Newly-developed De Cordova's Formula for Calculation of LDL Cholesterol in Bangladeshi Population

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ABSTRACT

Raised serum LDL cholesterol is an important modifiable risk factor for the development of atherosclerosis and cardiovascular disease. So the management of dyslipidaemia is mostly based on the concentration of LDL cholesterol. Friedewald's formula is commonly used method to estimate LDL cholesterol in most clinical laboratories. However, this formula cannot reflect the actual figure of LDL cholesterol. In 2013, de Cordova developed a new simple formula to calculate LDL cholesterol without using serum TG which is said to be more accurate than Friedewald's formula. The present study was designed to compare the formula-based calculated LDL cholesterol (Friedewald's formula and de Cordova's formula) with direct homogenous estimation. The objective of the present study was to evaluate of applicability of de Cordova's formula for calculation of LDL cholesterol. By using non-probability sampling technique, 460 individuals were enrolled in the study who were attending in the one-point collection centre of BSMMU for lipid profile estimation. Subjects were categorized as normolipidaemic individuals and dyslipidaemic patients. Serum TC, TG, HDL cholesterol and LDL cholesterol were measured by direct automated method. LDL cholesterol was also calculated by Friedewald's formula and de Cordova's formula. Results were expressed as mean ± SD. Comparison was done by Pearson's correlation test, agreement was done by Bland-Altman agreement test between measured and calculated LDL cholesterol. The mean ± SD of measured LDL cholesterol was 132.99 ± 36.65 mg/dL. LDL cholesterol calculated by Friedewald's formula and de Cordova's formula were 121.39 mg/dL and 116.81 mg/dL respectively. The limits of agreement between measured LDL cholesterol (direct method) and calculated LDL cholesterol by de Cordova's formula were lowest and agreement was better for all dyslipidaemic subjects. de Cordova's formula showed better agreement with measured LDL cholesterol (direct method) than Friedewald's formula for approximate calculation of LDL cholesterol without using triglycerides.

INTRODUCTION

The concentration of serum low-density lipoprotein cholesterol (LDL-C) is an independent risk factor for the development dyslipidaemia as well as coronary heart disease.[1, 2] Determination of the circulating level of LDL (low-density lipoprotein) cholesterol is important for the diagnosis and risk assessment for atherosclerosis and coronary artery disease (CAD).[3] Studies have shown the importance of blood lipids in the management and monitoring of patients with cardiovascular risk.[4, 5] As LDL cholesterol is the primary lipid agent for CAD risk prediction and therapeutic target, an accurate and precise determination of LDL cholesterol is very important for early identification of patients at risk.[6] Ultracentrifugation-polianion precipitation/ Beta Quantification (ßQ), is the reference method for measurement of LDL cholesterol concentration, which is expensive, laborious and not available everywhere.[7] The direct methods are costly and require expensive automation and are not affordable by most of the laboratories in the developing countries.[8] Several direct methods have been developed but all are expensive and not suitable for developing countries like ours, that is why Friedewald's formula is most commonly used for determining LDL cholesterol in the clinical laboratory.[9] This formula estimates LDL cholesterol from measurements of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) cholesterol [LDL cholesterol = TC − TG/5 − HDL cholesterol].[10] Also in Bangladesh, Friedewald's formula is the most commonly used procedure in clinical practice.[8] This formula

has several limitations and cannot be applied in hypertriglyceridemia (TG level > 400 mg/dL), in hyperchylomicronemia, patients with type III hyperlipoproteinemia.[11 − 14] Friedewald's formula should be used with precaution in several pathologic states (diabetes, hepatopathy and nephropathy), even if the TG concentrations are between 200 − 400 mg/dl.[1, 15] de Cordova et al. recently published a new, simpler and less expensive formula (LDL-C = ¾ [Total cholesterol- HDL-C]) independent of serum TG after analyzing lipid profiles of a large cohort of Brazilian population.[16] It opens a new door to calculate LDL cholesterol in non-fasting state.[17]

de Cordova's formula accurately estimates LDL cholesterol avoiding some of the limitations of currently published formulas, and it is an attractive alternative when direct estimation is not possible.[16] Direct measurement of LDL cholesterol is costly and Friedewald's formula cannot give accurate result.[18] Friedewald's formula is invalid when serum TG level is > 400 mg/dl and fasting blood sample is needed to calculate LDL cholesterol by Friedewald's formula.[11, 18] So we need to search out more accurate formula for calculation of LDL cholesterol for correct diagnosis and management of dyslipidaemia. This study was done to assess the applicability of de Cordova's formula for calculation of LDL cholesterol in Bangladeshi population. If this formula-based calculated LDL cholesterol is found to be more valid, this formula can be proposed to be used clinically for correct estimation of LDL cholesterol with minimum cost and time.

MATERIALS AND METHOD

This cross-sectional, analytical study was conducted in the department of Biochemistry, BSMMU, Shahbagh, Dhaka, during the period from January 2014 to December 2015. Fasting blood samples were collected from 460 study subjects who were attending in blood collection point centre of BSMMU for lipid profile estimation (serum triglyceride, HDL cholesterol, total cholesterol and LDL cholesterol). With

all aseptic precautions, 5 ml venous blood was drawn from anticubital vein after overnight fasting (about 10 − 12 h) in a disposable plastic syringe and delivered immediately into a clean dry tube. Then serum was prepared after centrifugation and stored in ultra freezer at −20°C and serum triglyceride, HDL cholesterol, total cholesterol and LDL cholesterol were measured by using the ARCHITECT auto analyzer System (Abbott Diagnostics, USA) at the department of Biochemistry, BSMMU. All kits, calibrators and quality control materials were obtained from Abbott Diagnostics, USA through local distributor. LDL cholesterol was also calculated by Friedewald's formula and de Cordova's formula. Subjects were categorized as normolipidaemic subjects, dyslipidaemic patients according to the definition of dyslipidaemia which was taken from third report of the National Cholesterol Education Program Adult Treatment Panel III.[19] Patients having TG ≥ 400 mg/dL were excluded when LDL cholesterol was calculated by Friedewald's formula. Statistical analysis will be performed by statistical package for Social Science (SPSS) Version 22. Results were expressed as mean ± SD. Comparison was done by Pearson's correlation test between estimated LDL cholesterol and formula-based LDL cholesterol. Agreement between estimated LDL cholesterol and formula-based LDL cholesterol was done by Bland-Altman agreement test.[20, 21] A p-value of < 0.05 was considered as statistically significant.

RESULTS

A total of 460 subjects were included in the study, the mean age of the study subjects was 45.32 ± 12.5, with 71 were normolipidaemic and 389 were dyslipidaemic. Out of 71 normolipidaemic subjects, 47 (66.2%) were male and 24 (33.8%) were female. Out of 389 dyslipidaemic subjects, 226 (58.1%) were male and 163 (41.9%) were female. The mean concentrations of TC, TG and HDL cholesterol were 129.11 mg/dL, 111.99 mg/dL and 47.87 mg/dL respectively in case of normolipidaemic and 204.06 mg/dL, 198.21 mg/dL and 37.26 mg/dL respectively

in case of dyslipidaemic subjects. The mean values of LDL cholesterol measured by direct method, Friedewald's formula and de Cordova's formula were 132.99 mg/dL, 121.39 mg/dL and 116.81mg/dL respectively. Patients having TG $\geq$ 400 mg/dL were excluded while calculating Friedewald's formula. Correlation of measured LDL-C (direct method) with calculated LDL-C in all normolipidaemic and dyslipidaemic subjects showed significant positive correlation between measured and calculated methods (see Table 1).

Table 1 Correlation of measured LDL-C (direct method) with calculated LDL-C

Calculated method	Total subjects (*n* = 460)		Normolipidaemic (*n* = 71)		Dyslipidaemic (*n* = 389)	
	r value	*p* value	*r* value	*p* value	*r* value	*p* value
Friedewald's formula	0.749[#]	<0.001	0.696	<0.001	0.635[#]	<0.001
de Cordova's formula	0.804	<0.001	0.652	<0.001	0.719	<0.001

[#]Patients having TG $\geq$ 400 mg/dL were excluded.

Bland-Altman agreement plot was done to see the agreement between the measured LDL-C (direct method) and calculated LDL-C by Friedewald's formula and de Cordova's formula for all (see Table 2 and Figure 1), dyslipidaemic (see Table 3 and Figure 2) and normolipidaemic subjects (see Table 4 and Figure 3) within 95% limit. For all study subjects, de Cordova's formula showed better agreement as limits of agreement were 102.01 and 87.75 respectively for Friedewald's formula and de Cordova's formula.

Table 2 Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for all study subjects

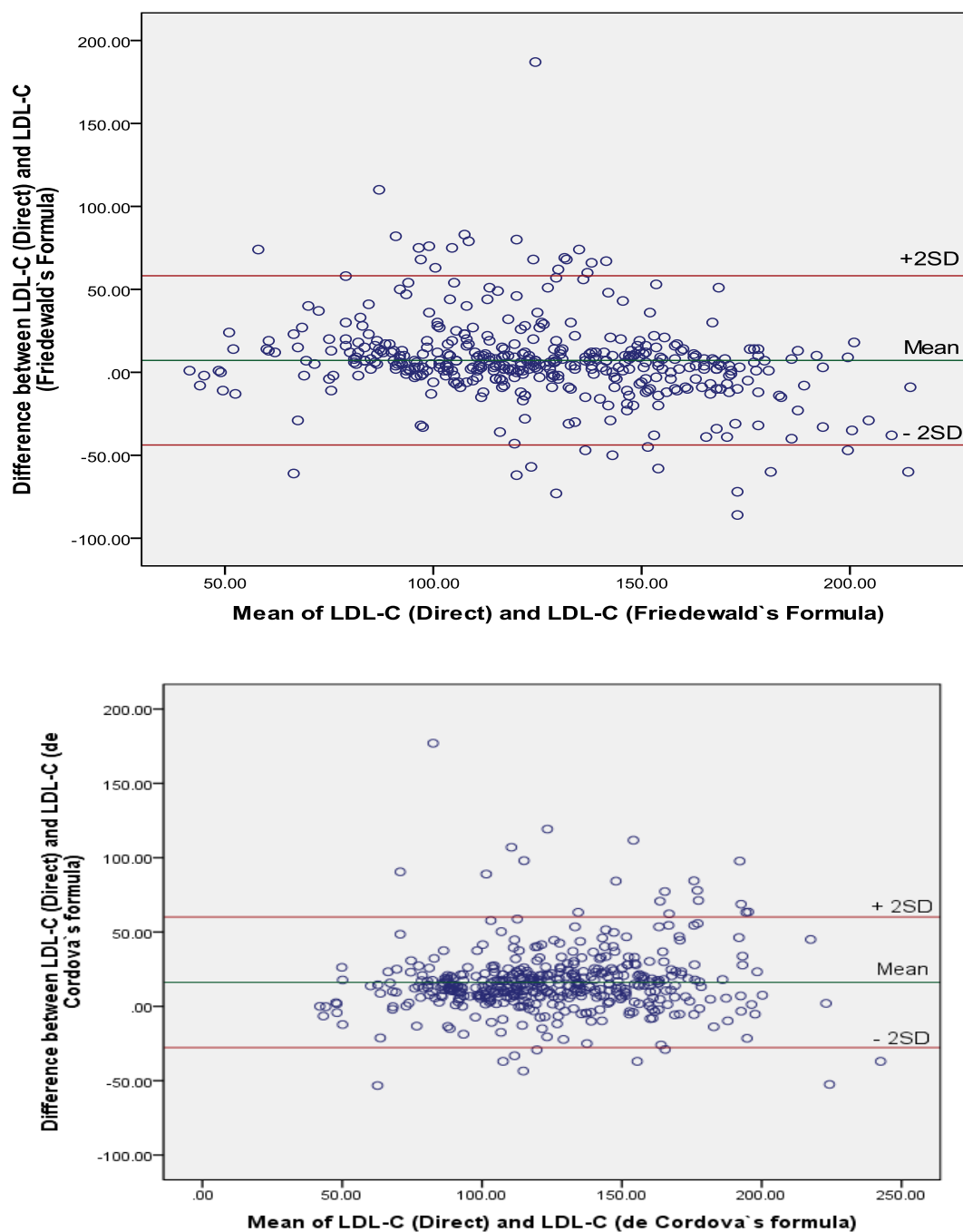|	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (direct) and LDL-C (Friedewald's formula)[#]	7.19	26.02	58.19	−43.82	102.01
Difference between LDL-C (direct) and LDL-C (de Cordova's formula)	16.18	22.38	60.05	−27.70	87.75

[#]Patients having TG $\geq$ 400 mg/dL were excluded.

Figure 1 Bland-Altman agreement plots between measured LDL-C and calculated LDL-C for all study subjects. (Patients having TG ≥ 400 mg/dL were excluded in case of Friedewald's formula.)

Bland-Altman plot showed better agreement of the de Cordova's formula than Friedewald's formula in dyslipidaemic subjects as limits of agreement was lower in case of de Cordova's formula (62.73, −29.56 vs 60.81, −47.95) within 95% limit (see Table 3 and Figure 2).

Table 3 Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for dyslipidaemic subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (direct) and LDL-C (Friedewald's formula) [#]	6.43	27.75	60.81	−47.95	108.76
Difference between LDL-C (direct) and LDL-C (de Cordova's formula)	16.59	23.54	62.73	−29.56	92.29

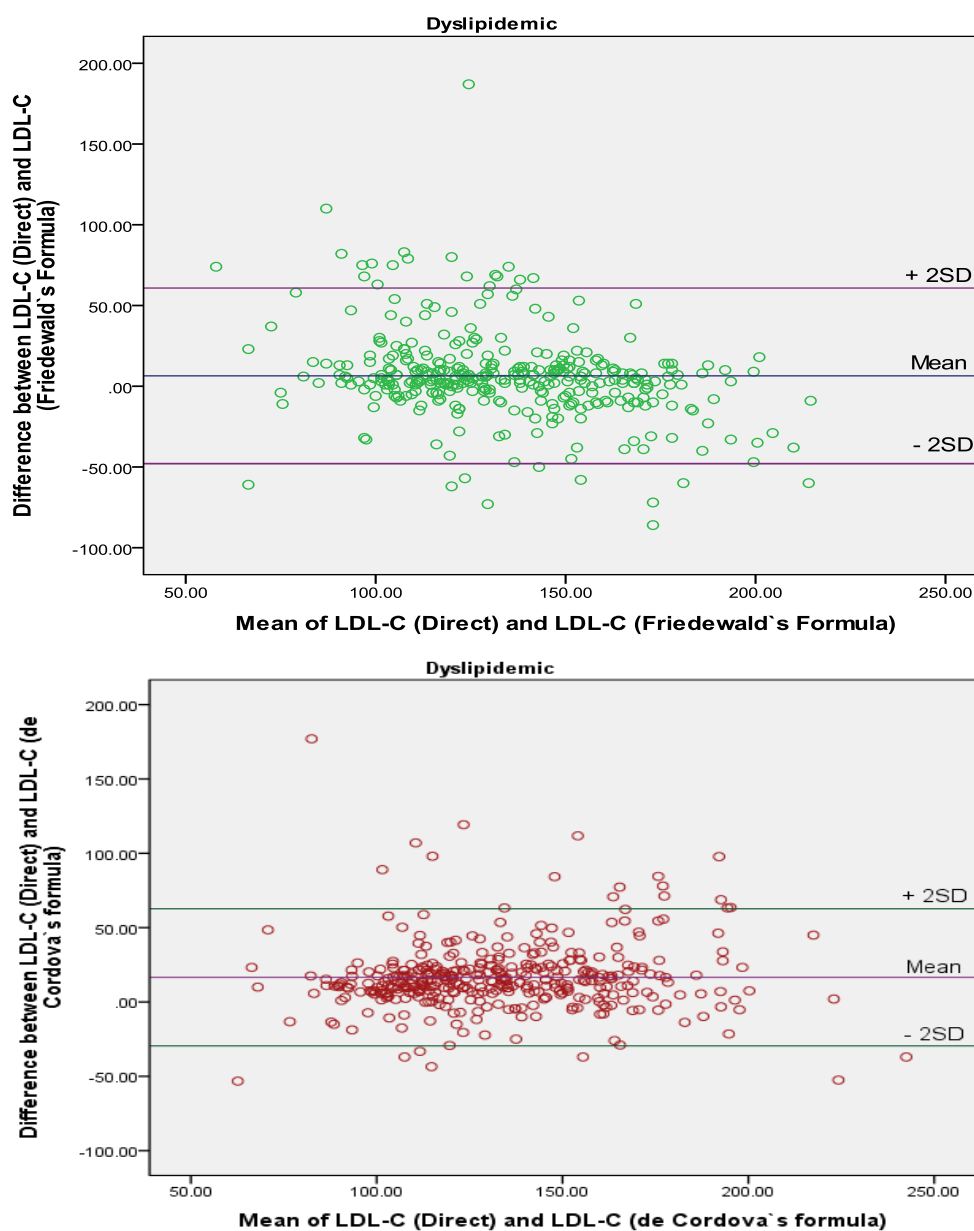[#]Patients having TG $\geq$ 400 mg/dL were excluded.

Figure 2 Bland-Altman agreement plots between measured LDL-C and calculated LDL-C for dyslipidaemic subjects. (Patients having TG $\geq$ 400 mg/dL were excluded in case of Friedewald's formula.)

For normolipidaemic subjects, limits of agreement were 53.99 and 56.41 respectively for Friedewald's formula and de Cordova's formula (see Table 4 and and Figure 3). Limits of agreement were lower and showed better agreement for Friedewald's formula.

Table 4 Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for normolipidaemic subjects

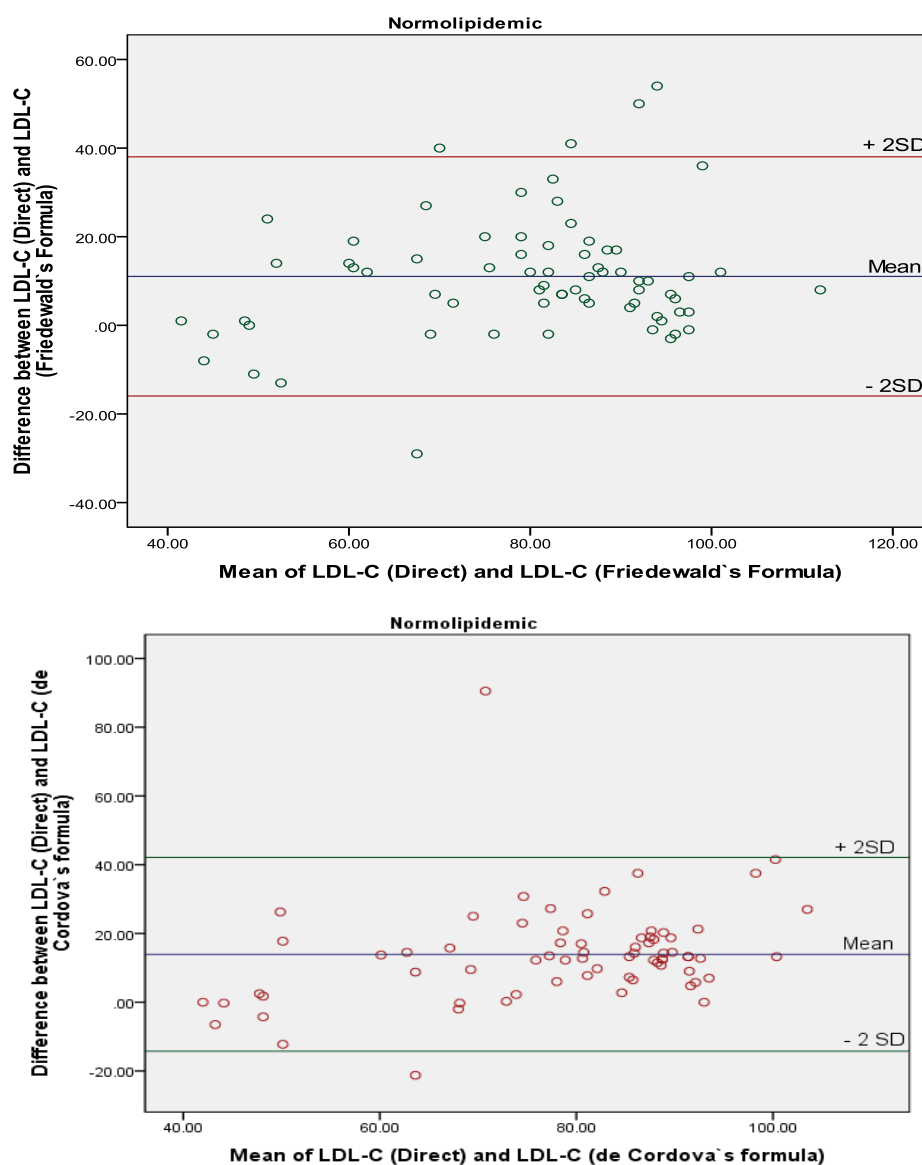|	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (direct) and LDL-C (Friedewald's formula)	11.04	13.77	38.04	−15.96	53.99
Difference between LDL-C (direct) and LDL-C (de Cordova's formula)	13.94	14.39	42.14	−14.27	56.41

Figure 3 Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for normolipidaemic subjects

DISCUSSION

The management of dyslipidaemia is largely based on the LDL cholesterol concentration, that is why both accuracy and precision of LDL cholesterol measurement are critically important.[7, 22] Direct homogenous assays for measurement of LDL cholesterol levels have shown reasonable accuracy but all are expensive.[23] In order to improve the accuracy of Friedewald's formula, many formula had been developed.[18, 24 – 26] However, none of these formulas can replace the original formula due to less evidence.[22, 27] Therefore, Friedewald's formula is the most commonly used method although it has several limitations.[8] In this study, the mean concentration of TC, TG and HDL cholesterol were 129.11 mg/dl, 111.99 mg/dl and 47.87 mg/dl respectively in case of normolipidaemic and 204.06 mg/dl, 198.21 mg/dl and 37.26mg/dl respectively in case of dyslipidaemic subjects. Saiedullah et al. conducted their study on 644 samples and found the mean values of TC, TG, HDL cholesterol and LDL cholesterol were 218.78 mg/dl, 383.59 mg/dl, 36.11 mg/dl and 120.01 mg/dl respectively.[2] In our study, the mean values of LDL cholesterol measured by direct method, Friedewald's formula and de Cordova's formula were 132.99 mg/dL, 121.39 mg/dL and 116.81 mg/dl respectively. Studies showed remarkable underestimation of LDL cholesterol calculated by Friedewald's formula in Bangladeshi population.[2, 8, 18] Boshtam et al. revealed a highly significant correlation between direct method and Friedewald's formula.[28] However, the Friedewald's formula overestimated the LDL cholesterol value compared to the direct method.[28] The results of our study did not support Boshtam et al.[28] In our study, de Cordova's formula gave better result than Friedewald's formula. In all dyslipidaemic study subjects, the correlation coefficient of LDL cholesterol calculated by de Cordova's formula with the measured LDL cholesterol was statistically high significant and better than the correlation coefficient of LDL cholesterol calculated by Friedewald's formula with the measured LDL

cholesterol (0.804 vs 0.749, 0.719 vs 0.635, $p < 0.001$). However, in normolipidaemic subjects the correlation coefficient of LDL cholesterol calculated by Friedewald's formula with the measured LDL cholesterol was statistically high significant and better than the correlation coefficient of LDL cholesterol calculated by de Cordova's formula with the measured LDL cholesterol (0.696 vs 0.652, $p < 0.001$). Siddique et al. found bias of calculated LDL cholesterol against measured LDL cholesterol −5.2% for de Cordova's formula and −9.6% for Friedewald`s formula.[17] de Cordova's formula revealed better performance than Friedewald's formula for approximate calculation of LDL cholesterol without using triglycerides and showed better agreement in Bland-Altman plot.[16] We also did Bland-Altman plot to see the agreement between measured LDL cholesterol (direct method) and calculated LDL cholesterol. Our study supported Siddique et al.,[17] we also found better agreement of the de Cordova's formula than Friedewald's formula. In all dyslipidaemic subjects, limits of agreement were lower in case of de Cordova's formula (60.05, −27.70 vs 58.19, −43.82 and 62.73, −29.56 vs 60.81, −29.56) within 95% limit (see Tables 2 and 3, Figures 1 and 2). In the case of normolipidaemic subject, limits of agreement were 53.99 and 56.41 respectively for Friedewald's formula and de Cordova's formula (see Table 4 and Figure 3) and showed better agreement for Friedewald's formula because limits of agreement were lower. Our study result supported the result of Nigam.[29] Nigam studied on de Cordova's formula and found this formula can be used in non-fasting specimen, validated in large number of Brazilian individuals with wide range of TC, HDL cholesterol and TG levels.[29] However, de Cordova's formula did not perform better than Friedewald's formula in healthy individuals.[29] Our study result differs from the result of Martin, et al.[30] They revealed that the Friedewald's formula has a better agreement with directly measured LDL cholesterol compared to the de Cordova's formula.[30]

CONCLUSION

From this study, it may be concluded that estimation of LDL cholesterol by de Cordova's formula shows better agreement with measured LDL cholesterol (direct method) than Friedewald's formula for all and dyslipidaemic subjects. de Cordova's formula can be used clinically for approximate calculation of LDL cholesterol without using triglyceride as well as in non-fasting states. However, more studies are recommended regarding the validity of this formula for calculation of LDL cholesterol both in fasting and non-fasting states in our country and neighbouring countries.

REFERENCES

1. Chen Y, Zhang X, Pan B, Jin X, Yao H, Chen B, et al. (2010). A modified formula for calculating low-density lipoprotein cholesterol values. Lipids in Health and Disease 9 (1), **52 – 55.**

2. Saiedullah M, Sarkar A, Kamaluddin SM, Begum S, Hayat S, Rahman MR, Khan MAH. (2011). Friedewald's Formula is applicable up to serum triacylglycerol to total cholesterol ratio of two in Bangladeshi population. J AKMMC 2 (2), 21 – 25.

3. Grundy SM, Bazzarre T, Cleeman J, D'Agostino RB, Hill M, et al. (2000). Prevention Conference V: Beyond secondary prevention; identifying the high-risk patient for primary prevention 101, 3 – 11.

4. Bayer P, Veinberg F, Couderc R, Cherfils C, Cambillau M, Cosson C, et al. (2005). Évaluation multicentrique de quatre méthodes de dosage direct du Cholestérol LDL. Annales de Biologie Clinique 63 (1), 27 – 41.

5. Orringer CE. (2013). Non-HDL Cholesterol, Apo-B and LDL Particle concentration in coronary heart disease risk prediction and treatment. Clin Lipidology 8 (1), 69 – 79.

6. Diallo F, Ndiaye A, Doupa D, Diatta A, Cissé F, Samba A, et al. (2014). Comparison of direct assay and Friedewald formula for determination of LDL cholesterol. International Research Journal of Biochemistry and Bioinformatics 4 (2), 20 – 23.

7. Anwar M, Khan DA, Khan FA. (2014). Comparison of Friedewald formula and modified Friedewald formula with direct homogeneous assay for low density lipoprotein cholesterol estimation. Journal of the College of Physicians and Surgeons Pakistan 24 (1), 8 – 12.

8. Parvin M, Saiedullah M, Khan MAH, Rahman MR, Islam MS. (2012). Validation of the modified Friedewald's formula to calculate low-density lipoprotein cholesterol in Bangladeshi Population. Journal of Bangladesh College of Physicians and Surgeons 30 (3), 141 – 144.

9. Friedewald WT, Levy RI, Fredrickson DS. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 18, 499 – 502.

10. Bachorik PS, Ross JW. (1995). National Cholesterol Education Program recommendations for measurements of low density lipoprotein cholesterol: Executive summary, national cholesterol education program working group on lipoprotein measurements. *Clin* Chem, 41, 1414 – 1420.

11. de Cordova CMM, Schneider CR, Juttel ID, de Cordova MM. (2004). Comparison of LDL cholesterol direct measurement with the estimate using the Friedewald formula in a sample of 10,664 patients. Arquivos Brasileiros de Cardiologia 83 (6), 482 – 487.

12. Nauck M, Warnick GR, Rifai N. (2002). Methods for measurement of LDL-cholesterol: A critical assessment of direct measurement by homogeneous assays versus calculation. Clinical Chemistry, 48 (2), 236 – 254.

13. Wägner AM, Sánchez-Quesada JL, Pérez A, Rigla M, Cortés M, Blanco-Vaca F, et al. (2000). Inaccuracy of calculated LDL-cholesterol in type 2 diabetes: Consequences for patient risk classification and therapeutic decisions. Clinical Chemistry 46 (11), 1830 – 1832.

14. Teerakanchana T, Puavilai W, Suriyaprom K, Tungtrongchitr R. (2007). Comparative study of LDL-cholesterol levels In Thai patients by the direct method and using the Friedewald formula Southeast Asian. J Trop Med Public Health, 38 (3), 519 – 527.

15. Pandeya A, Sharma M, Regmi P, Basukala A, Lamsal M. (2012). Pattern of dyslipidaemia and evaluation of non-HDL cholesterol as a marker of risk factor for cardiovascular disease in type 2 diabetes mellitus. Nepal Med Coll J 14 (4), 278 – 282.

16. de Cordova CMM, de Cordova MM. (2013). A new accurate, simple formula for LDL-cholesterol estimation based on directly measured blood lipids from a large cohort. Annals of Clinical Biochemistry 50, 13 – 19.

17. Siddique AH, Saiedullah M, Chowdhury N, Khan MAH. (2014). Evaluation of performance of the newly developed de Cordova's Formula for calculation of Low density Lipoprotein cholesterol without use of Triglycerides. Journal of Enam Medical College 1, 10 – 14.

18. Saiedullah M, Chowdhury N, Khan MAH, Hayat S, Begum S, Rahman MR. (2014). Comparison of Regression Equation and Friedewald's Formula with Direct Measurement of Low-density Lipoprotein Cholesterol in Bangladeshi Population. Journal of Scientific Research 6 (1), 143 – 152.

19. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III) executive summary of the third report. JAMA 2001 285 (19), 2486 – 2497.

20. Bland JM, Altman DG. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1, 307 – 310.

21. Bland JM, Altman DG. (1999). Measuring agreement in method comparison studies. Stat Methods Med Res 8, 135 – 160.

22. Kamal AHM, Hossain M, Chowdhury NS, Mahmud NU. (2009). A comparison of calculated with direct measurement of low density lipoprotein cholesterol level. JCMCTA 20 (2), 19 – 23.

23. Sahu S, Chawla R, Uppal B. (2005). Comparison of two methods of estimation of low density lipoprotein cholesterol: The direct versus Friedewald estimation. Indian Journal of Clinical Biochemistry 20 (2), 54 – 61.

24. Anandaraja S, Narang R, Godeswar R, Laksmy R, Talwar KK. (2005). Low density lipoprotein cholesterol estimation by a new formula in Indian population. International Journal of Cardiology 102, 117 – 120.

25. Balal M, Paydas S, Inal T, Demir E, Kurt C, Sertdemir Y. (2010). Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol in renal transplant recipients. Ren Fail 32, 455 – 458.

26. Tsai CH, Su JL, Wu MH, Huang CJ, Lin JL. (2013). Estimating low-density lipoprotein cholesterol by a new formula instead of the Friedewald formula in Adult Taiwanese. Taiwan J Fam Med 23, 1 – 14.

27. Vujovic A, Stevuljevic JK, Spasic S, Bujisic N, Martinovic J, Vujovic M, et al. (2010). Evaluation of different formulas for LDL-c calculation. Lipids in Health and Disease 9, 27.

28. Boshtam M, Ramezani MA, Naderi G, Sarrafzadegan N. (2012). Is Friedewald formula a good estimation for low density lipoprotein level in Iranian population? J Res Med Sci. 17 (6), 519 – 522.

29. Nigam PK. (2014). Calculated low density lipoprotein-cholesterol: Friedewald's formula versus other modified formulas. International Journal of Life Science and Medical Research 4 (2), 25 – 31.

30. Martins J, Olorunju SAS, Murray LM, Pillay TS. (2015). Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients. Clinica Chimica Acta, 444, 137 – 142.