for Friedewald's method than Anandaraja's method. Anandaraja's formula shows better correlation and lesser underestimation than Friedewald's formula when compared with Direct assay method. As Anandaraja's formula is more economical when compared to direct homogenous assay, it can be used in cases where Friedewald's formula cannot be used.

KEYWORDS

LDL cholesterol estimation, Friedewald's formula, Anandaraja's formula, Direct homogenous assay

INTRODUCTION

Serum low density lipoprotein (LDL-C) level is considered as the cornerstone in diagnosis and management of patients with hypercholesterolemia as per NCEP ATP III guidelines.[1] β-quantification process, which involves steps like ultracentrifugation and polyanion precipitation is the reference method for estimation of LDL-C.[2,3] But being cumbersome and time consuming, it is not routinely used for estimation of LDL-C. Serum LDL-C level in fasting blood sample is commonly calculated indirectly, assuming that total cholesterol (TC) consists of primarily cholesterol present in very low density lipoprotein (VLDL), LDL and High density lipoprotein (HDL).[4] Ever since Friedewald put forward a formula in his path breaking study published in 1972, LDL cholesterol level is routinely calculated from parameters included in lipid profile, namely Total cholesterol (TC), High density lipoprotein (HDL) and triglycerides (TG), using Friedewald's formula which is given by LDL = TC – HDL – (TG/5) mg/dL.[5] Friedewald's formula was put forward based on the assumption that in fasting serum samples, quantity of chylomicrons is negligible and triglycerides are seen mainly in VLDL, and the factor TG/5 was taken for calculating VLDL cholesterol, based on the average ratio of triglyceride to cholesterol in VLDL.[5] Thus, it cannot be used in a non-fasting sample as the amount of chylomicrons will be considerably high in such a sample.

The ratio of TG to cholesterol in VLDL which is assumed to be constant (TG/5) in all samples while using Friedewald's formula, need not be always correct. It does not take into account other lipoprotein fractions namely intermediate density lipoprotein (IDL) and lipoprotein (a) (Lp(a)). [4] Also, in clinical conditions like dysbetalipoproteinemia, type II diabetes mellitus, nephrotic syndrome or in chronic alcoholic patients, this formula will not be suitable for calculating LDL-C as the accompanying metabolic abnormalities in such conditions will produce erroneous results.[4,6,7]

In 1998, in an attempt to overcome the disadvantages of β-quantification and Friedewald's formula, direct homogenous assay kits were developed as the third generation of LDL-C measurement methods.[7] These methods utilise specific detergents and solvents making them more expensive.[3,8] Hence, homogenous assay kits are still not being used in clinical laboratories routinely in India.

High incidence of coronary heart disease (CHD) in India necessitates early, accurate and cost-effective estimation of LDL-C.[9] There are studies showing that homogenous assays and Friedewald's formula do not give comparable results. [10,11] These facts prompted modification of Friedewald's formula and also derivation of better formulas. [12 13,14,15] Anandaraja proposed a formula in 2005 which could be used in Indian population in place of Friedewald's formula.[16] Anandaraja's formula uses only 2 analytes - Total cholesterol (TC) and Triglycerides (TG) for calculating LDL-C which is more likely to reduce the chances of error when compared to Friedewald's formula which uses three analytes for calculation. It also reduces the total cost of LDL-C estimation by avoiding the expense of HDL estimation. However, studies comparing Anandaraja formula and Friedewald's formula have shown varying results.[17,18]

In this study, we estimated LDL-C by using Friedewald's formula and Anandaraja formula and compared them with that obtained by direct homogenous assay method. We also examined the categorisation of patients as per NCEP ATP III guidelines, by both calculated methods and direct assay method.

MATERIAL AND METHODS

A comparative study was conducted after obtaining approval from Institutional ethics committee. Data obtained from lipid profile analysis of 410 aseptically drawn venous blood samples were analysed. The specimens were allowed to clot for 30 minutes at room temperature. Serum separated by centrifugation at 3,000 rpm for 15 minutes were analysed for estimating TC, TG, HDL and LDL-C (D-LDL). The estimations were done using CE certified kits manufactured by Erba Mannheim ltd., on EM 360 Clinical Chemistry Analyzer. TC was estimated by Enzymatic endpoint CHOD- PAP method. TG was estimated by Enzymatic Glycerol Phosphate Oxidase/ Peroxidase method. HDL was estimated by Homogenous Enzymatic Direct Assay.

LDL direct assay kit was based on the principle of precipitation of LDL using PVS/PEGME complex, later release of LDL from the complex using specific detergent, enzymatic action producing hydrogen peroxide and its final quantification using Trinder reaction. Simultaneously in all samples, LDL-Cholesterol was calculated by using Friedewald's formula and Anandaraja formula also. LDL-C estimated by Homogenous Enzymatic Direct Assay was designated as D-LDL, LDL- Cholesterol obtained by Friedewald equation was designated as F-LDL, and LDL- Cholesterol obtained by Anandaraja formula was designated as A-LDL.

F-LDL = TC – HDL – (TG/5) mg/dL
A-LDL = (0.9 x TC) – (0.9 x TG/5) – 28 mg/dL
The mean percentage difference (%ΔLDL) defined as calculated LDL-C minus D-LDL-C compared to the direct measurement and was calculated using the formula:
%Δ calculated LDL = {(Calculated LDL) – (D-LDL)} / D-LDL x 100

Statistical analysis

Statistical analysis was done using Microsoft Excel 2007 and Medical statistical software free trial version with revision number {600337EE-AD37-4498-BB01-6B59DDE3896C}. Paired 't' test and Pearson's correlation coefficient were used for comparison. Two tailed P value of <0.05 was considered statistically significant.

RESULTS

Out of 403 samples analysed, there were 231 males and 172 females. Table 1 shows the baseline data of the samples studied.

Table 1. Basic demographic details of study samples

Total number of subjects included in the study	403
No. of males, females	231, 172
Mean Age of subjects in years	45.7+/- 12.7
Mean±SD Serum TC (mg/dL)	191.47 +/- 52.28
Mean±SD Serum TG (mg/dL)	149.55 +/- 70.38
Mean±SD Serum HDL-C (mg/dL)	52.93 +/- 14.54
Mean±SD Serum D-LDL (mg/dL)	119.38 +/- 41.40
Mean±SD Serum F-LDL (mg/dL)	108.62 +/- 43.11
Mean±SD Serum A-LDL (mg/dL)	113.61 +/- 43.44

Both F-LDL and A-LDL showed correlation with D-LDL, but A-LDL showed greater correlation (r - 0.8203, p<0.0001, CI for r- 0.7856 to 0.8499), as shown in Figure 1, Figure 2.

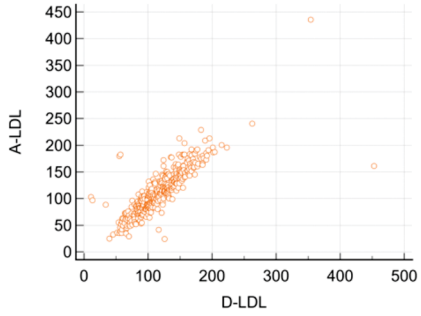

Figure 1: – Scatter plot showing A-LDL vs D-LDL

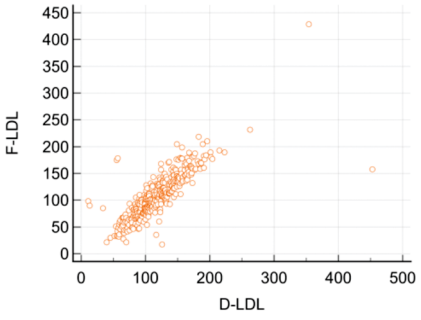

Figure 2: – Scatter plot showing F-LDL vs D-LDL

A-LDL gave lower values than D-LDL in 287 (71.22%), and higher values in 116 (28.78%) samples. F-LDL showed lower values in 309 (76.68%) and higher values in 94 (23.32%) samples. Both calculated methods underestimated LDL cholesterol levels when compared to D-LDL, but underestimation was greater when calculated by Friedewald's formula. %ΔLDL was lesser for Anandaraja's formula than Friedewald's formula as shown in Table 2.

Table 2. Mean % difference of Friedewald's formula and Anandaraja's formula when compared to Direct homogenous assay

	Mean difference in mg/dL (calculated LDL – D-LDL)	Mean Percentage difference (%)	Correlation (r)	Mean Percentage difference < 10%	Mean Percentage difference > 10%
F-LDL-C vs D-LDL-C	-10.75 +/- 1.71	-9.01	0.813 (p <0.0001)	151 (37.47%)	252 (62.53%)
AR-LDL-C vs D-LDL-C	-5.77 +/- 2.04	-4.83	0.82(p <0.0001)	182 (45.16%)	221 54.84%)

When patients were categorised as per NCEP ATP III guidelines, patients were placed in different categories based on LDL values as shown in Table 3.

Table 3. Categorisation as per NCEP ATP III guidelines, based on LDL cholesterol levels estimated by different methods

Categorisation as per NCEP ATP III guidelines	LDL Cholesterol level mg/dL	No. of patients by D-LDL level	No. of patients by F-LDL level	No. of patients by A-LDL level
Optimal	<100	127(31.44%)	177 (43.81%)	165 (40.94%)
Near optimal/above optimal	100-129	131 (32.43%)	104 (25.74%)	112 (27.79%)
Borderline high	130-159	95(23.51%)	73 (18.07%)	70 (17.37%)
High	160-189	38 (9.41%)	36 (8.91%)	43 (10.67%)
Very high	>190	12 (2.97%)	13 (3.22%)	13 (3.26%)

Out of the 403 patients, 171 samples (42.9%) showed calculated LDL values that could lead to placement of patients in a different category as per NCEP ATP III guidelines. F-LDL showed misclassification of 124 patients (30.77%) to the corresponding lower category. A-LDL values led to placement of 96 patients (23.82%) to the next lower category. A-LDL placed 46 patients (11.41%) into the corresponding higher category and F-LDL misplaced 35 patients (8.68%) into higher category (data not shown).

DISCUSSION

Studies comparing LDL cholesterol levels estimated by direct homogenous assay and various calculated methods have shown varying results.[19,20,21,22] Correlation was slightly better between A-LDL-C and D-LDL-C (correlation coefficient =0.82) than between F-LDL and D-LDL (correlation coefficient =0.81) in our study. Similar results were seen in other studies also.[17] Even though Friedewald's formula is commonly used for calculating LDL cholesterol level, in patients having lipoprotein abnormalities, TG/cholesterol ratio in VLDL can vary. Such abnormalities can make Friedewald's formula invalid for assessment of cardiovascular risk.[23] Underestimation of LDL by FF was reported by Gupta et al and Lindsey et al, similar to our study.[6, 23] But in a study, Sahu et al.,[12] have noted that LDL calculated by FF was significantly higher than the direct LDL measurement at TG between 1 and 300 mg/dl..[21] Such discrepancies in calculating LDL-C level by using Friedewald's formula must be owing to errors during measurement of TG, TC or HDL levels separately which add up during calculation.[24] Thus obtaining the recommended analytical quality of <12% total error in LDL estimation stipulated by NCEP will be difficult while using Friedewald's formula.[25] At the same time, the use of TG and TC in Anandaraja's formula reduces the chances for analytical errors than when Friedewald's Formula is used. Since the formula does not require HDL-C result for calculation, it is more economical also. Studies conducted by Gazi and Elisaf, Vujovic et al and Gasko et al have reported a higher %ΔLDL-C for F-LFL than A-LDL in Greek, Serbian and Brazilian populations respectively, gave similar results.[17, 26, 27]

LDL values calculated by Friedewald's or Anandaraja's formula can lead to misplacement of patients in either higher or lower category of management as per NCEP ATP III guidelines, as seen in our study. Calculation of LDL-C will place more patients in the "optimal" LDL-C category, leading to delay in initiation of therapeutic life style changes (TLC) which is benefitial if started early. Categorisation based on Direct assay of LDL-C may falsely place patients into "near/above optimal" category, leading to earlier introduction of TLC which is beneficial. Similar opinion was given by Nauck et.al and Mora et al in their studies.[8, 28] Thus, replacing calculation of LDL-C by direct assay using third generation homogenous assay kits can be considered in clinical laboratories, especially in patients having hyper triglyceridemia, where calculation by Friedewald's formula can be inaccurate. At the same time, overestimation of LDL-C leading to unnecessary pharmacological therapy also has to be avoided as opined by Kannan et al. [29]

In view of the varying results recorded, more detailed studies comparing these two formulas is necessary. The advantages of Anandaraja's formula makes it a better candidate for common use in laboratories.

CONCLUSION

Even though both Friedewald's method and Anandaraja's method underestimate LDL cholesterol values obtained by direct assay, Anandaraja's formula shows better correlation with direct method of LDL cholesterol estimation than Friedewald's method. Being more economical, Anandaraja's formula can be used in laboratories.

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