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ORIGINAL ARTICLE

Evaluation of Friedwald' and Anandaraja' formulae Against Direct Estimation of LDL in Fasting Samples Received in Aga Khan Multan Stat Lab

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ABSTRACT

Objective: To compare the Friedewald and Anandaraja's formulae with direct homogeneous assay for serum low density lipoprotein cholesterol (LDL-C) levels estimation.

Place and Duration of Study: A retrospective study was conducted from July 2012 to December 2012 at Multan Stat Laboratory of Aga Khan University Hospital

Method: The study assessed 1459 blood samples from out-patients of either gender sent to the Collection centers of Aga Khan university hospital, from southern Punjab for measurement of total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C)' VLDL and triglyceride (TG) levels. Total cholesterol, high-density lipoprotein cholesterol, TG and LDL-C were measured on Hitachi 902 chemistry analyzer (Roche). 68(4.66%) specimens with TG > 450mg/dl were excluded from further analysis .LDL-C levels were also calculated by Friedewald formula (FF) and Anandaraja's formula in1391 samples. The mean± SD (mg/dl) LDL-C levels were calculated for three methods. The percentage difference (%ΔLDL) defined as calculated LDL-C minus D-LDL-C compared to the direct measurement was calculated using the following formula: %Δcalculated LDL-C = [(calculated LDL-C)-(DLDL- C)]/D-LDL-C*100.(26).Linear regression analyses was done using Microsoft Excel 2007 to assess the regression and correlations between three methods.

Results: Out of the 1391 samples for which analysis was done, 876 (62.97%) were received from the male patients and 415 (29.83%) were from females. Mean ± SD of age of the total study subjects was 46.08± 12.73years. Mean and standard deviation of D-LDL-C (110.88 ±37.37) is lower than mean and standard deviation of F-LDL-C (112.25±40.93)and A-LDL-C (112.88± 39.77) **.** Mean percentage differences between Friedewald formula and Direct LDL-C values (ΔF-LDL-C%) were positive (12.25±40.93) and mean percentage differences between Anandaraja's formula and direct LDL-C values (ΔA-LDL-C%) were also positive (12.88±39.77). A comparison of D-LDL-C (x) versus F-LDL-C (y) and D-LDL-C (x) versus A-LDL-C(y) values resulted in the following regression equations: y=-0.93516+1.053(X), r = 0.9623 and y =2.656717 +0.993985(X) , r = 0.9340, respectively .

Conclusion: On the basis of these findings, the D-LDL assay appears to be superior to the F-LDL-C and A-LDL-c assay .LDL-C should be measured by direct homogeneous assay in routine clinical laboratories.

Key words: Low density lipoprotein cholesterol. Direct homogeneous assay. Friedewald formula. Anandaraja's formula.

INTRODUCTION

Coronary artery disease accounts for the greatest number of deaths of adult individuals worldwide[1].The National Cholesterol Education Programme's (NCEP) Adult Treatment Panel III (ATP III) recommended low density lipoprotein cholesterol (LDL-C) as the primary lipid agent for CAD risk prediction and therapeutic target, emphasizing the importance of accuracy and precision of LDL-C estimation[2]. The levels of LDL cholesterol recommended are: Optimal, <100mg/dl; near optimal or above optimal, 100-129mg/dl; Borderline high, 130-159 mg/dl; High, 160-189 mg/dl; very high,>190mg/dl[3]. High levels of low density lipoprotein cholesterol are correlated with atherosclerosis and coronay heart disease[4] Several studies have also shown that when high LDLC concentrations are decreased by means of diet and drugs, the subsequent incidence of CHD is diminished[5–9]. Each LDL particle contains one

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molecule of Apolipoprotein B-100 (apo B- 100), which is the main protein component of LDL, and the other minor apolipoproteins are apo E and apo C II[10].

The accepted "gold" standard method for blood LDL-C estimation is the beta - quantification (BQ-LDL) which is an expensive, labour intensive method and not generally available in routine laboratories[11,12]. Most clinical laboratories have therefore depended on calculations of LDL-C using the Friedewald equation {LDLc = total cholesterol − HDLc − [triglycerides (in mmol/L)/2.17 or triglycerides (in mg/dL)/5]}, the most frequently used method for the calculation of LDLc which is based on three independent measurements: total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG)[13,14,15].

Anandaraja et al.[16] published new formula for low density lipoprotein estimation from two other parameters, total cholesterol and triglycerides, as substitution to well known Friedewald's formula. Anandaraja's formula A-LDL-C (mg/dL) = 0.9*TC - 0.9*TG/5 - 28. is used in some countries to calculate LDL-C values. Anandaraja's formula has been approved for use in Brazilian and Greek population[17,18].

Several direct assays for LDL-C estimation have recently been developed and the kits are available for use by routine laboratories. There are reports of differences between LDL-C values calculated using the Friedewald's formula and those obtained by direct assays[19-22]. The Friedewald's report has become frequently cited and calculation has become the benchmark for routine LDL-C quantification[23]. A recent formula by Anandaraja and colleagues for LDL-C estimation still needs to be evaluated before it is extensively applied in diagnosis. There are no studies reporting use of this new formula in pakistan.

This study was aimed to compare LDL-C estimation in Multan Stat Lab by two different calculated methods (Friedwald formula and Anandaraja's formula) with direct homogeneous assay with the assumption that the results obtained by direct assay are the most accurate.

MATERIAL AND METHOD

Data was obtained from the lipid profile analysis performed in Multan Stat Lab .This study assessed the blood samples of 1459 patients received at collection centers of Aga Khan University Hospital Laboratory for lipid profile (total cholesterol, LDL-C, HDL-C, VLDL and triglyceride measurements) from July 2012 to December 2012. Hemolyzed, icteric and lipemic samples were excluded. Blood samples were collected after a 10- to 12-hour fast, and allowed to clot at room temperature. Serum was separated immediately after centrifugation at 3,000rpm for 8-10 minutes. Separated serum was transported from collection center to Multan Stat lab. The assays were performed on Roche/Hitachi 902 clinical chemistry autoanalyser using reagent, calibrator and recommendations of ROCHE.

The variables estimated in this study were cholesterol, triglycerides, HDL and LDL.Values in mg/dL were calculated. 68(4.66%) specimens with TG > 450mg/dl were excluded from further analysis .Total cholesterol (TC) and TG levels were measured enzymatically by CHOD-PAP and GPO-PAP methods (Roche Diagnostics GmbH, Mannheim, Germany), respectively according to the manufacturer's specifications. High-density lipoprotein cholesterol (HDL-C) was measured using a homogeneous assay without precipitation (Roche Diagnostics GmbH, Mannheim, Germany)[24,25]. Low-density lipoprotein cholesterol (LDL-C) was measured by using LDL-C plus 2nd generation homogeneous assay kit without pretreatment . (Roche Diagnostics GmbH, Mannheim, Germany). Because VLDL (very low density lipoprotein) carries most of the circulating triglycerides (TG), VLDL-C can be estimated reasonably well from the measured TG divided by 5 for mg/dl[25].

Direct LDL-C estimation and Friedewald's and Anandaraja's formulas were used for calculation of LDL-C (D-LDL-C,F-LDL-C and A-LDL-C, respectively) in remaining 1391 samples.The study compared directly the levels of F-LDL-C and A-LDL-C to direct measured LDL(D-LDL-C).The mean± SD (mg/dl) LDL-C levels were calculated for three methods. The percentage difference (%ΔLDL) defined as calculated LDL-C minus D-LDL-C compared to the direct measurement was calculated using the following formula: %Δcalculated LDL-C = [(calculated LDL-C)-(DLDL-C)]/D-LDL-C*100.[26]. Linear regression analyses was done using Microsoft Excel 2007 to assess the regression and correlations between three methods.

RESULTS

A total of 1391 lipid profiles were analyzed .Out of the 1391 samples for which analysis was done, 876 (62.97%) were received from the male patients and 415 (29.83%) were from females (Fig: 1)

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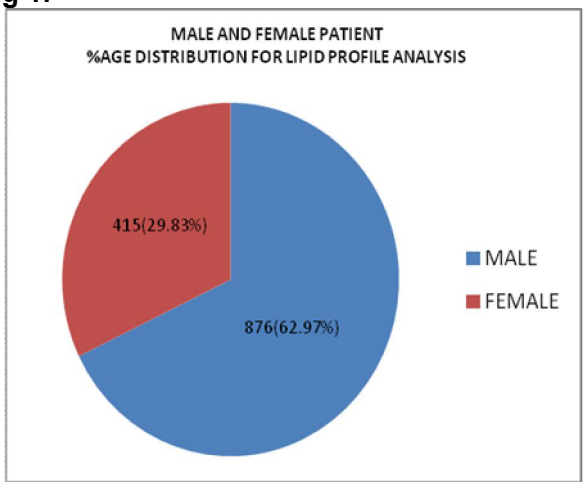
Fig 1:



Mean ± SD of age of the total study subjects was 46.08± 12.73years.Minimum age 04 years and maximum age was 90 years. Minimum and maximum values obtained by D-LDL-C (13mg/dl , 297mg/dl), F-LDL-C(-1mg/dl ,326mg/dl) and F-LDL-C(-1.9mg/dl,314mg/dl) for LDL concenteration. Mean and standard deviation of D-LDL-C (110.88 ±37.37) is lower than mean and standard deviation of F-LDL-C (112.25±40.93)and A-LDL-C (112.88± 39.77) . The mean level of F-LDL-C was approximately 1.37mg/ dl more than that of D-LDL-C and the mean level of A-LDL-C was approximately 2mg/dl more than that of D-LDL-C. Mean percentage differences between Friedewald formula and Direct LDL-C values (∆F-LDL-C%) were positive (12.25±40.93) and mean percentage differences between Anandaraja's formula and direct LDL-C values (∆A-LDL-C%) were also positive (12.88±39.77) (Table1). Coefficient variation for D- LDL-C, F-LDL-C and A-LDL-C are 33.70, 36.46 and 35.23 respectively.

Table 1: Mean And Standard Deviation Of Ldl Measurements And Mean Percentage Differences

	D-LDL-C mg/dl	**F-LDL-C mg/dl**	**∆F-LDL-C%**	**A-LDL-C mg/dl**	**∆A-LDL-C%**
MEAN	110.88	112.25	12.25%	112.88	12.88%
STANDARD DEVIATION	37.37	40.93	40.93	39.77	39.77
COEFFICIENT VARIATION	33.70	36.46	-	35.23	-
CORRELATION 'r'		r = 0.9623		r = 0.9340	

Directly measured LDL-C concentrations were found less in 54.99%(765) of samples when compared with F-LDL-C concentrations and also found less in 54.34%(756) of samples when compared with A-LDL-C concentrations.72.32% of samples have shown similar concentration trend by F-LDL-C formula and A-LDL-C formula as compared to D-LDL-C formula. A comparison of D-LDL-C (x) versus F-LDL-C (y) and D-LDL-C (x) versus A-LDL-C(y) values resulted in the following regression equations: y=-0.93516+1.053(X), r = 0.9623 and y =2.656717 +0.993985(X) , r = 0.9340, respectively .

DISCUSSION

The accuracy and precision of the method used to estimate blood LDL-C is very important. This study aimed at assessing the performance of a homogeneous method for direct LDL-C measurement, as compared with the LDL-C estimation by using the Friedewald formula and Anandaraja's formula. Strengths of the present study include the large number of participants from whom simultaneous concentrations of direct , Friedewald and Anandaraja's LDL-C were obtained.

The comparison of LDL-D and LDL-F has shown different findings in different studies. Fukuyama et al, gave higher F-LDL-C level than direct method in young Japanese females(27). Sahu *et al*. also reported that F-LDL-C was significantly higher than D-LDL-C(28). Mora *et al*. compared FF and direct assay in specimens from healthy female subjects. They reported that F-LDLC were significantly higher than DLDL-C(29). The direct assay used in the study correlated highly with Friedewald calculation but was generally lower by approximately 5–10 mg/dL.Kamazeki et al. have reported an underestimation of 5.9 mg/dl by FF compared to the directly measured LDL-C (30). Schanagl *et al*. reported lower level of F-LDL-C than D-LDL-C(31). Correlation between LDL-C measured by the direct method and estimated by FF has been reported by

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many authors(32,33,34,35,36). In the study by Gasko et al. (37) results by Anandaraja's formula were closer to direct measurement with a mean difference of -1 mg/dl. Vujovic et al. (38) have also reported higher values for D-LDL-C. They have found a percentage difference of -6.9 for F-LDL-C and -3.9% for A-LDL-C. Kamal and co-workers(39) reported that LDL-C calculated by FF, Anandaraja formula and another modified formula were significantly lower than the D-LDL-C ($p < 0.001$). In our study use of calculated LDL determined by Friedewald's formula and Anandaraja's formula overestimated the LDL-C level when compared with the Hitachi 902 homogenous assay.Parvin et al (40) reported the mean ± SD of age of the total study subjects was 48.28 ± 11.08 years,in which 65% subjects were males and 35% subjects were females.These observations were in accordance with the results of our study. Vujovic et al (38).,documented that directly measured LDL-C concentrations exceeded F-LDL-C and A-LDL-C concentrations in 82% and 65% of samples, respectively.A comparison of D-LDL-C (x) versus F-LDL-C (y) and D-LDL-C (x) versus A-LDL-C(y) values resulted in the following regression equations: $y = -0.17 + 0.980\times$, $r = 0.96$ and $y = 0.129 + 0{,}971\times$, $r = 0.89$, respectively . Tighe and colleagues (41) found good correlation between LDL-C calculated by Friedewald's formula and directly measured LDL-C ($r = 0.90$).Friedewald formula overestimated the LDL level compared to the direct method in the study by Boshtam et al (42). The mean level of LDL-F was approximately 7 mg/ dl more than that of LDL-D.

CONCLUSION

New direct homogeneous assays are accurate, precise, fully automated and cost effective. Therefore, for correct cardiac risk classification, direct homogeneous assay should be the method of choice to estimate LDL-C in routine clinical laboratories.

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DECLARATION OF INTERESTS

None.

LIMITATIONS OF STUDY

Clinical history and medications of patients were not considered in the study.

AUTHORS' CONTRIBUTIONS

JA and ZF contribute equally to the skillful editing of the manuscript and interpretation the results.

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