**Original Article**

# A Comparative And Correlative Study of Direct LDL Assay With Friedwald's Formula in Rural Kolar Population.

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**ABSTRACT**

***Aims:*** *There is a direct correlation between raised LDL levels and risk of developing heart disease and hence the aim of our study was to compare the LDL-C levels obtained by direct measurement with that calculated by Friedwald's formula.*

***Methods:*** *A total number of 80 serum samples from adult patients received at the R.L.Jalappa Hospital and Research Centre, Kolar was analyzed. Only those where full fasting lipid profile was requested were considered. Fasting Triglycerides concentration above 4.52 mmol/L and less than 0.33 mmol/L were excluded. Analysis of samples was performed on Minitechno semiautoanalyzer. Statistics by Pearson's correlation and scattered diagram showed significant correlation at the 0.01 level (2-tailed) between direct method of estimation of LDL-C and measurement of LDL-C using Friedwald's formula.*

***Results and Conclusions:*** *Our studies showed that there is a good correlation with Friedwald's formula and direct assay when TG levels are up to 3.39 mmol/L. Since, estimating direct LDL-C is a more expensive method it should be used in patients where Friedwald's estimation is limited as in patients where TG levels are>4.52 mmol/L.*

*Hence, the use of Friedwald's formula for estimation of LDL-C is a reliable alternative apart from being time saving and cost effective.*

***Key words:*** *LDL C, HDL, TG, TC, Friedwald's Formula.*

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## AIMS AND OBJECTIVES

LDL cholesterol is directly related to the risk of developing coronary heart disease (CHD) and other macro vascular complications. Hence, measure of LDL cholesterol is important. The method for measuring Low Density Lipoprotein (LDL) cholesterol assumes that total cholesterol (TC) is composed primarily of cholesterol in

very low density lipoprotein (VLDL), LDL and high density lipoprotein (HDL). LDL cholesterol can be measured by indirect methods of which the most widely used one is the calculation of LDL cholesterol using the empirical formula of Friedwald's which is as LDL-C = (TC)- (HDL -C) - TG/5

The factor (TG) / 5 is an estimate of cholesterol concentration in VLDL and is based on the average ratio of Triglycerides (TG) to cholesterol in it.

Studies have shown that there are limitations in using Friedwald's equation as it should not be used for estimation in samples where TG concentrations are more than 4.52 mmol/L. But studies conducted in Indian scenario particularly in rural parts of south India are neither inconclusive nor clear. The factor TG / 5 which gives an estimate of VLDL cholesterol concentrations cannot be applied as such because samples contain chylomicrons (CM), chylomicron remnants as well as VLDL remnants all of which, predict higher TG to cholesterol ratio than normal VLDL.

Since, there is a direct correlation between raised LDL levels and risk of developing CHD this has made us to take up a study with an aim to compare the LDL-C levels obtained by direct measurement with that calculated by Friedwald's formula. [1]

## REVIEW OF LITERATURE

LDL-C is key factor in the pathogenesis of premature CHD [2,3] Blood LDL-C concentration is used to assess cardiovascular risk and effectiveness of cholesterol lowering regimens. [4,5]

Epidemiological and clinical studies have demonstrated a strong positive correlation between LDL-C concentrations in serum and the incidence of CHD. [6,7] Pathological studies have shown that increased LDL-C concentrations correlate highly with the extent of atherosclerotic lesions.[8] A reduction of LDL-C decreases the risk and ameliorates the symptoms of CHD by causing a regression in the lesions.[9,10]

The diagnosis and management of adults with hypercholesterolemia are largely based on LDL-C concentration. In order to classify someone at risk of CHD, LDL-C must be measured within a total error of 12%. [11] Serum LDL-C concentrations used to classify adults at a risk of developing heart disease are as follows < 1.469 mmol/L desirable, 1.469-1.796 mmol/L as borderline high risk and >1.808 mmol/L as high risk. [12] The goal for subjects with two or more risk factors such as family history of diabetes, hypertension, cigarette smoking or low HDL-C should be to achieve LDL-C of 1.13 mmol/L. [13] Therefore, accurate and precise measurements of patients, LDL-C is necessary to identify the individual with hypercholesterolemia and also to monitor the response to diet and drug regime. [14]

## MATERIALS AND METHODS

Left over serum samples from adult patients received at the biochemistry lab of R.L.Jalappa Hospital and Research Centre, Kolar, where a full fasting lipid profile was requested were considered for our study. Samples with fasting Triglycerides concentrations above 4.52 mmol/L and less than 0.33 mmol/L were excluded as the equation has

been clearly shown to be invalid in hyper and hypotriglyceridaemic samples. [12,15] We have also excluded the patients with family history of diabetes or hypercholesterolemia as well as those patients in whom lipid profiles are known to get altered pathologically. The lowest age we have considered was 16 years and the highest age was 74 years irrespective of the sex. Total number of samples used for the study was 80. There was a male preponderance, males constituted the highest number of 48 (n=48) and females 32 (n=32).

**LIPID ANALYSIS**

Lipid profile analysis was performed using semi auto analyzer. Total cholesterol (TC) was measured by enzymatic determination of total serum cholesterol using cholesterol oxidase (CHOD) and peroxidase (POD) method of Allain CC, et al. [16] Triglycerides (TG) were estimated by enzymatic hydrolysis of triglycerides to glycerol and fatty acid using lipoprotein lipase (LPL) based on the method of Bucolo G David. [17] Here, the Glycerol in presence of ATP is phosphorylated by glycerol kinase (GK) and then is oxidized by glycerol -3-Phosphate oxidase (GPO) to produce hydrogen peroxide ($H_2O_2$) and dihydroxy acetone phosphate (DHAP). The $H_2O_2$ produced in the presence of peroxidase (POD) reacts with 4-aminoantipyrene and p-cholophenol to produce a colored compound that can be measured by a spectrophotometer at $_{max}$ 500 nm. HDL direct cholesterol (HDL-C) was estimated by Colorimetric end point test. Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated by the precipitating reagent. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction remains in the supernatant in this phase and is determined by an enzymatic method based on the method of Warnick G.R. and Wood P.D. [18]

LDL cholesterol was estimated based on the principle of first eliminating the non LDL fractions and then measuring LDL-C based on the method of Amayo AA, Kirera S and Okada M. [4,19]

The assay takes places in two steps. In the first step non LDL particles are eliminated. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non color forming reaction. In the next step, the remaining LDL particles are solubilized and a chromogenic complex brings about color development as shown.

1. Elimination of lipoprotein non LDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O_2 + O2$$

2. Measurement of LDL-C

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4. Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{TOOS} + \text{4-AA} \xrightarrow{\text{POD}} 2\,H_2O_2 + O_2$$

**Table 1 : Descriptive Statistics**

| | N | Minimum mmol/L | Maximum mmol/L | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Ff.ltr | 80 | 2.56 | 2.56 | 5.4917 | 2.01258 |
| Direct.ltr | 80 | 2.06 | 2.06 | 6.5253 | 1.92482 |

**Table 2: Comparison Between LDL-C Estimation By Friedwald's Formula and Direct Method using Paired't' Test**

| | | | | | |
|---|---|---|---|---|---|
| | | | ± | | |
| | | | ± | | |
| | | | ± | | |

**Table 3: Correlation Analysis of LDL-C Estimation by Friedwald's formula Vs. Direct Method**

| | | | | | |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |

FRIEDWALD

DIRECT

**Figure 1: Linear Regression Analysis of LDL-C Estimation by Friedwald's Formula and Direct Method**

## RESULTS AND DISCUSSION

The findings of our study show that, the mean calculated by Friedwald's formula is less compared to that calculated by direct method of LDL-C estimation (Table 1).

The statistical data was calculated using Paired 't' test and showed strong significance for 'p' value (Table 2). Correlation is significant at the 0.01 level (2 tailed) when correlation analysis of LDL-C estimation was done by Friedwald's formula vs. Direct method (Table 3).

The linear regression shows that the variables i.e, LDL-C calculated by Friedwald's formula and that obtained by direct method of estimation are linearly correlated by the linear regression equation (Fig 1).

Since, different methods of measurement of LDL-C by Friedwald's formula and direct estimation are both showing good correlation and taking into account the cost and performance, Friedwald's method is as good as other methods.

Friedwald's formula cannot be used when triglyceride levels are above 4.52 mmol/L (400 mg/dl) and below 0.33 mmol/L (30 mg/dl). Further studies are necessary to draw a conclusive line to prove its positiveness in a large study group.

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