MJCB
MALAYSIAN JOURNAL OF CLINICAL BIOCHEMISTRY

ORIGINAL ARTICLE

COMPARISON OF DIRECTLY MEASURED LDL-CHOLESTEROL WITH CALCULATED LDL-CHOLESTEROL USING FRIEDEWALD, DE CORDOVA, AHMADI AND VUJOVIC FORMULAS

Iman Nabilah Abd Rahim [1,2], Noor Alicezah Mohd Kasim [1,2*]
[1]Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor Kampus Sungai Buloh,47000 Sungai Buloh, Selangor, Malaysia
[2]Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, 47000 Sungai Buloh, Selangor, Malaysia

*Authors' Email Address: [1]iman.nabilah7@gmail.com, *[2]noor202@uitm.edu.my*

Received: 4 May 2024 / Revised: 23 May 2024 / Accepted: 27 May 2024 /
Published Online: 20 June 2024

*Corresponding Author

ABSTRACT

This study aimed to compare the measured LDL-C (LDLD) with the Friedewald (LDLF), de Cordova (LDLDC), Ahmadi (LDLA), and Vujovic (LDLV) formulas, and establish the formula that provides the most reliable LDL-C results. Seventy-two subjects were enrolled in this study. Fasting blood samples were collected, and the serum was utilized for the analysis of lipid profiles. The percentage difference between the calculated LDL-C and LDLD was established. The strength between LDLD and calculated LDL-C was measured through Pearson correlation analysis. The level of agreement was evaluated through Bland-Altman analysis. The study encompassed LDLD measurements ranging from 0.05 mmol/L to 36.91 mmol/L. The lowest percentage difference of mean LDL-C between calculated and LDLD is observed in LDLV (7.14%). The LDLD measurement showed a positive and strongest correlation to LDLDC in evaluating LDL-C ($r = 0.958$, $p<0.001$). However, only LDLV showed a certain level of agreement with LDLD, with no significant bias observed (mean difference ± SD = 0.54±2.69). Vujovic formula could be used to estimate LDL-C values in hypo/hypercholesterolemic and normolipidemic individuals. LDLV formula was the most accurate and reliable calculated LDL-C when compared to LDLD measurements ranging from 0.05 mmol/L to 36.91 mmol/L.

Keywords: *Ahmadi formula, de Cordova formula, direct LDL-C, Friedewald formula, Vujovic formula*

INTRODUCTION

Cardiovascular disease (CVD) has been the leading cause of death globally (WHO, 2021). An elevated level of serum low-density lipoprotein cholesterol (LDL-C) is an independent risk factor for the development of CVD (Jung et al., 2022). LDL-C is a particle that transports cholesterol from the liver to the peripheral tissues. It is made up of outer phospholipids, apolipoproteins, free cholesterol, inner triglycerides (TG), and cholesterol ester (Feingold, 2000).
The National Cholesterol Education Program (NCEP) Adult Treatment Panel III and other scientific societies have identified LDL-C concentrations as the main laboratory parameter used for cardiovascular risk assessment and the primary target for cholesterol control (Detection & Adults, 2002;

Grundy et al., 2004). Hence, it is critically important to ensure precise and accurate measurement of LDL-C levels. Patients may be incorrectly classified in the wrong risk category as a result of inaccurate LDL-C estimation.

There are several methods that is commonly used to measure LDL-C levels such as beta quantification method, direct method, and calculation of LDL-C using formulas (Wolska & Remaley, 2020). Among them, beta quantification method is the gold standard reference method for LDL-C measurement (Martin et al., 2018). However, the beta quantification method is a highly manual technique that requires significant laboratory skill and expensive equipment, making it difficult to be widely used in clinical practice and it is primarily reserved for research settings.

Homogeneous enzyme assays, also referred to as direct LDL-C measurements have been developed recently. However, they are not widely used in most laboratories worldwide because they are expensive, time-consuming, and poorly standardized across laboratories (Martin et al., 2018).

To circumvent these issues, various formulae to calculate LDL-C have been developed. Friedewald formula is the most commonly used method to estimate LDL-C in medical laboratories. It is developed based on fasting serum measurements of TG, high density lipoproetin cholesterol (HDL-C), and total cholesterol (TC) (Friedewald et al., 1972). However, the Friedewald equation has some limitations, such as analytical variability and invalidity of the results in samples with TG greater than 4.51 mmol/L and certain types of hyperlipidemias (Lindsey et al., 2004; Nauck et al., 2002). Besides, subjects need to be fasted prior to blood collection in order to achieve reliable results as it does not account the cholesterol formed postprandially in chylomicrons or in the intermediate-density lipoproteins or in lipoprotein (a) (Contois et al., 2011). Moreover, Friedewald formula could lead to a false interpretation of the LDL-C estimation with a low serum TG of less than 100 mg/dL or 1.13 mmol/L (Ahmadi et al., 2008).

Other than the Friedewald formula, de Cordova, Ahmadi, and Vujovic formulas were developed to calculate LDL-C. Some of these formulas showed higher accuracy than the Friedewald formula in certain conditions (Ahmadi et al., 2008; de Cordova & de Cordova, 2013; Vujovic et al., 2010). Therefore, this study aimed to compare the measured LDL-C (LDL_D) with the calculated LDL-C by the Friedewald (LDL_F), de Cordova (LDL_{DC}), Ahmadi (LDL_A), and Vujovic (LDL_V) formulas, and establish the best calculated LDL-C formula that provides the most reliable LDL-C results.

METHODS

Study Population

A data set of 72 subjects was included in the study. This study was approved by the Ethics Committee of Universiti Teknologi MARA (326/2020). A total of 4 ml blood samples were obtained in the morning after an overnight fast of 8 hours from all subjects. The blood samples were collected in plain vacutainer vials and processed within 2 hours of collection. All the samples were allowed to clot at room temperature and the serum is separated by centrifugation at 3500 rpm for 10 min. Gross hemolytic or icteric serum was rejected. The samples were then stored at −80◦C refrigerator until analysis.

Lipid Profile Analysis

All samples were analyzed in terms of lipid profiles comprising high-density lipoprotein cholesterol (HDL-C), LDL-C, triglycerides (TG), and total cholesterol (TC) using an automatic chemistry analyzer, cobas c 501, Roche®. The calibrating and internal controls were provided by RNZ MARKETING Sdn Bhd. Measurements of direct LDL-C (LDL_D) were performed using LDL-Cholesterol Gen.3 (LDLC3) reagent by cobas®. Direct LDL-C was quantified photometrically by

homogenous enzymatic colorimetric assay using cholesterol esterase and cholesterol oxidase enzymes with surfactants, which selectively solubilizes LDL. The surfactants and a sugar compound inhibit the enzyme reactions to the lipoproteins other than LDL. Measurements of HDL-C, TC, and TG were performed using reagents by Roche Diagnostics, according to the specifications of the manufacturers using the cobas c 501, Roche® by the enzymatic colorimetric method.

Low-density Lipoprotein Cholesterol Calculation

For each subject, the LDL-C was calculated using the following four formulas:

(1) Friedwald formula (LDL_F): LDL-C (mmol/L) = TC – HDL – TG/2.2 (Friedewald et al., 1972)
(2) de Cordova formula (LDL_{DC}): LDL (mmol/L) = 0.7516 (TC – HDL-C) (de Cordova & de Cordova, 2013)
(3) Ahmadi formula (LDL_A): LDL (mmol/L) = TC /1.19 + TG /0.81 – HDL-C /1.1 – 0.98 (Ahmadi et al., 2008)
(4) Vujovic formula (LDL_V): LDL-C (mmol/L) = TC – HDL – TG/3 (Vujovic et al., 2010)

Statistical analysis

Data was entered into Microsoft® Excel® for Microsoft 365 MSO (Version 2307 Build 16.0.16626.20086) and the statistical analysis was performed using IBM® Statistical Package for Social Sciences (SPSS) version 27.0. The normality test was conducted using the Kolmogorov-Smirnov test. Discrete data were expressed as a mean and standard deviation. One sample *t*-test was used to analyze the difference between LDL-C concentrations measured by the direct method compared with the various formulas. The mean percentage difference was calculated using the formula: Mean percentage difference = (calculated LDL-C – direct LDL-C) / (calculated LDL-C + direct LDL-C) / 2) × 100. The degree of correlation between the results of the two methods was calculated by using Pearson's correlation test. The Bland-Altman plot was employed to analyze the degree of agreement between each calculated formula with the direct LDL-C. *P* values of < 0.05 were considered statistically significant.

RESULTS

The study encompassed LDL_D measurements ranging from 0.05 mmol/L to 36.91 mmol/L. The mean ± SD (mmol/L) lipid profiles were: LDL_D = 7.69±9.25, TC = 8.09±8.84, HDL-C = 0.65±0.28 and TG = 0.87±0.58 (Table 1). The mean ± SD of calculated LDL-C levels (mmol/L) were 7.69±9.25, 7.05±8.75, 5.60±6.55, 6.31±7.27, and 7.16±8.74 for LDL_D, LDL_F, LDL_{DC}, LDL_A, and LDL_V respectively (Table 2). The mean difference (mmol/L) between LDL-C concentrations measured by the LDL_D with various formulas is shown in Table 2. The lowest percentage difference of mean LDL-C between calculated and LDL_D is observed in LDL_V (7.14%), followed by LDL_F (8.68%), LDL_A (19.71%), and LDL_{DC} (31.45%) (Table 2).

Pearson correlation coefficient for LDL_F, LDL_{DC}, LDL_A, and LDL_V was 0.957 ($p < 0.001$), 0.958 ($p < 0.001$), 0.954 ($p < 0.001$), and 0.957 ($p < 0.001$) respectively (Table 2). The LDL_D measurement showed a positive and strongest correlation to LDL_{DC} in evaluating LDL-C ($r = 0.958$, $p<0.001$), followed by LDL_V and LDL_F ($r = 0.957$, $p<0.001$), and LDL_A ($r = 0.954$, $p<0.001$). The regression equation for each of the formulas when calculated LDL-C is plotted in X axis and LDL_D is plotted in Y axis was: LDL_F ($y=1.011x + 0.562$), LDL_{DC} ($y=1.352x + 0.126$), LDL_A ($y= 1.214x + 0.038$), and LDL_V ($y=1.013x + 0.445$) (Figure 1 – 4).

The Bland-Altman plot of LDL_F, LDL_{DC}, LDL_A, and LDL_V against LDL_D showed a mean bias (mmol/L) of 0.64±2.70, 2.10±3.52, 1.39±3.17 and 0.54±2.69 respectively. However, only LDL_V showed a certain

level of agreement with LDL_D, with no significant bias observed (mean bias ± SD = 0.54±2.69) (Figure 5 – 8).

DISCUSSION

To the best of our knowledge, this is the first study to compare the calculated LDL-C levels using four different formulas: LDL_F, LDL_{DC}, LDL_A, and LDL_V to LDL_D levels with LDL_D measurements ranging from 0.05 mmol/L to 36.91 mmol/L.

It is very crucial to obtain accurate measurements of the LDL-C levels since it plays an essential role in determining the treatment plans for the patients (Expert Panel on Detection, 2001). From the LDL-C results, dietary adjustments, drug therapy such as lipid-lowering therapy, and advance monitoring could be determined so that LDL-C levels could be reduced by at least 50% (Ginsberg et al., 2022). Underestimation or overestimation of LDL-C levels may result in misdiagnosis and incorrect disease management, resulting in poor patient outcomes. To circumvent this issue, several methods to determine LDL-C levels have been developed. These methods, however, have their own set of limitations (Table 3).`

Friedewald formula was published in 1972 by Friedewald et al. (Friedewald et al., 1972). However, the Friedewald formula has several drawbacks in which it is not valid in those with a high level of TG more than 4.5 mmol/L, in non-fasting sample (Lee et al., 2020; Rim et al., 2016) and in individuals with very low levels of LDL-C lower than 2.4 mmol/L (Karkhaneh et al., 2019). Besides, it is not suitable for individuals with type III hyperlipidemia and suffering from several pathological states such as liver and renal failure, diabetes mellitus and other metabolic diseases ((Chen et al., 2010; Hattori et al., 1998)). Despite its numerous shortcomings, the Friedewald formula is still widely used by routine laboratories globally.

Recently, several authors have developed various formulas in specific populations to overcome the limitations of the Friedewald formula ((Ahmadi et al., 2008; de Cordova & de Cordova, 2013; Vujovic et al., 2010)). These authors advocated for the validation of their formulas in populations other than those studied. The advantages of each formula are summarized in Table 4. The results from our study revealed that all formulas underestimate the LDL-C level as compared to LDL_D. The least percentage difference of mean LDL-C is observed when using the Vujovic formula. This is consistent with a previous study that discovered the Vujovic formula had the lowest percentage error when compared to other formulas (Choi et al., 2016).

There were positive and strong correlations between LDL_D and calculated LDL-C with all four formulas. The de Cordova formula, however, produced the strongest correlation. This finding is consistent with previous research, which found that calculated LDL-C using de Cordova's formula is a close approximation to direct estimation when compared to other newly derived formulas (Tomo et al., 2022). This was also investigated in a previous study, which found that the de Cordova formula had better agreement with LDL_D than the Friedewald formula in both healthy and dyslipidemic subjects (Nasrin, 2017). This could be due to the lack of TG value in the de Cordova's formula.

Since high correlation coefficients do not always indicate the agreement of methods, the Bland-Altmann plot was used to evaluate the agreement and concordance between LDL_D with each calculated LDL-C. The de Cordova and Ahmadi formulas differed significantly from LDL_D in the presence of proportional bias. This is consistent with previous research, which found that the de Cordova formula has no significant advantage over the Friedewald formula in Indian populations (Wadhwa & Krishnaswamy, 2016). Wadhwa et al. also discovered that the Ahmadi formula performed poorly at TG levels greater than 1.70 mmol/L (Wadhwa & Krishnaswamy, 2016). Furthermore, a recent study on a population of over 100,000 people in Italy discovered that the Vujovic formula is one of the most accurate formulas when compared to LDL_D (Piani et al., 2021).

Surprisingly, the Friedewald formula demonstrated a statistically significant difference with LDL_D, despite the absence of proportional bias. A low level of LDL-C could be one of the reasons that contribute to the significant difference to the LDL_D values. Only the Vujovic formula showed significant agreement and no significant difference from the LDL_D formula, with no evidence of bias. Overall, our findings suggest that the Friedewald formula could be used as an alternative cost-effective tool to measure LDL-C in healthy individuals with a normal range of LDL-C if a direct measurement cannot be afforded. In the case of a person with known hypocholesterolemia or hypercholesterolemia, the Vujovic formula could be used instead of the Friedewald formula to estimate LDL-C values.

There are several limitations in this study. Firstly, calculated LDL-C was compared to direct LDL-C, i.e., homogenous enzyme assay, rather than the reference method, ultracentrifugation-polyanions precipitation. Furthermore, only one specific assay was used for TG, TC, and HDL-C (Roche) to calculate the LDL-C value. Other assays were not considered. Despite the small sample size used in this study, it is large enough to yield a significant result. Finally, several other equations for calculating LDL-C besides the ones described in this study were not considered.

Figures and Tables

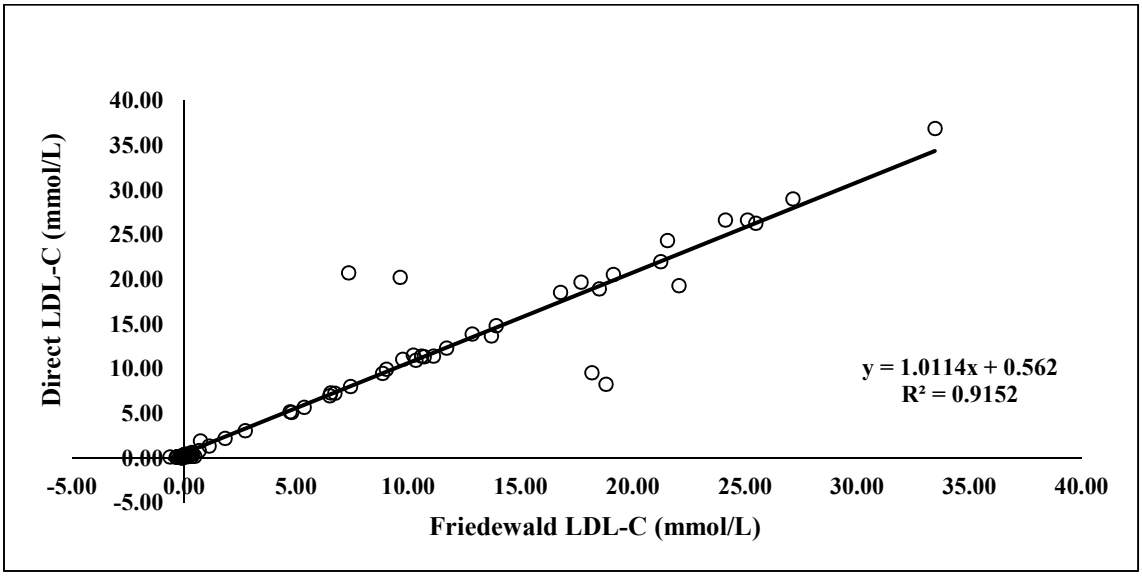

Figure 1: Correlation between calculated LDL-C using Friedewald formula and direct LDL-C

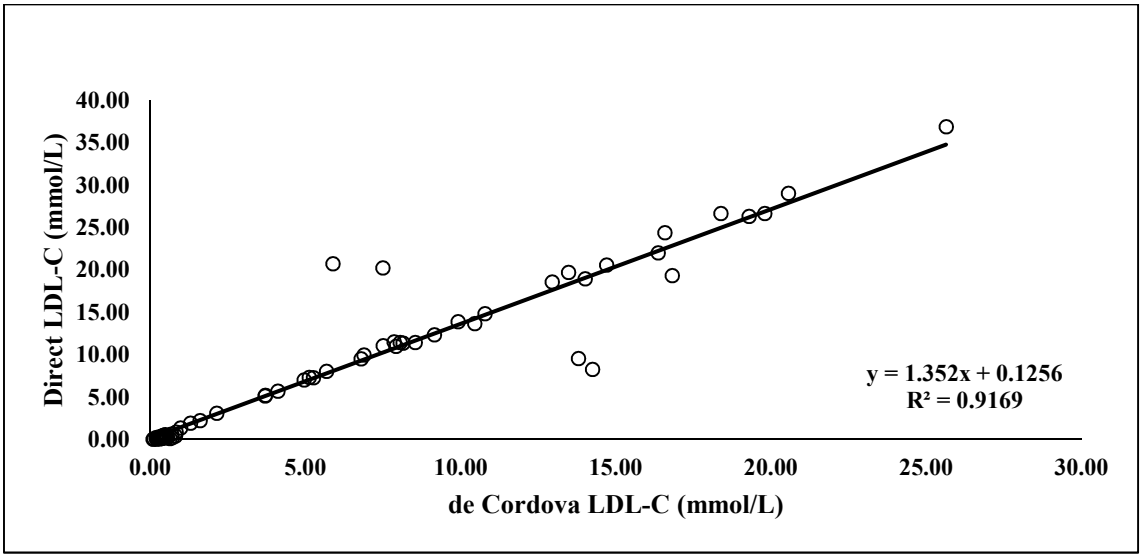

Figure 2: Correlation between calculated LDL-C using de Cordova formula and direct LDL-C

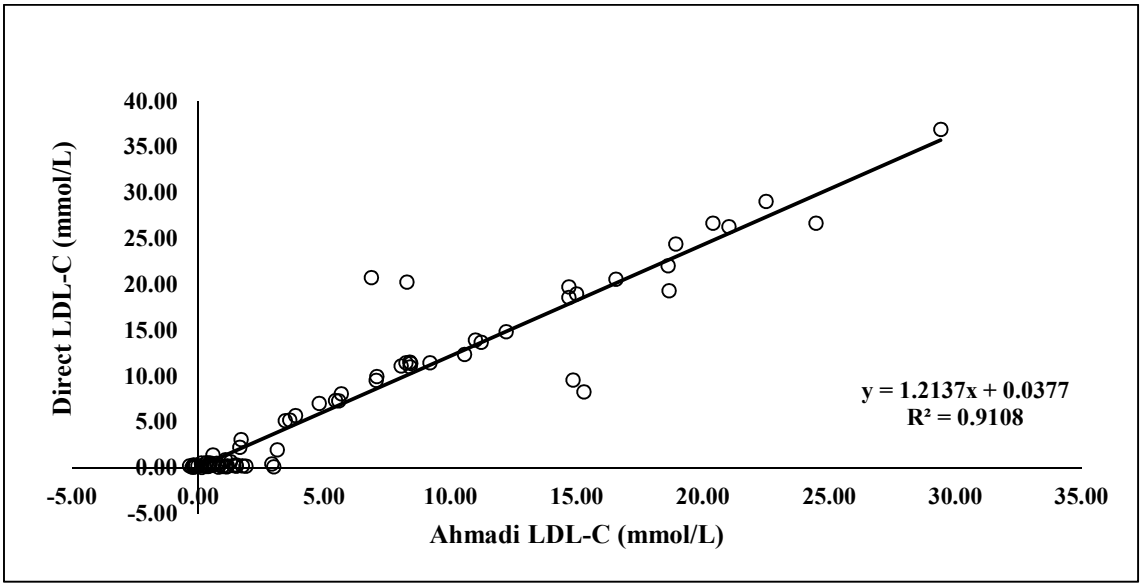

Figure 3: Correlation between calculated LDL-C using Ahmadi formula and direct LDL-C

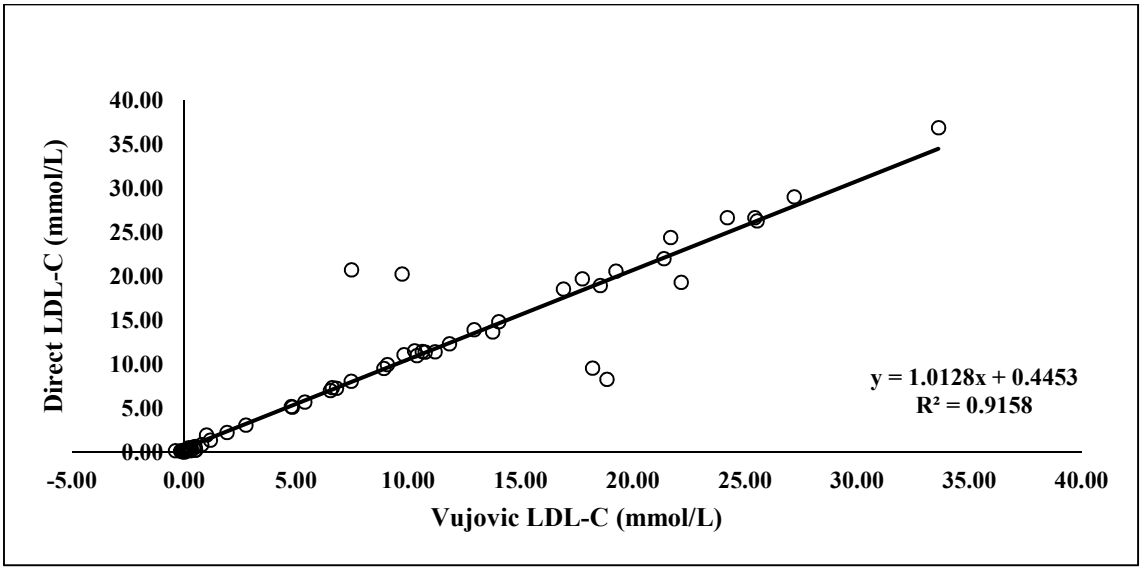

Figure 4: Correlation between calculated LDL-C using Vujovic formula and direct LDL-C

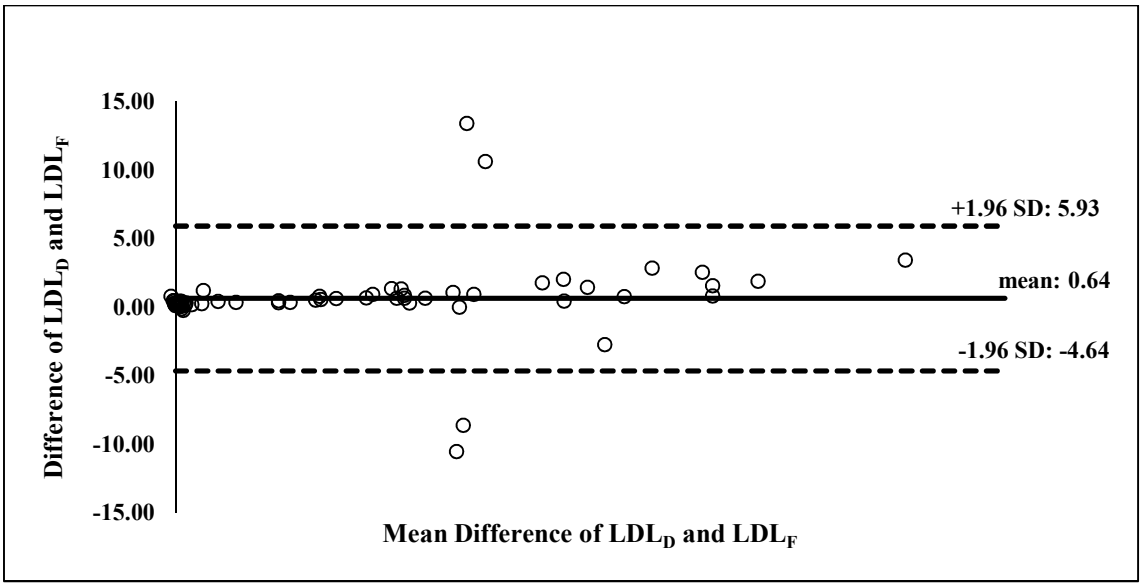

Figure 5: Bland-Altman plots of direct LDL-C and calculated LDL-C (Friedewald)

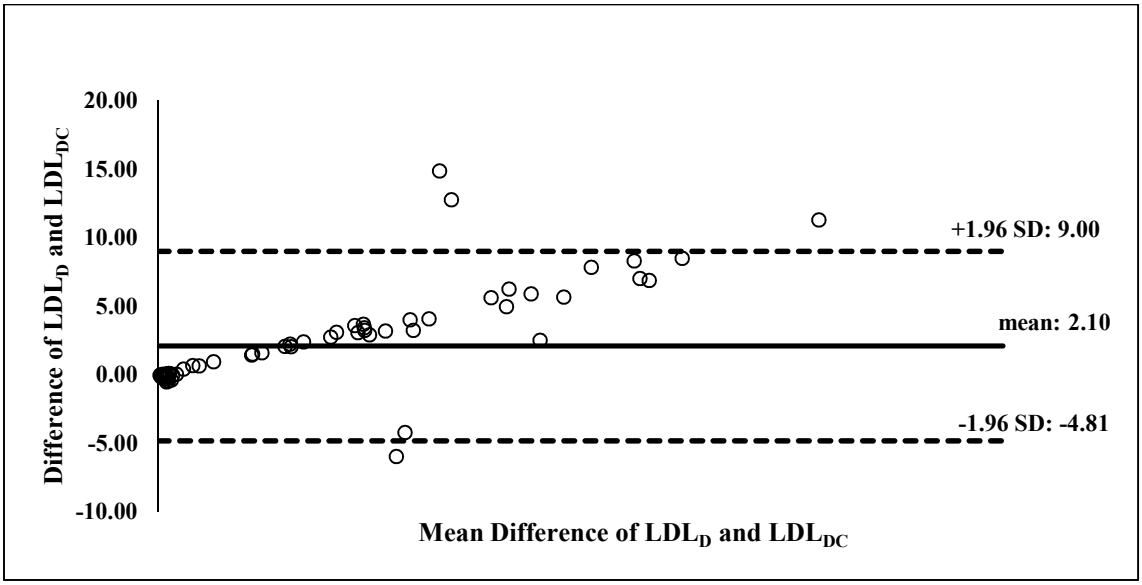

Figure 6: Bland-Altman plots of direct LDL-C and calculated LDL-C (de Cordova)

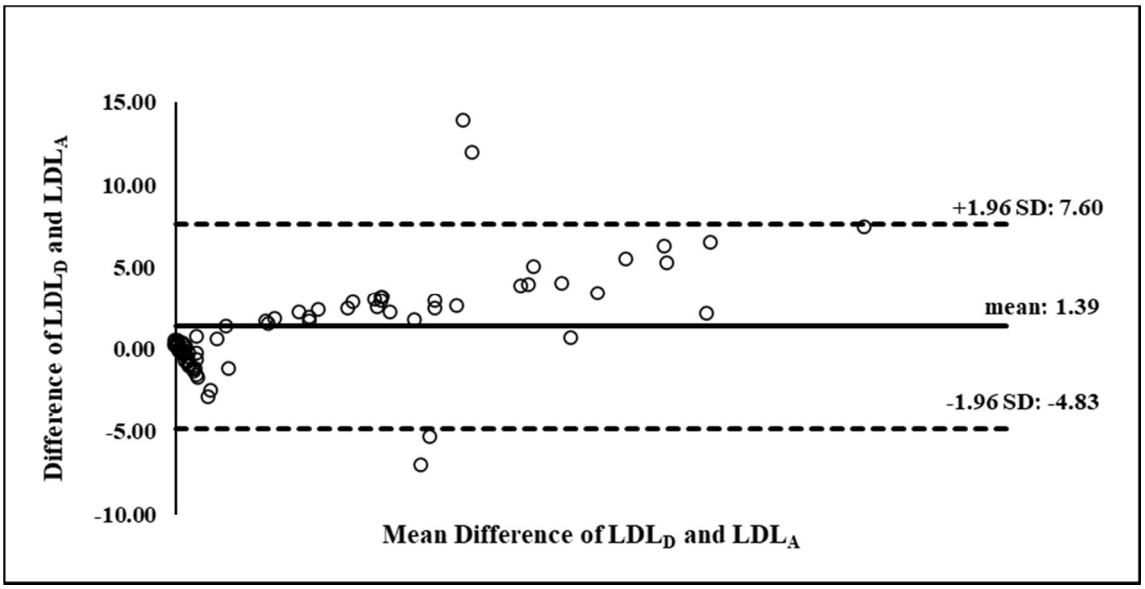

Figure 7: Bland-Altman plots of direct LDL-C and calculated LDL-C (Ahmadi)

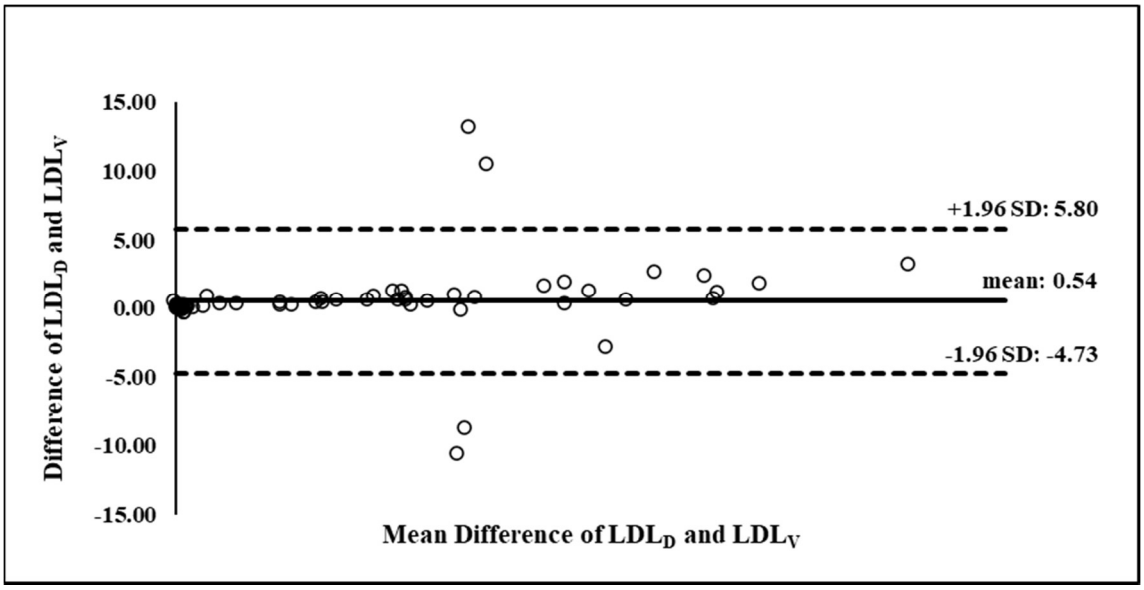

Figure 8: Bland-Altman plots of direct LDL-C and calculated LDL-C (Vujovic)

Table 1: The mean lipid profiles of study population expressed as mean ± SD (n=72)

Parameter	Mean ± SD (mmol/L)
Direct LDL-C	7.69±9.25
HDL-C	0.65±0.28
TC	8.09±8.84
TG	0.87±0.58

Table 2: The data of calculated LDL-C and their mean difference in comparison to direct LDL-C

Formula	Mean ± SD (mmol/L)	Mean difference (mmol/L)	Mean percentage difference (%)	Correlation (*r*)	*p* value
Friedewald LDL-C	7.05±8.75	0.64±2.70	8.68	0.957	$p < 0.001$
de Cordova LDL-C	5.60±6.55	2.10±3.52	31.45	0.958	$p < 0.001$
Ahmadi LDL-C	6.31±7.27	1.39±3.17	19.71	0.954	$p < 0.001$
Vujovic LDL-C	7.16±8.74	0.54±2.69	7.14	0.957	$p < 0.001$

Table 3: Limitations of several methods to determine LDL-C levels

Analytes	Methods	Limitations
Beta (ß) quantification	Ultracentrifugation-polyanions precipitation (Gold-standard reference method)	- Not convenient for routine use - Expensive - Labour intense - Requires a large number of sample batches - Can only be done in specialized laboratories
Direct LDL-C	Homogenous enzyme assay	- Lack of standardization across laboratories - The performance varies across methods and reagents used - Time intervals between ordering the test are inappropriate compared with proposed guidelines - Variation of results using different analyzer - Less reliable in mild hypertriglyceridemia > 4.5 mmol/L (Miller et al., 2010) - Time consuming - Not cost effective
Friedewald equation	Calculation	- Not valid in high level of TG > 4.5mmol/L (Lee et al., 2020; Rim et al., 2016) - Requires fasting blood samples - Not suitable for type III hyperlipidemia - Lose accuracy with TG above 1.69 mmol/L (Martins et al., 2023) - Not accurate with very low level of LDL-C < 1.8 mmol/L (Martins et al., 2023) - The study based on a very small sample size (448 samples) - Data included is from a population with familial hypercholesterolemia - Should be used with precaution in several pathological states.

Table 4: Advantages of de Cordova, Vujovic and Ahmadi formulas compared to Friedewald formula

Formula	Year	Advantages
de Cordova	2013	- Independent from serum TG - Does not require fasting samples - Based on large database of 10,000 individuals with LDL-C values ranging from 0.62 to 10.94 mmol/L
Vujovic	2010	- Proposed to be used over a wide TG range - Could be used for calculation of LDL-C in reseource-poor setting or small sample size
Ahmadi	2008	- Advisable to use in samples with low TG levels

CONCLUSION

In conclusion, among four formulas, the Vujovic formula was the most accurate and reliable formula to calculate LDL-C ranging from 0.05 mmol/L to 36.91 mmol/L. Future studies with larger sample sizes are warranted to verify the findings from the present study.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Institute of Medical Molecular Biotechnology (IMMB) of Faculty of Medicine and the Department of Clinical Diagnostic Laboratories (CDL) of Hospital Al-Sultan Abdullah (HASA), Universiti Teknologi MARA for providing the facilities to complete this study.

FUNDING

This research was fully funded by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme, grant number (FRGS) 600-IRMI/FRGS 5/3 (366/2019).

AUTHORS' CONTRIBUTION

Mohd Kasim, N.A. designed the research study. Abd Rahim, I.N. performed the research. Mohd Kasim, N.A. provided help and advice on conception, acquisition of data, and supervision. Abd Rahim, I.N. analyzed the data. Abd Rahim, I.N. wrote the manuscript. All authors actively contributed to providing critical feedback, shaping the research, guiding the analysis, and refining the manuscript.

CONFLICT OF INTEREST DECLARATION

We affirm that there is no Conflict of Interest among the authors concerning the subject matter or materials discussed in this manuscript. We further certify that the article represents the original work of the Authors and Co-Authors. The manuscript has not been previously published and is not currently under consideration for publication elsewhere. This research/manuscript has neither been submitted for publication nor published in whole or in part elsewhere. We attest that all authors have made significant contributions to the work, ensuring the validity and legitimacy of the data and its interpretation, thereby warranting its submission to the Malaysian Journal of Clinical Biochemistry.

REFERENCES

Ahmadi, S. A., Boroumand, M.-A., GOUHARI, M. K., Tajik, P., & Dibaj, S.-M. (2008). The impact of low serum triglyceride on LDL-cholesterol estimation.

Chen, Y., Zhang, X., Pan, B., Jin, X., Yao, H., Chen, B., . . . Chen, H. (2010). A modified formula for calculating low-density lipoprotein cholesterol values. *Lipids in health and disease*, *9*, 1-5.

Choi, H., Shim, J.-S., Lee, M. H., Yoon, Y. M., Choi, D. P., & Kim, H. C. (2016). Comparison of formulas for calculating low-density lipoprotein cholesterol in general population and high-risk patients with cardiovascular disease. *Korean circulation journal*, *46*(5), 688-698.

Contois, J. H., Warnick, G. R., & Sniderman, A. D. (2011). Reliability of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B measurement. *Journal of clinical lipidology*, *5*(4), 264-272.

de Cordova, C. M. M., & de Cordova, M. M. (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*(1), 13-19. https://doi.org/10.1258/acb.2012.011259

Detection, N. C. E. P. E. P. o., & Adults, T. o. H. B. C. i. (2002). *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)*. The Program.

Expert Panel on Detection, E. (2001). 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*, *285*(19), 2486-2497.

Feingold, K. R. (2000). Introduction to Lipids and Lipoproteins. In K. R. Feingold, B. Anawalt, M. R. Blackman, A. Boyce, G. Chrousos, E. Corpas, W. W. de Herder, K. Dhatariya, K. Dungan, J. Hofland, S. Kalra, G. Kaltsas, N. Kapoor, C. Koch, P. Kopp, M. Korbonits, C. S. Kovacs, W. Kuohung, B. Laferrère, M. Levy, E. A. McGee, R. McLachlan, M. New, J. Purnell, R. Sahay, A. S. Shah, F. Singer, M. A. Sperling, C. A. Stratakis, D. L. Trence, & D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc.

Copyright © 2000-2023, MDText.com, Inc.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), 499-502.

Ginsberg, H. N., Rosenson, R. S., Hovingh, G. K., Letierce, A., Samuel, R., Poulouin, Y., & Cannon, C. P. (2022). LDL-C calculated by Friedewald, Martin-Hopkins, or NIH equation 2 versus beta-quantification: pooled alirocumab trials. *Journal of Lipid Research*, *63*(1).

Grundy, S. M., Cleeman, J. I., Merz, C. N. B., Brewer Jr, H. B., Clark, L. T., Hunninghake, D. B., . . . Stone, N. J. (2004). Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. *Circulation*, *110*(2), 227-239.

Hattori, Y., Suzuki, M., Tsushima, M., Yoshida, M., Tokunaga, Y., Wang, Y., . . . Ryomoto, K. I. (1998). Development of approximate formula for LDL-chol, LDL-apo B and LDL-chol/LDL-apo B as indices of hyperapobetalipoproteinemia and small dense LDL. *Atherosclerosis*, *138*(2), 289-299.

Jung, E., Kong, S. Y., Ro, Y. S., Ryu, H. H., & Shin, S. D. (2022). Serum Cholesterol Levels and Risk of Cardiovascular Death: A Systematic Review and a Dose-Response Meta-Analysis of Prospective Cohort Studies. *Int J Environ Res Public Health*, *19*(14). https://doi.org/10.3390/ijerph19148272

Karkhaneh, A., Bagherieh, M., Sadeghi, S., & Kheirollahi, A. (2019). Evaluation of eight formulas for LDL-C estimation in Iranian subjects with different metabolic health statuses. *Lipids in health and disease*, *18*(1), 1-11.

Lee, J., Jang, S., Jeong, H., & Ryu, O.-H. (2020). Validation of the Friedewald formula for estimating low density lipoprotein cholesterol: the Korea National Health and Nutrition Examination Survey, 2009 to 2011. *The Korean Journal of Internal Medicine*, *35*(1), 150.

Lindsey, C. C., Graham, M. R., Johnston, T. P., Kiroff, C. G., & Freshley, A. (2004). A Clinical Comparison of Calculated versus Direct Measurement of Low-Density Lipoprotein Cholesterol Level. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *24*(2), 167-172.

Martin, S. S., Giugliano, R. P., Murphy, S. A., Wasserman, S. M., Stein, E. A., Češka, R., . . . Tikkanen, M. J. (2018). Comparison of low-density lipoprotein cholesterol assessment by Martin/Hopkins estimation, Friedewald estimation, and preparative ultracentrifugation: insights from the FOURIER trial. *JAMA cardiology*, *3*(8), 749-753.

Martins, J., Steyn, N., Rossouw, H. M., & Pillay, T. S. (2023). Best practice for LDL-cholesterol: when and how to calculate. *Journal of Clinical Pathology*, *76*(3), 145-152.

Miller, W. G., Myers, G. L., Sakurabayashi, I., Bachmann, L. M., Caudill, S. P., Dziekonski, A., . . . Leary, E. T. (2010). Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clinical chemistry*, *56*(6), 977-986.

Nasrin, N. (2017). Newly-developed De Cordovaâ€™ s Formula for Calculation of LDL Cholesterol in Bangladeshi Population. *Borneo Journal of Medical Sciences (BJMS)*, *11*(3), 25-34.

Nauck, M., Warnick, G. R., & 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.

Piani, F., Cicero, A. F., Ventura, F., Dormi, A., Fogacci, F., Patrono, D., . . . D'Addato, S. (2021). Evaluation of twelve formulas for LDL-C estimation in a large, blinded, random Italian population. *International Journal of Cardiology*, *330*, 221-227.

Rim, J. H., Lee, Y.-h., Lee, M. H., Kim, H. Y., Choi, J., Lee, B.-W., . . . Lee, S.-G. (2016). Comparison and validation of 10 equations including a novel method for estimation of LDL-cholesterol in a 168,212 Asian population. *Medicine*, *95*(14).

Tomo, S., Sankanagoudar, S., & Sharma, P. (2022). Cordova Formula for Low-Density Lipoprotein-Cholesterol Estimation: Not Only the Simplest of All But Also Superior to Other Formulae at a Higher Range of Triglyceride. *Anatolian Journal of Cardiology*, *26*(7), 520.

Vujovic, A., Kotur-Stevuljevic, J., Spasic, S., Bujisic, N., Martinovic, J., Vujovic, M., . . . Pajic, D. (2010). Evaluation of different formulas for LDL-C calculation. *Lipids in health and disease*, *9*(1), 27. https://doi.org/10.1186/1476-511X-9-27

Wadhwa, N., & Krishnaswamy, R. (2016). Comparison of LDL-Cholesterol Estimate using Various Formulae with Directly Measured LDL-Cholesterol in Indian Population. *J Clin Diagn Res*, *10*(12), Bc11-bc13. https://doi.org/10.7860/jcdr/2016/22272.9018

WHO. (2021). Cardiovascular diseases (CVDs) [Fact sheets]. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

Wolska, A., & Remaley, A. T. (2020). Measuring LDL-cholesterol: what is the best way to do it? *Current opinion in cardiology*, *35*(4), 405.