Research Article

# Evaluating Dry Chemistry Estimated Direct Low Density Lipoprotein (LDL), Against Calculated LDL from Different Formulae

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**ABSTRACT:**

**Background-**Lipid profile is an important investigation for many diseases especially cardiovascular diseases. LDL cholesterol is an important component of lipid profile and direct LDL cholesterol is enzymatic whereas indirect or calculated LDL cholesterol is based on other components of lipid profile and devised by formula. The most well-known is Friedewald's formula. However apart from Freidewald's formula which often doesn't correlate with direct estimation we have many other formulae. This study attempts to correlate Direct LDL cholesterol estimated on Dry chemistry with different formulae.

**Materials & Methods**-The 202 samples received during study period were correlated with different formulae like Friedewald, Cordova and Cordova, Vujovic et al, Ahmadi et al, Anandaraja et al, Puavilai et al, Hattori et al. and analyzed for the same. The lipid profiles were analyzed on QuidelOrtho's Vitros 4600 Dry chemistry.

**Results**-Our results showed a negative correlation indicting that Friedewald's formula underestimates LDL cholesterol in comparison to direct estimation. Calculated LDL cholesterol with Friedewald's formula after 400 mg/dL of triglycerides (TGs) was found high undesirable . Our results show that Cordova's formula does not provide any significant advantage over Friedewald's formula and Ahmadi's formula didn't show close correlation.Anandaraja's formula did not provide any advantage over Friedewald's formula for LDL cholesterol estimation.

**Conclusion**- Direct LDL cholesterol measured using dry chemistry shows good correlation with calculated LDL cholesterol derived from Vujovic's formula in the Indian population and can be reliably used in resource-limited settings across a wide range of TGs concentrations.

**KEY WORDS:** Direct LDL cholesterol, Friedewald's formula, Dry chemistry, Vujovic's formula, Cardiovascular disease.

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## INTRODUCTION:

A lipid profile or lipid panel is a panel of blood tests used to find abnormalities in lipids, such as cholesterol and Triglycerides (TGs) for the diagnosis of cardiovascular disease, certain forms of pancreatitis, and other diseases.[1,2] Of these tests Low- density lipoprotein (LDL) cholesterol is one of the five major groups of lipoprotein directly associated with cardiovascular risk. Therefore, the accurate measurement of LDL cholesterol is crucial. The Lipid profile can be analyzed directly or by calculated way using the Friedewald's formula. This formula requires

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fasting plasma high density lipoprotein cholesterol (HDL), total cholesterol (TC), and TGs, and is calculated as LDL = TC – HDL – (TG/5) for mg/dL(TG/2.2 in mmol/l).[3,4]

But The Friedewald's calculation has also got limitations. If triglyceride levels are 400 mg/dL or higher, LDL cholesterol cannot be calculated, and direct LDL cholesterol assays have to be performed .[5,6] Over years several other formulae were proposed for calculation of LDL. These are Cordova and Cordova et al, Vujovic et al, Ahmadi et al, Anandaraja et al, Puavilai et al, and Hattori et al. [7,8,9,10,11,12] Similar to Friedewald's formula, these formulae also use TGs, TC and HDLto calculate LDL cholesterol but have not been validated in varied populations. In our clinical lab experience we have observed that Direct LDL cholesterol and indirect calculated LDL cholesterol do not often correlate with Friedewald's formula and this often creates confusion among clinicians who know only Friedewald's formula. By considering this ambiguity in reporting, the present study was undertaken with the objectives to calculate LDL cholesterol using various formulae and to compare with direct LDL cholesterol assay, so as to obtain an acceptable formula at our setting in replacement for the direct measurement, hence, making lipid profile estimation cost effective. Usually, all studies comparing direct LDL cholesterol from indirectly calculated LDL cholesterol methods are done on wet chemistry mostly. We evaluated direct LDL Measurementby Dry Chemistry Method Versus calculated LDL Using Various Estimation Formulae.

## MATERIALS & METHODS:

The prospective observational study was carried out at a tertiary care, super-speciality of central India, for a duration of 1 week. A total of 202 samples were received during study period and were studied for correlation with different formulae like Friedewald's, Cordova and Cordova, Vujovic et al, Ahmadi et al, Anandaraja et al, Puavilai et al, Hattori et al. and analyzed for the same. The lipid profiles were analyzed on QuidelOrtho's, Vitros 4600 Dry chemistry. Total cholesterol was estimated by Method: Cholesterol Oxidase, Esterase, Peroxidase, HDL by Method: Direct Measure, PTA/Mg Cl2 Enzymatic, LDL by Method: Direct measure, Enzymatic, Triglycerol by Method: Enzymatic – Lipase /Glycerol Kinase. Quality assurance is followed as per norms. LDL cholesterol values were categorized as follows as per ATP III classification[1]Optimal: <100 mg/dL; Near Optimal/Above Optimal: 100-129 mg/dL; Borderline High: 130-159 mg/dL;High: 160-189 mg/dL; Very High: >190 mg/dL. The study was approved by Institutional ethical committee. Statistical analysis was performed by SPSS 20. Mean and standard deviation was calculated for quantitative variable. One way ANOVA test was applied for more than 2 group comparisons and Pearson correlation test was applied for correlation between two variables. If *p*-value was<0.05, it was considered as statistically significant result and if *p*-value was >0.05, then it was statistically insignificant.

## RESULTS:

Table 1 depicts the range, mean and SD of various components of lipid profile included in the study. In Table 2 Friedewald's formula showed highest SD (107.1), which means its LDL cholesterol estimates are the most widely scattered and potentially less reliable in heterogeneous population. Cordova, Vujović and Anandaraja formulas all have SDs in the high-20s to low-30s (relative SD ≈ 0.23–0.27), indicating much tighter clustering of estimates around their means. Ahmadi, Puavilai and Hattori, with SDs ~36–50 (relative SD ≈ 0.25–0.31), suggest moderate variability. Table 3 elaborates comparison between LDL cholesterol with different formulae. *p*-value of 0.001 is statistically significant and all the formulae have shown statistical significance results. However, one-way Anova showed a higher F value with of Vujovic et al. A higher F-value indicates that the variability between groups is substantially greater than the variability within groups, suggesting significant differences among the group means. F value was low for Friedewald's formula. Table 4 and Figure 1 showed that Friedewald's formula has negative correlation with direct LDL whereas Vujović et al. (r = 0.706) showed

**Table 1:** Descriptive statistics of lipid profile variables.

| Variable | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|
| TC | 96.00 | 268.00 | 196.68 | 33.844 |
| HDL | 25.00 | 110.00 | 43.16 | 12.002 |
| LDL | 16.00 | 197.49 | 111.98 | 26.740 |
| TG | 18.00 | 476.00 | 149.63 | 52.755 |

**Table 2:** Descriptive statistics for different formulas calculated.

| Variable | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|
| Friedewald's et al. (6) - LDL c=TC-HDLc-TG/5 | -199.40 | 190.20 | -73.2072 | 107.09156 |
| Cordova and Cordova (9) - LDL c=3/4 (TC - HDLc) | 18.25 | 259.50 | 105.5866 | 28.41278 |
| Vujovic et al. (11) - LDL c= TC-TG/6. 85 - HDL | 49.10 | 221.20 | 132.8525 | 30.20198 |
| Ahmadi et al.(11)- LDL c= TC/1.19 + TG/1.9 - HDLc/1.1 | 74.31 | 410.16 | 205.2765 | 50.58078 |
| Anandaraja et al. (12)- LDL c= 0.9 TC-0.9/5 -28 | 16.10 | 183.14 | 121.5791 | 27.73759 |
| Puavilai et al.(13) - LDL c= TC-HDLc - TG/6 | -214.00 | 187.30 | 127.1892 | 38.42059 |
| Hattori et al..(16) LDL c=0.94TC-0.94 HDLc -0.19TG | -214.96 | 170.36 | 114.8345 | 36.09543 |

**Table 3:** Compare between different categories of Direct LDL with Indirect LDL with different formulae.

| Formula | LDL Categories | N | Mean | Std. Deviation | F-test (One way ANOVA test) | *p*-value* |
|---|---|---|---|---|---|---|
| Friedewald's et. al. | Optimal | 39 | 9.48 | 86.57 | 14.99 | 0.0010 |
| | above optimal | 135 | -103.39 | 86.86 | | |
| | borderline high | 14 | -62.96 | 136.17 | | |
| | High | 14 | -22.79 | 162.13 | | |
| Cordova and Cordova | Optimal | 39 | 62.17 | 18.75 | 98.302 | 0.0010 |
| | above optimal | 135 | 113.99 | 14.31 | | |
| | borderline high | 14 | 115.34 | 13.82 | | |
| | High | 14 | 135.71 | 41.03 | | |
| Vujovic et al.. | Optimal | 39 | 84.86 | 19.59 | 112.053 | 0.0010 |
| | above optimal | 135 | 142.18 | 16.86 | | |
| | borderline high | 14 | 150.92 | 17.58 | | |
| | High | 14 | 158.55 | 29.44 | | |
| Ahmadi et al.. | Optimal | 39 | 144.54 | 57.07 | 36.032 | 0.0010 |
| | above optimal | 135 | 218.89 | 31.65 | | |
| | borderline high | 14 | 214.58 | 37.83 | | |
| | High | 14 | 233.94 | 65.65 | | |
| Anandaraja et al.. | Optimal | 39 | 87.03 | 28.78 | 42.447 | 0.0010 |
| | above optimal | 135 | 127.95 | 18.22 | | |
| | borderline high | 14 | 138.27 | 29.46 | | |
| | High | 14 | 139.70 | 23.02 | | |
| Puavilai et al.. | Optimal | 39 | 80.32 | 18.33 | 39.103 | 0.0010 |
| | above optimal | 135 | 138.90 | 17.21 | | |
| | borderline high | 14 | 148.02 | 17.47 | | |
| | High | 14 | 123.99 | 99.96 | | |
| Hattori et al.. | Optimal | 39 | 75.96 | 23.73 | 28.264 | 0.0010 |
| | above optimal | 135 | 124.40 | 17.08 | | |
| | borderline high | 14 | 136.80 | 17.15 | | |
| | High | 14 | 108.93 | 95.70 | | |

**Table 4:** Correlation between direct LDL and different formulas of given patients.

| Formula | Direct LDL (Correlation coefficient r) | *p*-value |
|---|---|---|
| Friedewald's et al. | -0.340 | 0.001 |
| Cordova and Cordova | 0.639 | 0.001 |
| Vujovic et al. | 0.706 | 0.001 |
| Ahmadi et al. | 0.479 | 0.001 |
| Anandaraja et al. | 0.594 | 0.001 |
| Panavillai et al. | 0.665 | 0.001 |
| Hattori et al. | 0.601 | 0.001 |

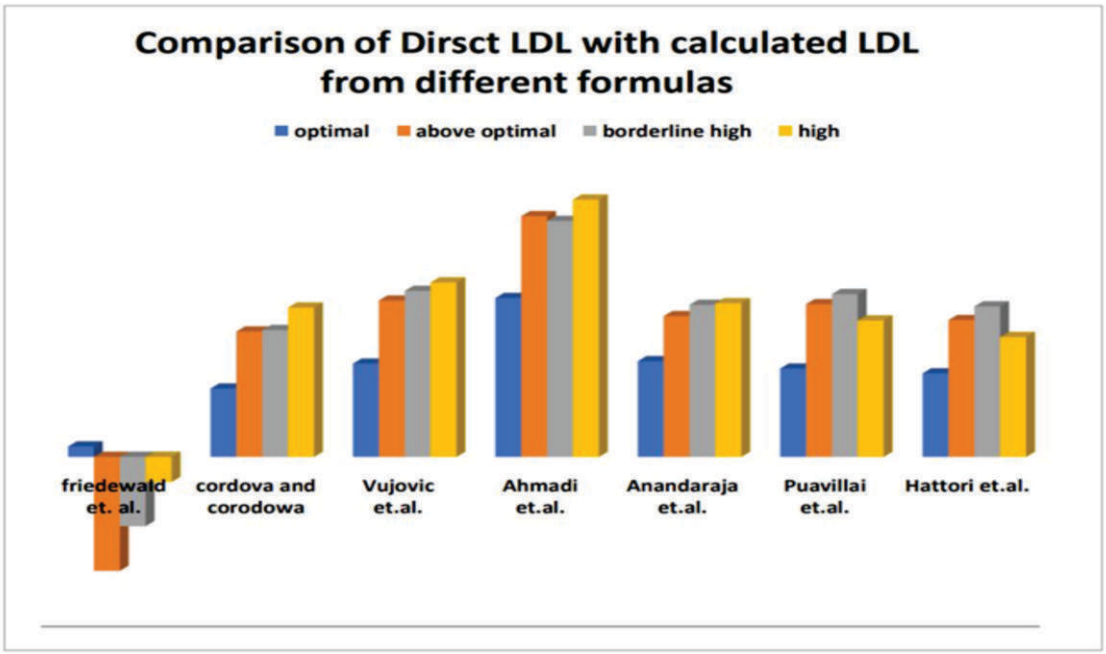


**Figure 1**: Comparison of Direct LDL cholesterol with Calculated LDL cholesterol from different Formulae.

**Table 5:** Comparison between values of Direct and indirect LDL cholesterol with TAG levels

| Variable | LDL Categories | N | Mean | Std. Deviation | F-test (one way ANOVA test) | *p*-value* |
|---|---|---|---|---|---|---|
| TG | Optimal | 39 | 111.2821 | 76.83268 | 11.526 | 0.001 |
| | above optimal | 135 | 158.3704 | 34.53070 | | |
| | borderline high | 14 | 140.2857 | 50.85035 | | |
| | High | 14 | 181.5714 | 67.09318 | | |

TG- Triglyceride.

very strong correlation indicating in agreement with direct LDL, followed by Puavilai (r = 0.665) and Cordova (r = 0.639.) Anandaraja (r = 0.594) and Hattori ® = 0.601). Table 5 showed low variance of calculated LDL with respect to TG concentrations with Vujovic et al based calculated LDL.

## DISCUSSION:

Increased LDL cholesterol levels is associated with an increase in coronary artery disease (CAD). A 1% greater LDL cholesterol value isassociated with slightly more than a 2% increase in CAD over 6 years.[13]

Most laboratories across the world use Friedewald's formula for LDL cholesterol calculation. However many studies have shown its limitation and some have shown that other equations perform better for certain groups of populations only. Hence in our current study we compared several other formulae being used along with Friedewald's formula with direct LDL cholesterol performed on Vitros Dry Chemistry. Our results showed that negative correlation indicating that Friedewald's formula underestimates LDL cholesterol in comparison to direct estimation. Also, the correlation was better with Vujovic et al.[8]

Our study is in concordance with Wadhwa et al, who had performed a similar study but the platform used was Wet chemistry.[14]

Credibility of calculated LDL cholesterol with Friedewald's formula for values > 400 mg/dL of TG was found highly undesirable. However, Sha MFR et al., in Bangladeshi population concluded that Friedewald's formula can be used up to serum TG concentration of 700mg/dL.[15]

Cordova and Cordova suggested a new formula that performed better than Friedewald's formula in Brazilian population.[7] Our results show that Cordova formula does not provide any significant advantage over Friedewald's formula in Indian population (Table 3, 4; Figure 1). Similarly Ahmadi's formula has been validated in Iranian subjects at TG<300mg/dL.[9] However, our study didn't show strong correlation. Hence, it is not suitable to be used.

Our study is in concordance with the study done by Gupta S et al., which concluded that Anandaraja formula does not provide any advantage over Friedewald's formula for LDL cholesterol estimation in Indian population[16]. This is contradictory to the study done by Anandaraja et al., which found their formula more accurate than Friedewald's formula for TG <350mg/dL[10]. The modified Friedwald's equation developed by Cordova et al, Puavilai et al, and Hattori et al all showed almost similar correlation with direct estimations of LDL[7,11,12]. Our study supports the study done by Vujovic et al, which validated a modifiedformula in Serbian population with TG<400mg/dL[8]. In our study it was correlating with wide range of TG levels as well.

## CONCLUSION:

Direct LDL cholesterol from Dry chemistry better correlates with calculated LDL cholesterol from Vujovic formula for Indian population when compared to Friedewald's formula and when needed can be used in resource poor settings over a wide range of triglyceride concentration.

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**Conflicts of Interest**
There are no conflicts of interest.

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