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Friedewald's Method underestimates LDL-Cholesterol even at Lower Range of Triglyceride.

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ABSTRACT

Although Friedewald's method is routinely used and convenient for clinical practice in measuring serum cholesterol level, it is not recommended for use in non-fasting blood samples or the presence of hypertriglyceridemia (>400 mg/dL) or type III hyperlipoproteinemia. To compare LDL-cholesterol (LDL-C) determined by direct homogeneous method with LDL-C determined by the Friedewald's formula. It was a hospital based cross sectional study. A total of 1018, 10-12 hours fasting serum samples were taken. Triglyceride (TG) and total cholesterol (TC) were assessed by enzymatic colorimetric method; direct LDL-C and high density lipoprotein (HDL-C) by homogeneous enzymatic colorimetric assay in Cobas c 311 of Roche. LDL-C was calculated using Friedewald's method.Pearson's correlation and paired t-test. The mean LDL-C showed significant difference ($p<0.001$) when measured by two different methods, direct homogeneous and Friedewald's estimation at all different TG ranges <100, 101-200, 201-300, 301-400 and >400 mg/dL. There was significant correlation between direct and Friedewald's calculated LDL-C ($r=0.966$). We also found -5%, -8.7%, -17.9%, -23.7% and -39% negative error in calculated LDL-C with direct LDL-C at TG ≤100, 101-200, 201-300, 301-400 and >400 mg/dL respectively. Significantly higher percentage (38.2%) of subjects were classified having >130mg/dL LDL-C by direct homogenous method as compared with Friedewald's method (24.9 %), with odds ratio of 1.87. Friedewald's method for LDL-C estimation can't be used for assessment of patients having hypertriglyceridemia.

Keywords: Direct LDL, Friedewald's method,

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INTRODUCTION

According to the third report of the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP), LDL-C is the primary target for the diagnosis and treatment of hypercholesterolemia [1]. The common approach for determining LDL-C concentration in the clinical laboratory is the Friedewald's method, which derives LDL-C from total cholesterol, HDL-C, and TG in the fasting samples. Although this method is routinely used and convenient for clinical practice, it is not recommended for use in nonfasting blood samples or the presence of hypertriglyceridemia (>400 mg/dL) or type III hyperlipoproteinemia. For these reasons, an expert panel of NCEP recommended in 1995 developing direct methods for the measurement of LDL-C. In addition, the Friedewald's method primarily requires assessment of TC, HDL-C, and TG, for calculation of LDL-C. These three parameters potentially decreasing the accuracy and precision of the derived cholesterol concentration [2]. Therefore this study was undertaken to compare the performance and limitation of Friedewald's method with direct homogenous method.

MATERIALS AND METHODS

This is a cross-sectional study carried out in Department of Biochemistry B.P. Koirala Institute of Health Sciences, Dharan, Nepal from February to May 2013. 1018 patients advised for lipid profile, were recruited on the basis of convenient sampling method. Data were obtained from the lipid profile analysis of 10-12 hours fasting venous blood samples collected in plain vial. Serum was separated by centrifugation at 3000rpm for 10 minutes and following Lipid parameters were analysed by Roche, cobas c 311 chemistry auto-analyser.

Patient having impaired hepatic function were excluded from the study as abnormal liver function affects lipid metabolism. TC was analysed by enzymatic endpoint cholesterol oxidase peroxidase method (CHOD- PAP) method where the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye.4 amino antipyrine . TG by Enzymatic Glycerol Phosphate Oxidase/ Peroxidase method, and HDL-C by Homogenous Enzymatic Direct Assay. LDL-C was analysed by homogenous Enzymatic Direct Assay.

The assay was performed according to manufacturer's specifications. We used a lyophilized calibrator provided by the manufacturer. The assay contains two ready-to-use reagents. The Reagent 1 contains $MgCl_2$, dye, buffer (pH 6.75), and α-cyclodextrin sulphate, which has a highly concentrated negative charge to mask cholesterol in chylomicrons and VLDL in the presence of magnesium ions.Reagent 2 contains the enzymes; cholesterol oxidase, cholesterol esterase, peroxidase, dye, buffer (pH 6.75), and a polyoxyethylene polyoxypropylene block polyether (POE-POP) to block cholesterol, especially in HDL. The assay is CDC CRMLN (Cholesterol Reference Method Laboratory Network) certified and meets the 1995 NCEP goals of < 4 % total CV, bias ≤ 4 % versus reference method, and ≤ 12 % total analytical error. Indirectly LDL-C values were calculated using Friedewald's formula.Paired t-test and Pearson's correlation coefficient were used for statistical significance.

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RESULTS

In our study we found significant difference ($p<0.001$) in LDL-C measured by direct homogeneous and Friedewald's method at different TG ranges as shown in Table 1. At higher cholesterol levels, >300 mg/dL, LDL-C measured by direct homogenous and Friedewald's method was not significantly different whereas the homogenous LDL-C was significantly higher than Friedewald's calculated LDL-C at TC level of 101-200 and 201-300 mg/dL (Table 2).

It was found -5%, -8.7%, -17.9%, -23.7% and -39% negative error in calculated LDL-C with direct LDL-C at TG level ≤100 mg/dl, 101-200 mg/dl, 201-300 mg/dl, 301-400 mg/dl and >400 mg/dL respectively. Table 4 shows that significantly higher percentage of subjects (38.2%) were classified under high risk group by direct homogenous method as compared with Friedewald's method (24.9 %), with an odds ratio of 1.87. Besides these discrepancies in measuring the LDL-C levels, there was a strong positive correlation between LDL-C levels obtained by both the methods at different TG ranges as shown in figure 1.

Figure 1: Correlation between Direct assay and Friedewald's estimate of LDL cholesterol at different triglyceride ranges:(A) ≤100 mg/dL, r= 0.968; (B) 101-200 mg/dL, r=0.966; (C) 201-300 mg/dL, r=0.960; (D) 301-400 mg/dL, r=0.960; (E) >400 mg/dL, r=0.600

DISCUSSION

The present study was carried out to assess the performance of homogenous method of LDL-C estimation with that of Friedewald's calculated method. There has been a general consensus not to use the Friedewald's method to measure LDL-C for the samples containing triglyceride (TG) levels > 400 mg/dL [3]. However, in this study there was a substantial difference in LDL-C levels obtained by both the methods even at lower TG levels (<100, 101-200, 201-300, and 301-400 mg/dL) and the bias was found to be negative for the Friedewald's method at these TG levels. This can be explained as estimated VLDL-C (TG/5) becomes a larger part of the equation at higher triglyceride concentrations, and its systematic overestimation in this setting generates LDL-C underestimation [4]. In addition, the Friedewald,s method to measure for LDL-C requires three primary measurements (TC, HDL-C and TG), potentially decreasing the accuracy and precision of the derived cholesterol concentration [2]. Nevertheless, few studies [5,6] have reported similar findings as ours. In contrast, some other studies have shown the Friedewald's method to have a positive deviation or bias in regard to the direct method [2, 7-9]. On the other hand, when assessing the results at different levels of TC, LDL-C was similar in both the methods at low and high cholesterol levels shown in Table 2.

The diagnosis and management of adults with hypercholesterolemia are largely based on LDL-C. In order to classify someone correctly into the National Cholesterol Education Program cut-points (desirable LDL-C limit < 130 mg/dl), LDL-C must be measured with a total error of ≤12%. This direct homogeneous assay was found to meet the current NCEP requirements for LDL-C testing for precision (CV <4%) and accuracy (bias <4%) with an analytical error of ≤12% as certified by the Cholesterol Reference Methods Laboratory Network [9]. In our study, 136 (13.3%) subjects were underestimated to be classified as

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hypercholesterolemic (LDL-C ≥ 130 mg/dL) by the Friedewald's estimation, thereby missing the large number of patients to be correctly diagnosed for early management. This particularly becomes more pronounced for the high risk individuals having diabetes, atherosclerotic disease, or metabolic syndrome [1].

CONCLUSION

To conclude Friedewald's method for LDL-C estimation can't be used for assessment of patients having hypertriglyceridemia. Now with advancement in technology, direct homogenous assay becomes more accessible and economical as it classifies more patients at high risk and hence improves the patient care with early diagnosis and management.

Table 1: Direct and Friedewald's LDL-C (mg/dL) at different Triglyceride ranges (N = 1018)

TG Range (mg/dL)	N	TC (Mean±SD)	HDL-C (Mean±SD)	Direct LDL-C (Mean ±SD)	Friedewald's LDL-C (Mean±SD)	P value
≤100	218	156.2±39.0	51.3±17.2	94.4±32.8	89.9±30.9	**<0.001***
101-200	362	185.9±45.8	44.2±14.9	122.4±40.5	112.6±38.9	**<0.001***
201-300	295	198.5±57.1	38.8±12.9	132.1±53.5	112.0±53.9	**<0.001***
301-400	84	212.5±58.8	35.4±11.3	134.0±52.5	108.3±51.4	**<0.001***
>400	59	231.5±60.0	32.9±8.4	119.4±47.1	85.9±51.8	**<0.001***

* Statistically significant, Paired t test between Direct and Friedewald's LDL

Table 2: Direct and Friedewald's LDL-C (mg/dL) at different total cholesterol ranges (N = 1018)

TC Range (mg/dL)	N	TG (Mean±SD)	HDL-C (Mean±SD)	Direct LDL-C (Mean±SD)	Friedewald's LDL-C (Mean±SD)	P value
≤100	27	111.4±75.5	25.6±14.0	40.6±13.3	40.1±13.9	0.776
101-200	613	170.3±98.0	41.0±13.7	98.5±25.93	85.8±25.5	<0.001*
201-300	359	243.8±141.1	46.3±15.8	155.1±27.0	136.2±29.2	<0.001*
>300	19	364.6±281.9	54.5±27.1	261.9±123.0	260.9±113.5	0.953

* Statistically significant, Paired t test

Table 3: Percentage error of means between Direct and Freidewald's LDL-C (mg/dL)

TG Range (mg/dl)	N	Direct LDL-C (Mean)	Friedewald's LDL-C (Mean)	% Error
≤100	218	94.4	89.9	-5.0
101-200	362	122.4	112.6	-8.7
201-300	295	132.1	112.0	-17.9
301-400	84	134.0	108.3	-23.7
>400	59	119.4	85.9	-39.0

Table 4: Assessment of risk according to NCEP, ATP III classification of LDL-C (N = 1018)

LDL-C	Direct Homogenous Assay (%)	Friedewald's method (%)	Odds Ratio (95%CI)
≥ 130	389(38.2%)	253 (24.9%)	1.87 (1.55-2.26)
<130	629 (61.8%)	765 (75.1%)	

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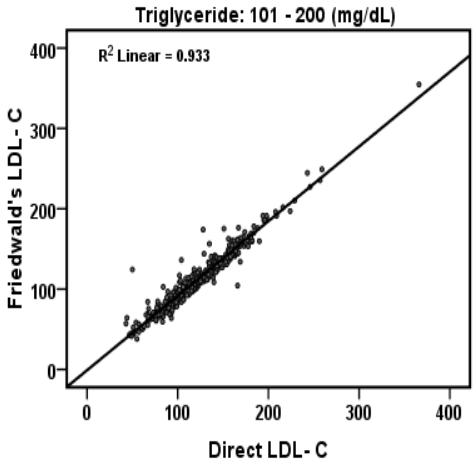
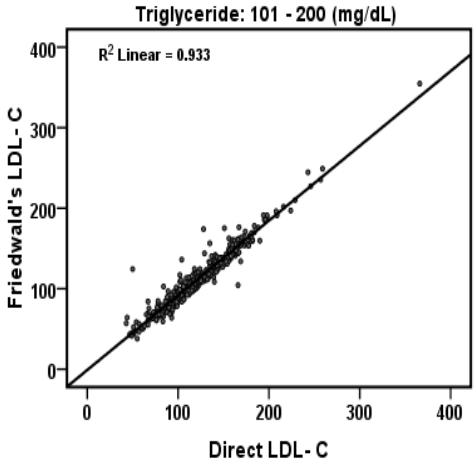
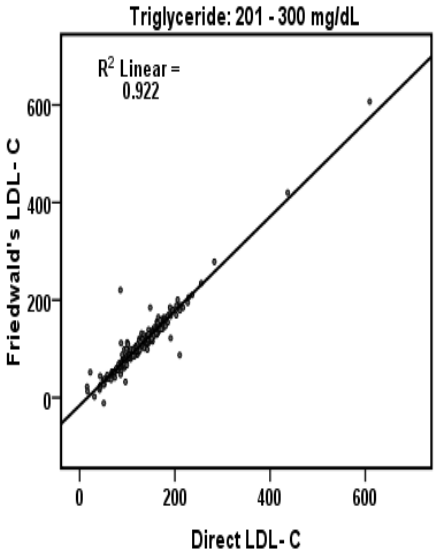


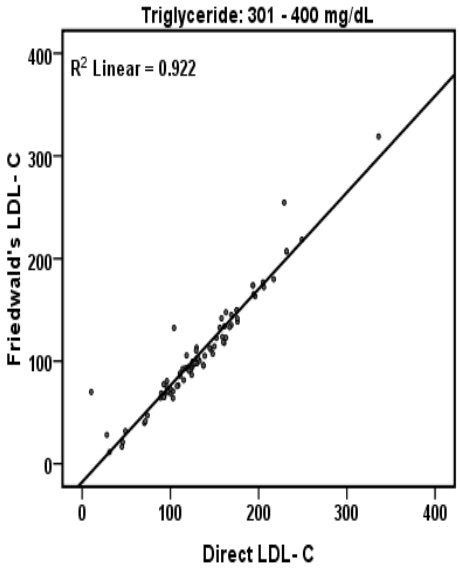
(A) ≤100 mg/dL, r= 0.968



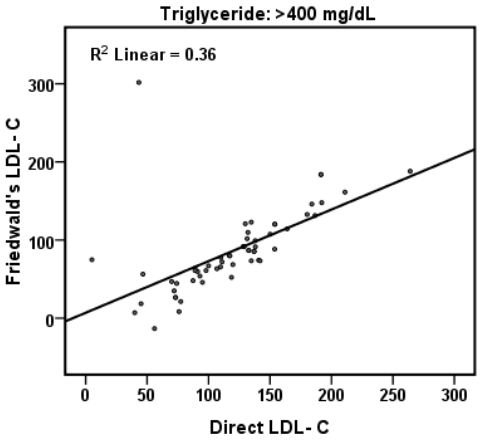
(B) 101-200 mg/dL, r=0.966



(C) 201-300 mg/dL, r=0.960



(D) 301-400 mg/dL, r=0.960



(E) >400 mg/dL, r=0.600

Figure 1: Correlation between Direct assay and Freidewald's estimate of LDL cholesterol at different triglyceride ranges (A), (B), (C), (D) and (E).

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