Research Letter

Calculated low density lipoprotein cholesterol: Its relevance in Indian perspective

Low density Lipoprotein Cholesterol (LDLc) is considered as a major risk factor in the development of cardiovascular disease and is the primary basis for diagnosis, treatment and risk classification in patients with hyperlipidemia. This makes it very important to estimate serum LDLc with great precision and accuracy.

The ultracentrifugation procedure or β-quantification (BQ) is considered as the reference method for LDLc. It is time consuming, expensive and requires a large serum volume. The two commonly used methods of LDLc estimation are – Direct Homogenous Assay, and the Indirect Calculation Method.1 Direct assays are found to be reasonably specific and free from endogenous interference. However, these direct methods are not perfect, as they have some disadvantages such as over/under estimation of LDLc, failure of many commercial methods to meet the NCEP total error goals especially in diseased individuals, and high cost.2

The indirect method of LDLc estimation is by calculation using the landmark equation proposed by Friedewald et al., using other lipid parameters viz. total cholesterol(TC), high density lipoprotein cholesterol(HDLc) and triglycerides(TG). This equation is

\[
\text{LDLc (mg/dl)} = \text{TC} - \text{HDLc} - \frac{\text{TG}}{5}
\]

However, Friedewald et al. themselves found that this formula was not applicable to plasma samples containing chylomicrons, patients with Type III hyperlipoproteinemia and plasma containing TG >400 mg/dl. Furthermore, each component’s (TC,TG and HDLc) analytical error is added, hence it is difficult to achieve the recommended total analytical error goal of ±12%. Additionally, the equation requires fasting serum sample. Despite these limitations this formula is the method of choice for routine quantification of LDLc by most of the clinical laboratories due to its simplicity, reliability and cost effectiveness, especially in developing countries.

To overcome the foresaid limitations of Friedewald formula (FF), several modified equations have been suggested and their advantages and disadvantages have been discussed.3 These modified formulas did provide some improvement in overcoming the limitations of Friedewald equation but many modified formulas failed to give better estimate of LDLc in sera containing TG >400 mg/dl. The different modified formulas have been validated in different populations and each formula was found suitable for a particular population.4 Some recent studies have shown Friedewald equation to be still better than several modified formulas.4 Therefore, it is important to evaluate these modified formulas in Indian population as the studies on the validation of the modified formulas and even Friedewald formula in Indian population are only a few.

1. Indian studies

Anandaraja et al.,5 suggested a formula which incorporated TC and TG values (LDLc = 0.9 TC – 0.9 TG(5 – 28)). This formula was tested in 1008 Indian patients’ sera and compared with FF and direct LDLc (dLDLc) and its performance was found to be better than FF and correlated very well with dLDLc. The limitations of the Anandaraja’s study were not including the sera with TG >350 mg/dl, no comparison with BQ and no inclusion of healthy subjects. However, it’s economical since it incorporates only two variables (TC and TG). Unfortunately, Gupta et al.,6 found that FF gave better agreement with dLDLc as compared to Anandaraja formula by comparing FF and Anandaraja formula with dLDLc in 515 Indian subjects. Moreover, Anandaraja’s LDLc gave higher percentage error in this study and did not perform well at low HDLc and TC whereas FF LDLc showed maximum error at higher HDLc and TG 201–300 mg/dl. However, the sample size was small in this study and there were no records of normal and diseased subjects.

Recently Krishnaveni and Gowda7 also studied FF and Anandaraja’s formula and compared with dLDLc in 370 subjects. Here also FF LDLc correlated maximally with dLDLc at all levels of TG except TG <100 mg/dl, while Anandaraja’s LDLc worked better at TG <100 mg/dl. Neha et al.,8 also got variable results using FF and Anandaraja’s formula at different levels of TG, TC and HDLc as compared to direct LDLc and the Pearson coefficient of correlation between FF LDLc and dLDLc was better than that between Anandaraja’s LDLc and dLDLc.

In a study by Sahu et al.,9 893 subjects’ serum LDLc was determined by direct method and FF. They found a significantly higher FF LDLc values as compared to dLDLc at TG <200 and TC >150 mg/dl. FF LDLc classified 23.5% of patients at higher cardiac risk whereas by direct assays it was 17.58%. However, there was no record of patients and healthy individuals and the sample size was also small. On the other hand, Sudha et al.,10 found no difference between FF LDLc and dLDLc at TG <150 mg/dl but at other levels of TG the dLDLc was significantly higher than FF LDLc in 260 subjects. Likewise, Warade et al.,11 also found FF LDLc significantly lower than dLDLc in 1768 Indian subjects.

The only study with large number of subjects was by Kannan et al.,12 who compared FF with dLDLc in 14620 samples and found that the FF correlated best with dLDLc when TG was 100–150 mg/dl but FF underestimated LDLc when TG was >200 mg/dl and LDLc was <70 mg/dl. However, when LDLc was >130 mg/dl, no comparison with BQ and no inclusion of healthy subjects. Unfortunately, there was no record of clinical characteristics of patients’ clinical outcome and statin therapy.

Recently, Kapoor et al.,13 studied 480 patients’ serum LDLc by FF, Puavilai modified formula and Anandaraja’s formula and compared with dLDLc. They found that Anandaraja’s formula gave maximum negative bias. Puavilai modified formula correlated best with dLDLc at all levels of TG as compared to FF and Anandaraja...
proposed models for calculating LDLc. Although correlation between all the three formulas and dLDLc was almost equal (0.93–0.95) a significant difference was found between dLDLc and Anandaraja LDLc at TG <200 mg/dl and 201–400 mg/dl.

Thus in these limited Indian studies, only three formulas viz. FF, Anandaraja’s and Puavilai modified were evaluated. Besides, these Indian studies had certain limitations: (1) The number of subjects were quite less except the study by Kannan et al., 12 which validated only FF. (2) These studies mentioned no record of normal healthy and diseased subjects hence can not be generalized to general population, (3) None of the study compared calculated LDLc with the reference method (BQ).

2. Future research

As evident from the foregoing text, only a few studies on validation of calculated LDLc have been done and variable results have been found for FF and other modified formulas in Indian subjects. Because of the simplicity and cost effectiveness, the calculated LDLc is still the method of choice in developing countries like ours. However, due to the limitations of FF, a suitable new formula or the most suitable among those already reported for correct estimation of LDLc is needed for prediction, classification, therapy and outcome in patients with cardiovascular disease in Indian population. However, certain factors are required to be considered in search of a suitable formula for calculation of LDLc for Indian population. First, the study should be multi-centric covering most of the parts of our country as our country has diverse population and each of the formulas proposed so far has been found to be suitable for a particular population. Contradictory results have been reported with FF and other modified formulas even in studies reported from different regions of India. Secondly, the factors such as age, gender, race/ethnicity, obesity, diabetes, BMI, TC, TG and insulin resistance may affect the outcome of proposed modified equations for calculated LDLc. 14 Hence these factors should be taken into account. Thirdly, the modified equations should also be validated by comparing with the reference method (BQ) for LDLc at least in some representative samples (due to limitations of BQ) as the direct homogeneous LDLc assay methodology has not yet been perfectly standardized. Fourthly, for the equations incorporating HDLc the procedure for HDLc (direct/precipitation) should also be taken into consideration as bias have been reported even in the directly assayed HDLc values by different direct assays as compared to reference HDLc values. 4,15 Lastly, these formulas should be validated in large number of healthy as well as diseased subjects.

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