Evaluation of directly measured LDL-Cholesterol in Assessing the Cardiovascular Risk Defined by Total Cholesterol/HDL-Cholesterol Ratio

Banerjee P¹, Krishnamurthy U²*

¹Consultant Biochemist, Saroj Gupta Cancer Centre & Research Institute, Kolkata 700063, India
²Associate Professor, Department of Biochemistry, M S Ramaiah Medical College, Bangalore 560054, India

ABSTRACT

Introduction: Commonly, LDL-cholesterol levels are estimated indirectly by Friedewald equation and called as calculated LDL (cLDL). Assays for direct determination of LDL-C have come into vogue to overcome the limitations of Friedewald equation and are called as direct LDL-C (dLDL). The total cholesterol to High Density Lipoprotein cholesterol (TC: HDL-C) ratio is a powerful predictor of cardiovascular disease (CVD). Therefore, this study was undertaken to evaluate dLDL in assessing CVD risk defined by the TC: HDL-C ratio. Materials and Methods: The lipid profile data of 734 patients with triglyceride values between 100 and 400 were obtained. Patients were grouped and analysed on the basis of TC: HDL-C ratio cut-off. Results: This study showed dLDL values were significantly increased in CVD risk groups defined by TC: HDL-C ratio. Study also revealed that dLDL values were more than the cLDL values and hence identified more number of CVD risk individuals compared to cLDL. Directly measured LDL had high specificity and calculated LDL had high specificity. TC: HDL-C ratio and dLDL identified more number of CVD risk individuals. Conclusion: LDL-C measured by direct method not only assesses CVD risk defined by the TC: HDL-C ratio but also identifies more number of CVD risk individuals when compared to cLDL.

KEYWORDS: High cholesterol, triglyceride, lipoprotein, Friedewald

INTRODUCTION

Low Density Lipoprotein - Cholesterol (LDL-C) is a modifiable risk factor for cardiovascular disease (CVD). Its routine measurement is recommended in the evaluation and management of hypercholesterolemia.[1] LDL-C levels are estimated indirectly from measurements of total cholesterol (TC), triglyceride (TG), and high density lipoprotein cholesterol (HDL-C) using Friedewald equation [LDL-C = (TC) – (HDL-C) – (TG/5)] which is termed as calculated LDL (cLDL). Assays for direct determination of LDL-C are coming into vogue to overcome the limitations of Friedewald calculation and are called as direct LDL-C (dLDL). [2] The statutory bodies like American Heart Association (AHA), National Lipid Association (NLH) and National Cholesterol Education Program (NCEP) provide a set of CVD risk stratification guidelines for fasting LDL-C levels which do not differentiate for the methods of estimation i.e. either cLDL or dLDL.[3, 4, 5]

TC: HDL-C ratio is an index to predict CVD risk. The number is obtained by dividing TC from HDL-C. A ratio of ≥5 indicates a higher risk of heart attack. [6] Even though this ratio is a powerful predictor of CVD it is not used as a sole indicator for therapy. The treatment choices are based upon LDL-C levels. [7] Since, cLDL calculation is based on the TC and HDL-C; it obviously draws a parallel value with the TC: HDL-C ratio and further the risk. But, dLDL is an independent measurement its association with TC: HDL-C ratio is less addressed. Therefore, this study was undertaken to evaluate dLDL in assessing CVD risk defined by the TC: HDL-C ratio. This study was also extended to find the association of dLDL and cLDL values with other lipid profile parameters to find their independent association.
MATERIALS AND METHODS

The lipid profile data of 734 patients with triglyceride values between 100 and 400 mg/dl were obtained from the M S Ramaiyah Hospital laboratory. The Friedewald limitation was applied to enable the comparison between cLDL and dLDL. All the lipid profile parameters (TC, TG, HDL-C & dLDL) were estimated by enzymatic colorimetric method. In addition, LDL-C was also calculated in difference by Friedewald equation. CVD risk was assessed by the TC: HDL-C ratio. Based on the TC: HDL-C ratio, patients were grouped as Group A and Group B. The ratio with <5 were considered as Group A and with the ratio ≥5 were considered as Group B.

Statistical Analysis: Data was tabulated on Microsoft Excel and analysed using SPSS version 18.0. Fisher’s Exact Test was used to test the significance in the proportions between the groups. Quantitative data summarized to test the difference in mean values for lipid profile parameters between the groups and student’s t-test was used to test the significance; p value < 0.05 is considered as the level of significance.

Further, Pearson’s correlation was used to find the correlation between the various lipid profile parameters. Receiver operating characteristic (ROC) curve was plotted and area under the curve (AUC) was determined to find the association between cLDL and dLDL for CVD risk defined by TC: HDL-C ratio. Regression analysis was also performed to find the association between the dLDL and cLDL.

RESULTS

This study revealed the sensitivity and specificity of dLDL for CVD risk by TC: HDL-C ratio as 71.39% and 50.63% respectively, where as cLDL had 69.91% and 62.28%. Study also found that 78.1% of the patients had dLDL values greater than cLDL. Results obtained were shown in tables 1 & 2 and as figures 1-3.

Comparison of the Mean ±SD of lipid profile parameters between the two groups is shown in Table 1. It shows that there is significant difference in the mean values of all the lipid profile parameters between the Groups A and B including dLDL, the parameter of interest. Table 2 shows the Pearson’s correlation between all the parameters of the lipid profile. It can be noted that there is significant correlation between dLDL and cLDL. It can also be noted that cLDL and dLDL shows similar pattern of correlation with other lipid profile parameters except with TC: HDL-C ratio where only cLDL has significant correlation but not the dLDL.

Table 1: Comparison of the Mean ±SD of lipid profile parameters between the two groups

|                      | Group A      | Group B      | P value |
|----------------------|--------------|--------------|---------|
| Total cholesterol    | 165.3 ± 36.9 | 185.5 ± 44.8 | < 0.01  |
| Triglycerides        | 147.2 ± 46.5 | 192.5 ± 64.7 | < 0.01  |
| dLDL (mg/dl)         | 100.2 ± 31.4 | 119.5 ± 44.0 | < 0.01  |
| cLDL (mg/dl)         | 92.3 ± 30.6  | 117.7 ± 37.8 | < 0.01  |
| HDL (mg/dl)          | 43.6 ± 11.1  | 29.3 ± 9.8   | < 0.01  |
| TC:HDL               | 3.9 ± 0.7    | 7.2 ± 37.8   | < 0.01  |

Student’s t test

Table 2: Pearson’s correlation between various lipid profile parameters among the study subjects

|                      | Total cholesterol | Triglycerides | dLDL | cLDL | HDL | TC:HDL |
|----------------------|-------------------|---------------|------|------|-----|--------|
| Total cholesterol    | -                 | r: 0.19       | r: 0.91 | r: 0.93 | r: 0.42 | r: 0.04 |
|                      | p<0.01            | p<0.01       | p<0.01 | p<0.04 | p<0.01 | p<0.21 |
| Triglycerides        | r: 0.19           | -             | r: 0.07 | r: -0.003 | r: -0.28 | r: 0.31 |
|                      | p<0.01            |               | p: 0.04 | p<0.01 | p<0.01 | p<0.01 |
| dLDL                 | r: 0.91           | r: 0.07       | -    | r: 0.90 | r: 0.35 | r: -0.06 |
|                      | p: 0.04           |               |      | p<0.01 | p<0.01 | p: 0.06 |
| cLDL                 | r: 0.93           | r: -0.003     | r: 0.90 | -    | r: 0.23 | r: 0.16 |
|                      | p<0.01            | p: 0.917      | p<0.01 |      | p<0.01 | p<0.01 |
| HDL                  | r: 0.42           | r: -0.28      | r: 0.35 | -    | r: 0.23 | r: -0.60 |
|                      | p<0.04            | p<0.01       | p<0.01 |      | p<0.01 | p<0.01 |
| TC:HDL               | r: 0.04           | r: 0.31       | r: -0.06 | r: 0.16 | r: -0.60 | -     |
|                      | p: 0.21           | p<0.01       | p<0.01 | p<0.01 | p<0.01 |         |

Figure 1 shows the distribution of the CVD risk patients based on definitions for TC: HDL-C ratio and LDL-C with the intersecting population. TC: HDL-C ratio ≥5, dLDL ≥100 mg/dl and cLDL ≥100 mg/dl was observed in 339 (46.1%), 437 (59.5%) and 386 (52.5%) patients respectively. It also revealed that 225 (30.6%) patients were at risk by TC: HDL-C ratio and by both method of estimation for LDL-C. Stand alone TC: HDL-C ratio identified more number of CVD risk (85 patients) verses dLDL (48 patients). Figure 2 shows the ROC for cLDL and dLDL. Accuracy measured by the AUC for dLDL and cLDL was found to be 0.65 and 0.70 respectively. Figure 3 shows the regression analysis to find the association between cLDL and dLDL, and R² found to be 0.815.
Figure 1: Distribution of the CVD risk patients based on various definitions.

Figure 2: ROC for cLDL and dLDL

Figure 3: Association between the dLDL and cLDL

\[
y = 0.267x + 8.465 \\
R^2 = 0.815
\]
DISCUSSION

LDL-C a modifiable risk factor for CVD is measured by either Friedewald calculation or by direct measurement. CVD risk guidelines issued by the statutory bodies do not differentiate between cLDL and dLDL. Currently dLDL is in the market requiring more studies for validation. Therefore, this study defined the CVD risk by TC: HDL-C ratio and the analysis were performed to find the association of dLDL with it.

Initially risk proportions were estimated. This showed that dLDL by its own definition for CVD risk i.e. patients with values with ≥100 mg/dl identified highest number of CVD risk patients (437, 59.5%). This study observed that dLDL values were greater than cLDL values in majority of the patients (78.1%). Also, the mean dLDL value was higher than the cLDL. This shows that Friedewald calculation underestimates the LDL-C in majority of the patients. Similar finding has been observed by Lindsey CC et al, where half of the patients lost LDL goal attainment for LDL-C when measured by cLDL. [8]

Various statistical parameters were derived for dLDL and cLDL against CVD risk defined by TC: HDL-C ratio. It was observed that sensitivity of dLDL was higher than the cLDL; whereas specificity of cLDL was higher than the dLDL. Therefore, dLDL avoids false negatives and may have added advantage as more individuals are covered in the risk category.

Pearson’s correlation was performed between all the parameters of the lipid profile. It can be noted that there is significant correlation between dLDL and cLDL. It can also be noted that cLDL and dLDL shows similar pattern of correlation with other lipid profile parameters except with TC: HDL-C ratio where only cLDL has significant correlation but not the dLDL. This shows that dLDL is an independent parameter. Association between the dLDL and cLDL was found by regression analysis and the coefficient was found to be 0.815. Though the triglyceride levels are between 100 to 400mg/dl the strength of association between dLDL with cLDL is not substantial, probably because of the higher dLDL values.

From the figure 1 it can be inferred that 225 (30.6%) are the number of patients identified as risk by TC: HDL-C ratio, dLDL and by cLDL. The number increases to 534 (72.7%) when TC: HDL-C ratio and dLDL are combined. Therefore, the predictive value increases when TC: HDL-C ratio and dLDL are combined than used as isolated parameters.

CONCLUSION

LDL-C measured by direct method not only assesses CVD risk defined by the TC: HDL-C ratio but also identifies more number of CVD risk individuals when compared to cLDL. Also, TC: HDL-C and dLDL together identify more number of CVD risk individuals.

ACKNOWLEDGEMENTS:

We wish to acknowledge M S Ramaiah Medical College & Hospital for supporting this study.

REFERENCES

1. Kannel WB, Castelli WP, Gordon T, et al. Serum cholesterol, lipoproteins and risk of coronary heart diseases. The Framingham study. Ann Intern Med 1971; 74:1-12.
2. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clinical chemistry 2002; 48(2): 236-254.
3. Stone NJ, Robinson J, Lichtenstein AH, et al; 2013 ACC/AHA Guideline on the treatment of blood cholesterol to reduce atherosclerotic CVD risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014; 63:2889-2934.
4. NLA Recommendations for Patient-Centered Management of Dyslipidemia. Available at https://www.lipid.org/sites/default/files/PIIS1933287415000598.pdf. Accessed September 1, 2015.
5. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001; 285:2486-2497.
6. Quispe R, Manalac RJ, Faridi KF, Blaha MJ, Toth PP, Kulkarni KR, Nasir K, Virani SS, Banach M, Blumenthal RS, Martin SS, Jones SR. Relationship of the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio to the remainder of the lipid profile: The Very Large Database of Lipids-4 (VLDL-4) study. Atherosclerosis. 2015; 242(1):243-50.
7. Kones R. Primary prevention of coronary heart disease: integration of new data, evolving views, revised goals, and role of rosuvastatin in management. A comprehensive survey. Drug Des Devel Ther. 2011; 5:325-80.
8. Lindsey CC, Graham MR, Johnston TP, Kiroff CG, Freshley A. A clinical comparison of calculated versus direct measurement of low-density lipoprotein cholesterol level. Pharmacotherapy 2004; 24(2):167-72.

*Corresponding author: Dr. Krishnamurthy U
E-Mail: kmurthyu@gmail.com