ORIGINAL ARTICLE

Is Friedewald formula a good estimation for low density lipoprotein level in Iranian population?

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Objective: Serum low density lipoprotein (LDL) level is an important biomarker for coronary artery disease (CAD). As direct LDL measurement is expensive and not cost effective, especially in a large population, it is estimated by Friedewald formula. Therefore, we decided to compare the direct LDL measurement method with LDL measured by Friedewald formula in a large general population for the first time in Iran. Furthermore, we examined the association of total cholesterol (TCh), triglyceride (TG), and high density lipoprotein (HDL) with LDL. **Subjects and Methods:** This study was conducted on the subjects, aged 11–97 years, in the third phase of Isfahan Healthy Heart Program (IHHP) from three cities: Isfahan, Najafabad, and Arak. A fasting blood sample was taken from all subjects and referred to Isfahan Cardiovascular Research Center (ICRC) laboratory (central laboratory of IHHP) to assess TCh, TG, HDL, and LDL directly. Also, the LDL level was calculated by Friedewald formula, in addition. **Results:** The mean level of LDL by direct method was lower than that calculated by Friedewald formula. The mean difference between the two methods was significant, which was 6.6 ± 15.5 mg/dl difference (t = -42.925, P < 0.0001). There was strong correlation between direct and calculated LDL levels (adjusted R^2 = 80.4%). Using regression model, a new formula was found for the estimation of LDL. **Conclusion:** It is concluded that the Friedewald formula overestimates the LDL level compared to the direct method in general Iranian population. It is suggested that LDL measurement be carried out directly, especially in high-risk people. If a formula is necessary for LDL estimation, it is better to obtain an especial formula for each population.

Key words: Direct measurement, Friedewald formula, Iiran, low density lipoprotein

INTRODUCTION

High level of low density lipoprotein (LDL) cholesterol is a major risk factor for ischemic heart diseases (IHDs), and its relation to premature coronary artery disease (CAD) has been demonstrated.[1-4] Insofar as each 1% reduction in LDL can reduce the risk of CAD by 1%.[5] The standard and acceptable method to measure LDL is β-quantification method including two steps: ultracentrifugation and chemical precipitation. Even there are other automatic methods for direct LDL analysis. These methods are time-consuming and/or require costly equipments and trained personnel.[6,7] But Friedewald and colleagues designed a formula to estimate the LDL level (LDL-F) based on total cholesterol (TCh) and triglyceride (TG).[8] Because of its low cost and convenience in measurement, calculation of LDL using Friedewald formula is an important component of the CAD risk assessment. However, there are some restrictions in using the formula. It has been delineated that if serum TG level exceeds 500 mg/dl, the formula cannot be accurate.[8] In addition, the formula for calculating LDL gives erroneously high results in patients with some types of cholesterol and lipoprotein disorders. On the other hand, the Friedewald formula underestimates LDL when it is in low concentration.[9] Because of the importance of LDL in CAD risk assessment, the measurement of LDL should be accurate. Yet, it should be cost effective for the general population.

In the present study, we decided to compare the direct LDL measurement method with Friedewald formula in a large general population for the first time in Iran. It was a multicenter study. Furthermore, we examined the association of TCh, TG, and high density lipoprotein (HDL) with LDL.

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SUBJECTS AND METHODS

Setting

The samples were recruited from Isfahan Healthy Heart Program (IHHP), which was a multicenter, interventional community-based program to reduce the cardiovascular

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diseases. IHHP was conducted in three phases: (1) basic evaluation; (2) interventions and repeated survey; and (3) final evaluation by Isfahan Cardiovascular Research Center (ICRC) which is a World Health Organization (WHO) collaborating center in non-communicable diseases in Eastern Mediterranean region (EMRO). It has been described previously in detail.[10] We selected the samples from the third phase, which ranged in age between 11 and 97 years, from three cities: Isfahan, Najafabad, and Arak.

Measurements

Demographic data were gathered using a questionnaire and trained nurses took the venous blood samples from antecubital vein into tubes containing K3-EDTA (1 mg/ml final concentration). All samples were referred to ICRC laboratory (central laboratory of IHHP). It meets the Iranian National Reference Laboratory criteria and is under external quality control of Labquality, Finland.

The blood samples underwent centrifugation at 3000 rpm for 10 min to separate serum. Then, the serum was directly analyzed by chemical assay kits from Pars Azmoon, Iran, with Hitachi 902 auto analyzer to analyze TCh, TG, HDL, and LDL.

Statistical analysis

We used the SPSS software (version 15.0) to analyze the data. Comparison of means of two groups was done by *t*-test. Pearson correlation coefficients were determined to identify the significance of associations between lipids level.

We used receiver operating characteristic (ROC) analysis to evaluate the clinical utility of Friedewald formula in finding LDL treatment level. This curve assesses how well LDL-F discriminates individuals into those with high and low LDL levels in 130 mg/dl boundary. Then, sensitivity and specificity of Friedewald formula in finding high LDL level were assessed.

At the end, to create a new formula for predicting the LDL value upon the other serum lipids, we used the multiple regression model. LDL with direct method was considered as the dependent variable, and TCh, TG, and HDL were independent variables. TG value was transformed as logarithmic because of un-normalized distribution. We formed two regression models. In the first one, TG level up to 400 mg/dl was omitted. The second model was based on all TG levels. The *P* value below 0.05 was considered as significant level in all tests.

RESULTS

In this study, we analyzed 10,151 samples; 50.1% of these were males and 49.9% were females. The mean age of samples was 35 ± 16.9 years and 70.5% of these lived in urban areas and the remaining lived in rural areas (29.5%).

The distribution of plasma lipid levels is presented in Table 1. As shown in the table, the mean of LDL by direct method (LDL-D) was lower than LDL-F. The mean difference between the two methods was significant, which was 6.6 ± 15.5 mg/dl difference (t = –42.925, P < 0.0001). However, the correlation between direct and calculated LDL levels was high,(adjusted R2=80.4%) [Figure 1]. In addition, ROC analysis determined the appropriate sensitivity for LDL measurement with the appropriate sensitivity for LDL estimation with Friedewald formula at cut-off point 130 mg/dl. The area under ROC curve was calculated to be 75% (95% confidence interval 71.4–78.7%). Figure 2 shows this relation.

The correlation between serum lipid levels was significant. Thus, we designed two regression models to understand whether we can predict LDL from the other serum lipids. Because of un-normalized TG, the logarithm of TG was carried out in the models. In the first regression model, all TG measured was entered, whereas in the second one, only TG <400 mg/dl was used to predict LDL. As shown in Table 2, TC, log TG, and HDL in both models could estimate the LDL level significantly. The prediction formula for each level of TG was:

LDL = 0.702TC − 23.834 log TG − 0.337HDL + 40.262

And the formula for TG <400 mg/dl was:

LDL = 0.709TC - 14.208 log TG - 0.347HDL + 20.056

DISCUSSION

Table 1: The mean distribution of serum lipids levels in the study

Lipid	Mean	Standard deviation
Total cholesterol (mg/dl)	185.9	42.1
Triglycerides (mg/dl)	142.9	99.4
Direct LDL cholesterol (mg/dl)	105.9	29.6
Formula LDL cholesterol (mg/dl)	112.6	34.9
HDL cholesterol (mg/dl)	44.7	10.7

Table 2: The regression model to predict LDL cholesterol from the other lipids

	β	95% CI	
The first model with triglycerides <400 mg/dl			
Total cholesterol	0.709	0.701–0.716	R^2 = 84.2%
HDL cholesterol	–0.347	–0.37 to -0.321	
Log triglycerides	–14.208	–15.71 to -12.71	
The second model with all triglycerides data			
Total cholesterol	0.702	0.69–0.71	R^2 = 80.6%
HDL cholesterol	–0.337	–0.365 to -0.31	
Log triglycerides	–23.834	–25.39 to -22.28	

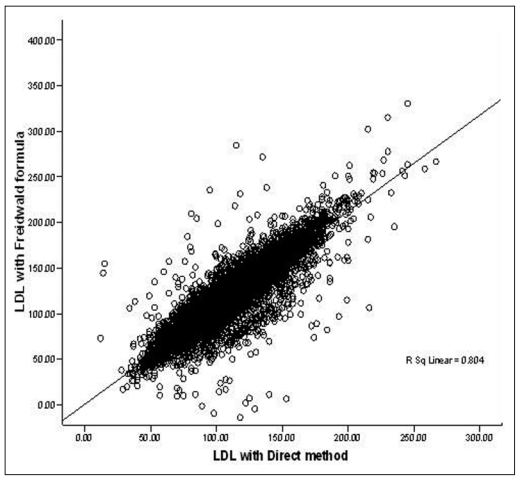

Figure 1: The correlation between low density lipoprotein cholesterol with direct method and with Friedewald formula

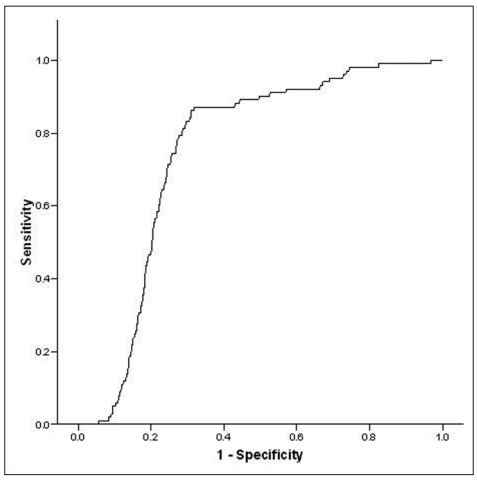

Figure 2: The receiver operating characteristic diagram to validate Friedewald method from direct method

Our results demonstrated a highly significant correlation between LDL-F and LDL-D. However, the Friedewald formula overestimated the LDL level compared to the direct method. The mean level of LDL-F was approximately 7 mg/dl more than that of LDL-D.

Adult Treatment Panel III of the National Cholesterol Education Program (NCEP ATP III) has recommended that LDL level is the major factor for initiating drug treatment, and the accurate measurement of LDL is very important to assess the clinical response to lipid-lowering therapy. The LDL cut-off values for initiating appropriate management recommended in NCEP ATP III are based on Friedewald formula.[10] However, the accuracy of the Friedewald formula has been questioned recently.[9,11] The comparison of LDL-D and LDL-F has shown different findings. In some studies, Friedewald formula underestimated the LDL. For example, Schanagl *et al*. reported lower level of LDL-F than LDL-D.[9] The study results of Can and colleagues are contrary to our findings. According to Can's study, the Friedewald formula has a negative bias in regard to the direct method.[12] Also, a study from Spain confirmed the findings of the two aforementioned studies.[13]

In contrast, some studies have reported the reverse finding which is in agreement with our finding. The Friedewald equation gave higher LDL level than direct method in young Japanese females.[14] Another study from Japan compared the different methods of LDL evaluation, Friedewald formula estimated higher level of LDL than the direct assay.[11] Anandaraja and co-workers carried out such a research in Indian population, but they presented a new formula to estimate LDL accurately.[15] Direct measurement of LDL in children is the same as in adults, and direct method is more accurate than Friedewald formula.[16]

Our literature review demonstrated the reasons behind the controversy existing between studies in comparing direct method with Friedewald formula. The first important cause of these differences is with regard to other serum lipid levels, especially TG. Also, the difference is due to the influence of fasting and non-fasting samples on lipid measurement. The performance of Friedewald formula in non-fasting samples is not more accurate. Friedewald formula is required to define the lipid phenotype.[16]

Also, quality control of the biochemical tests is most important for the accuracy of results in each laboratory.

We collected the samples from a community randomly, and all samples fasted 12–14 h before blood sampling. Our laboratory is qualitatively controlled for serum lipids and lipoproteins annually. In the third phase of IHHP, quality of our central laboratories' tests was compared with Iranian Reference Laboratory in Tehran and also Labquality of Finland. So, it seems that difference in lipid phenotype of each population is the main reason for the difference observed in some studies.

CONCLUSION

In conclusion, the Friedewald formula overestimated the LDL level compared to direct method in the general Iranian population. It is suggested that LDL measurement is carried out directly, especially in high-risk people. If a formula is necessary for LDL estimation, it is better to find an especial formula for each population.

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