# Is direct method of low density lipoprotein cholesterol measurement appropriate for targeting lipid lowering therapy?

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All experimental and clinical trials indicate that the elevated LDL cholesterol (LDL-C) is the major cause of coronary heart disease (CHD) [1–2]. In addition, recent clinical trials have clearly shown that LDL-C lowering therapy reduces risk for CHD [3]. For these reasons, the last guideline for cholesterol testing and management, National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) has provided to identify elevated LDL-C as the primary target of cholesterol lowering therapy [4]. For LDL-C detection, β-quantification is reference method that consists of ultracentrifugation and precipitation [5]. Disadvantages of this method are; long turn around time, higher cost effectivity and requirement of the large volume. Therefore, this method is not used in routine laboratories. Simplified methods have been implanted in these laboratories such as electrophoresis[6], Friedewald Formula [7], HPLC [8] and homogeneous methods [9]. Most clinical laboratories use the Friedewald formula to evaluate LDL-C because of its feasibility [7]. However there are several limitations on its use: it requires fasting sample, and it is unacceptable in type III hyperlipidemias and triglyceride concentrations above 4.52 mmol/L [10–11]. In our study, we compared the Friedewald formula with direct homogeneous LDL-C assay for the detection of LDL-C.

This study includes fasting serum samples of 1001 sequential patients who proceeded to our laboratory from March 2006 to September 2006. The LDL-C was calculated using the equation LDL-C = TC − (HDLC + TG/2.2) expressed in mmol/L, excluding samples with TG concentrations ≥ 4.52 mmol/L. The direct LDL-C, direct HDL-C, cholesterol and triglycerides measurement was performed by using the Roche commercially assay kit with Hitachi Modular P800 analyzer. Pearson correlation coefficients were determined to identify the significance of associations between these variables. Comparisons between the methods were analyzed using the Bland-Altman difference plots.

This study evaluated 1001 subjects; 40.6% of these were males with a mean age of 50.9 ± 15.3 and 59.3% were females with a mean age of 51.2 ± 14.9. None of the patient had blood triglyceride >4.52 mmol/L. The lipid profiles of individuals are shown in Table 1.

The correlation between measured LDL-C levels and calculated LDL-C levels was statistically significant ($r = 0.964$, $P < 0.001$). The distribution of the differences between measured LDL-C and calculated LDL-C levels are shown in Fig. 1. The bias of measured and calculated LDL is 0.40 ± 0.28 with the limits −0.98 to 1.19.

The LDL-C levels of Friedewald formula and direct measurement modified were shown according to cutoff values recommended in NCEP-ATPIII in Table 2.

Increase/decrease percentage of the patients who might be treated with LDL-C cutoffs recommended by NCEP-ATPIII shown in Table 3. As the treatment goal of 2.56 mmol/L, 12.3% of total patient would be treated with direct measurement who would not have been treated when using the Friedewald formula. For the cutoff 3.36 mmol/L, the ratio increase to 18.8% in direct measurement. The ratios are decreased to 8.3% in direct measurement when using a 4.14 mmol/L cutoff for treatment.

According to the results of our patients, the LDL-C estimated by the Friedewald formula observed an extremely significant correlation with the direct method. However, the Friedewald formula has a negative bias in regard to the direct method. One explanation for these higher LDL-C levels obtained by the direct method might be the difference in the triglyceride/cholesterol ratio in the VLDL particles. It is known that cholesterol rich VLDL

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Table 1
The lipid profiles of individuals.

| | LDL-C Friedewald (mmol/L) | LDL-C measured (mmol/L) | Triglyceride (mmol/L) | Total Cholesterol (mmol/L) | HDL-C (mmol/L) |
|---|---|---|---|---|---|
| Men (407) | 2.98±1.00 | 3.44±1.05 | 1.64±0.76 | 4.95±1.23 | 1.23±0.31 |
| Women (594) | 3.10±0.98 | 3.59±1.04 | 1.59±0.82 | 5.28±1.22 | 1.44±0.36 |

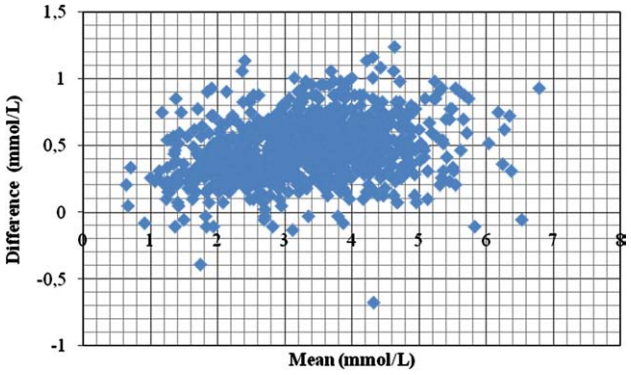


Fig. 1. Comparison of measured and calculated LDL from Friedewald formula for determination of LDL-C.

particles could induce a positive bias and show variations in different populations [12]. Second explanation would be the possibility of measuring the cholesterol present in the particles of intermediate density lipoproteins (IDL) through some direct methods [13].

LDL-C level has been recommended by the NCEP ATPIII as the major factor for initiating dietary and drug treatment. The accurate measurement of LDL-C at low LDL-C levels is very important in assessment of the clinical response to lipid lowering medications. Now it is clear that the direct LDL-C procedure is more precise and accurate than the estimated LDL-C by the Friedewald formula because of three analytical variables (TC, triglyceride, and HDL-C) compared to one (LDL-C). Nevertheless, the LDL-C cutoff values for initiating appropriate management recommended in NCEP ATPIII are based on Friedewald formula. However, the accuracy of the Friedewald formula at low LDL-C concentrations has been questioned recently, [14]. Our study demonstrate that, treatment decisions based on the direct method results as if not have been treated in many patients (8.0% to 18.8) when using the Friedewald formula. Therefore, measured LDL-C levels could not replace calculated levels if one wants to use the LDL-C cutoff values recommended in NCEPIII as a guide for management of the patients with dyslipidemia.

In the present study, we also showed that the levels of HDL-C were in reference range in our population. Onat et al have investigated lipid profiles of 2472 person in the TEKHARF study from the survey 1997/98 with Reflotron system [15]. They suggested that HDL-C levels encountered in Turks are lower by 20% than in Americans and Germans in either gender [16]. In a recent study Yuksel et al have reported

Table 2
LDL-C levels (mean±SD) according to cutoff values recommended in NCEP ATPIII.

| | Patient number (*n*) | Friedewald formula (mmol/L) | Direct measurement (mmol/L) |
|---|---|---|---|
| <2.56 mmol/L | (n=320) | 1.93±0.44 | 2.35±0.51 |
| 2.56–3.36 mmol/L | (n=277) | 2.95±0.21 | 3.44±0.32 |
| 3.36–4.11 mmol/L | (n=264) | 3.83±0.37 | 4.18±0.30 |
| 4.14–4.89 mmol/L | (n=106) | 4.44±0.22 | 4.92±0.34 |
| >4.92 mmol/L | (n=34) | 5.41±0.42 | 5.90±0.48 |

Table 3
Percentage of the patients according to LDL-C cutoffs for treatment recommended by NCEP-ATP III.

| LDL-C cutoffs for treatment | Patient number (*n*) | Friedewald formula (mmol/L) | Direct measurement (mmol/L) |
|---|---|---|---|
| <2.56 mmol/L | (n=320) | 67.3% | 79.6% |
| <3.36 mmol/L | (n=597) | 37.4% | 56.2% |
| <4.14 mmol/L | (n=861) | 18.9% | 27.2% |

that HDL-C levels both in men (45.8±11.04 mg/dl) and women (55.3±11.6 mg/dl) were with in the normal reference range [17]. Our results are in agreement with Yuksel et al described previously. Bijtster reported that HDL-C concentrations measured with the Reflotron system were about 10% lower than those obtained with the two routine precipitation methods, using different instruments with different cholesterol standardization procedures [18]. In contrast to Onat et al. [15], we did not think Turks have low HDL-C levels and the method of the measurement is considered to be one of the main causes of error in the estimation of HDL-C levels in TEKHARF study.

In conclusion, laboratories have a very limited use of direct method as a substitute for Friedewald formula because direct method has not been standardized in large populations and increase cholesterol assay costs.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [19].

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# The beneficial effects of angiotensin-converting enzyme inhibitors on serum asymmetric dimethylarginine levels in the patients with cardiovascular disease

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We have read with great interest the study published in the recent issue of the Journal, by Kawata et al. [1]. In that study measurements of serum asymmetric dimethylarginine (ADMA) and coronary flow velocity reserve (CFVR) using transthoracic Doppler echocardiography were performed at baseline and after 4 weeks of temocapril therapy in 18 patients with type 2 diabetes. Although blood pressure, fasting blood sugar and lipid profiles remained unchanged, serum ADMA concentrations decreased significantly and CFVR increased significantly after the treatment. Moreover, a strong correlation was observed between the difference of ADMA and that of CFVR. Temocapril reduced serum

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