Clinica Chimica Acta 495 (2019) 487–492

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Evaluation of Martin's equation for LDL-C estimation in type 2 diabetes mellitus Egyptian patients

Marwa M. Esawy[a,*], Marwa A. Shabana[a], Mahmoud M. Magdy[b]

[a] Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt
[b] Internal Medicine Department, Faculty of Medicine, Zagazig University, Egypt

ARTICLE INFO

Keywords:
Diabetes mellitus
LDL-cholesterol
Friedewald equation
Martin equation

ABSTRACT

Introduction: Type 2 Diabetes Mellitus has characteristic dyslipidemia. Low-density lipoprotein cholesterol (LDL-C) measurement plays a role in cardiovascular risk assessment and management. Friedewald equation (FE) has several limitations. This study aims to evaluate the effectiveness of Martin equation (ME) in Egyptian patients, especially those with type 2 diabetes.

Methods: A cross-sectional study was conducted on 454 diabetic and non-diabetic patients who were referred to the internal medicine outpatient clinic. Lipid profile was assessed by Cobas 8000 Modular Analyzer.

Results: The LDL-C was estimated by both FE and ME. In diabetic patients, LDL-C estimated by FE was underestimated with a bias of -3.9 ± 5.3 mg/dL ($p = .04$). But LDL-C estimated by ME was not significantly different compared to directly measured LDL-C. FE underestimate LDL-C with a bias of -4.6 ± 6.4 mg/dL ($p = .042$) in uncontrolled diabetic patients. A non-significant difference in both uncontrolled patients and controlled ones was detected by ME. FE had lower sensitivity and specificity (80% and 88.9 respectively) compared to the ME (95.9% sensitivity, and 95.6% specificity). ME was not influenced by triglyceride levels ($p = .34$).

Conclusion: The ME improves concordance of calculated LDL-C with a direct LDL-C assay in Egyptian diabetic patients.

1. Introduction

Type 2 Diabetes Mellitus has characteristic dyslipidemia [high triglycerides, normal or slightly increased total cholesterol, low level of high-density lipoprotein cholesterol (HDL-C), a predominance of low-density lipoprotein cholesterol (LDL-C), and abnormal very low-density lipoprotein (VLDL-C)] [1,2]. These changes cause the development of premature atherosclerosis and coronary heart disease [3]. LDL-C measurement plays a role in cardiovascular risk assessment. Also, LDL-C measurement is applied in case of risk management decision and therapy monitoring [4–6].

Direct methods for LDL-C measurement are available [7], but many laboratories use the equation of Friedewald et al. [8] for estimating LDL-C. Generally, the Friedewald equation (FE) works well for most of the patients. But it is still inaccurate when it is applied to some patients including (diabetes, metabolic syndrome, liver and kidney disease) [9–13].

FE has several limitations. FE requires fasting blood samples, and it is not recommended if triglyceride level > 400 mg/dL [14]. FE underestimates LDL-C at low levels of LDL-C ([15] and with high triglyceride levels [16]. Inaccurate estimation of LDL-C can be problematic as it may delay treatment or impose unnecessary medication. For these reasons, many studies attempted to modify FE [17].

Martin et al. [18] have developed a new equation for estimating LDL-C. Martin equation (ME) uses an adjustable factor for the triglyceride/VLDL-C ratio. This factor determines the possibility of using triglyceride and non-HDL-C concentrations. ME showed a more accurate LDL-C estimation [19,20].

This study attempts to evaluate the effectiveness of ME and FE for LDL-C estimation in Egyptian patients, especially those with type 2 diabetes. The accuracy of these equations was evaluated in comparison with that of the direct method.

Abbreviations: AUC, Area under curve; FE, Friedewald equation; HbA1c, hemoglobinA1c; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; ME, Martin equation; ROC, Receiver operating characteristic; VLDL-C, Very Low-Density Lipoprotein

* Corresponding author at: Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt.
E-mail address: dr.marwaesawy@ymail.com (M.M. Esawy).

https://doi.org/10.1016/j.cca.2019.05.025
Received 8 April 2019; Received in revised form 18 May 2019; Accepted 25 May 2019
Available online 27 May 2019

2. Subjects and methods

2.1. Subjects

This cross-sectional study was conducted on 454 diabetic and non-diabetic patients who were referred to the internal medicine outpatient clinic, Zagazig University Hospital. The study was carried out in Clinical Pathology Department, Faculty of medicine, Zagazig University (from August 2018 to January 2019). This study was approved by the Institutional Research and Ethics Committee of Faculty of Medicine, Zagazig University. All patients were informed about the study and written consents were obtained.

2.2. Method

After 12 h of fasting, blood samples were collected by venipuncture into BD Vacutainer® plastic EDTA and plain tubes. Whole blood HbA1c testing was done on the Cobas 6000-C501 (Roche Diagnostics, Mannheim, Germany). Serum levels of Cholesterol, Triglycerides HDL-C and LDL-C were measured using the Cobas 8000 Modular Analyzer series/C702 (Roche Diagnostics, Mannheim, Germany). All patients were restricted to triglyceride levels below 400 mg/dL. LDL-C was directly measured by Roche LDL-Cholesterol plus 2nd generation reagent. It is a homogenous enzymatic cholesterol assay by using cholesterol esterase and cholesterol oxidase/peroxidase. The reaction was modified in order to increase its selectivity. This was made by adding magnesium, non-ionic detergent, and sugar compound. The calibration of the tests was performed according to the manufacturer requirements using the calibration materials from Roche Diagnostics. The daily quality control was performed in accordance with controls provided by the manufacturer.

LDL-C was calculated by FE = Cholesterol − (HDL-C + Triglyceride/5). LDL-C was calculated by ME = Cholesterol − (HDL-C + Triglyceride/adjustable factor). ME was coded into an online calculator (http://ldlcalculator.com/).

2.3. Statistical analysis

Shapiro-Wilk test shows normal data distribution, so continuous variables are expressed as mean and standard deviations (SD). In this regard, categorical variables are expressed as numbers and percentages. Student *t*-test and one-way ANOVA test were used to evaluate the level of significance difference in two and multiple comparisons respectively. The agreement between measured and estimated LDL-C was evaluated using Bland-Altman plots, and Bias was calculated as the mean difference between estimated and measured LDL-C. The calculation of agreement limits is done in terms of (bias ± 1.96 SD). Linear regression analyses were also performed to detect the significance of the estimation of LDL-C. Receiver operating characteristic (ROC) curve determines the clinical utility of equations in finding LDL-C treatment level (LDL-C levels at 100 mg/dL). The area under the ROC curve (AUC) assesses the performance. Then, sensitivity and specificity were calculated for each formula. Statistical analysis was carried out using SPSS software "version 17.0" (SPSS Inc., Chicago, IL, USA). Significance was considered at p-value lower than 0.05.

3. Results

Overall, 47.4% of the study subjects had type 2 diabetes mellitus. The mean hemoglobinA1c (HbA1c) levels among diabetic patients were 6.2 ± 1%. The characteristics of study subjects and laboratory results are shown in Table 1. Triglyceride levels were 161.6 ± 65.1 and 117.5 ± 50.1 mg/dL, in diabetic and non-diabetic patients respectively. Diabetic patients showed a cholesterol level of 206.1 ± 54.9 mg/dL and non-diabetic patients showed a cholesterol level of 187 ± 64.9. HDL-C levels were 43.5 ± 11.5 and 43 ± 6.3 mg/dL in diabetic and non diabetic patients respectively. LDL-C levels were 134.2 ± 47.4 and 120.8 ± 57.8 mg/dL in diabetic and non diabetic patients respectively.

Table 1
Characteristics of the subjects.

Parameter	Subjects (No. = 454)
Age (years)	45.4 ± 9.9
Sex: Male/Female	203/251 (44.7/55.3)
Diabetic patients	215 (47.39)
HbA1c (%)	6.2 ± 1
Cholesterol (mg/dL)	196.2 ± 61.2
Triglyceride (mg/dL)	138.7 ± 62.2
HDL-C (mg/dL)	43.2 ± 9.2
LDL-C (mg/dL)	
LDL (D)	127.3 ± 53.48
LDL (F)	125.3 ± 53.52
LDL (M)	127.2 ± 53.51

No.: number of subjects; HbA1c: Hemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; LDL(D): directly measured LDL-C; LDL(F): LDL-C level estimated by Friedewald equation; LDL(M), LDL-C level estimated by the Martin equation.
Data are presented as No. (%) or mean ± SD.
HBA1C values were only measured in diabetic patients.
Conversion for cholesterol, HDL-C and LDL-C from mg/dL to SI (in mmol/L): multiply by 0.0259. Conversion for triglyceride from mg/dL to SI units (in mmol/L): multiply by 0.0113.

In diabetic patients, the LDL-C estimated by FE was systematically underestimated (Fig. 1). Bias was −3.9 ± 5.3 mg/dL (p = .04) and −0.3 ± 2.2 mg/dL (p = .09) in patients with or without diabetes respectively. LDL-C estimated by ME was not significantly different since the bias was −0.2 ± 1.3 mg/dL (p = .96) in patients with diabetes and −0.1 ± 0.5 mg/dL (p = .22) in patients without diabetes.

In diabetic group, 91 patients had HbA1c above 6.5% and were considered an uncontrolled patient group (7.3 ± 0.44). LDL (F) was underestimated when compared with LDL (D) and had bias of −4.6 ± 6.4 mg/dL (p = .042) and −3.6 ± 4.3 mg/dL (p = .56) in uncontrolled and controlled patients respectively. A non-significant p value of LDL (M) in both uncontrolled and controlled patients was detected. Bias was −0.3 ± 1.8 mg/dL (p = .54) and −0.1 ± 0.7 mg/dL (p = .13) respectively (Fig. 2). There was no statistically significant difference between triglyceride levels in controlled and uncontrolled patients (167.2 ± 65.1 and 155.7 ± 66.7 mg/dL respectively, p = .25). Moreover, no statistically significant difference between HDL-C levels in controlled and uncontrolled patients was detected (43.1 ± 12.2 and 43.8 ± 10.6 mg/dL respectively, p = .86).

ROC analysis determined the performance of LDL measurement with FE and ME in diabetic patients at cut-off point 100 mg/dL (Fig. 3). A higher AUC for LDL (M) [AUC: 0.992; 95%CI: 0.981–1.001] when compared to LDL (F) [AUC: 0.968; 95%CI: 0.946–0.990] was found (p < .0001). LDL (F) had lower sensitivity and specificity (80% and 88.9% respectively) in comparison with the LDL (M) (95.9% sensitivity, 95.6% specificity). The analysis at the cut-off point 70 mg/dL (the therapeutic target) LDL (F) had sensitivity and specificity (98.2% and 100%, respectively) while the LDL (M) had sensitivity and specificity both of 100%.

As both equations use triglyceride and HDL-C levels as variables, comparison of the means and SDs of differences in LDL estimations at different HDL-C and triglyceride values, which were categorized by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines, was presented in Table 2. When triglyceride increased, the difference of LDL estimated by FE tended to increase significantly (p < .0001), but ME was not influenced by triglyceride levels (p = .34). Both FE and ME were not influenced by HDL-C levels (p = .47 and p = .44, respectively).

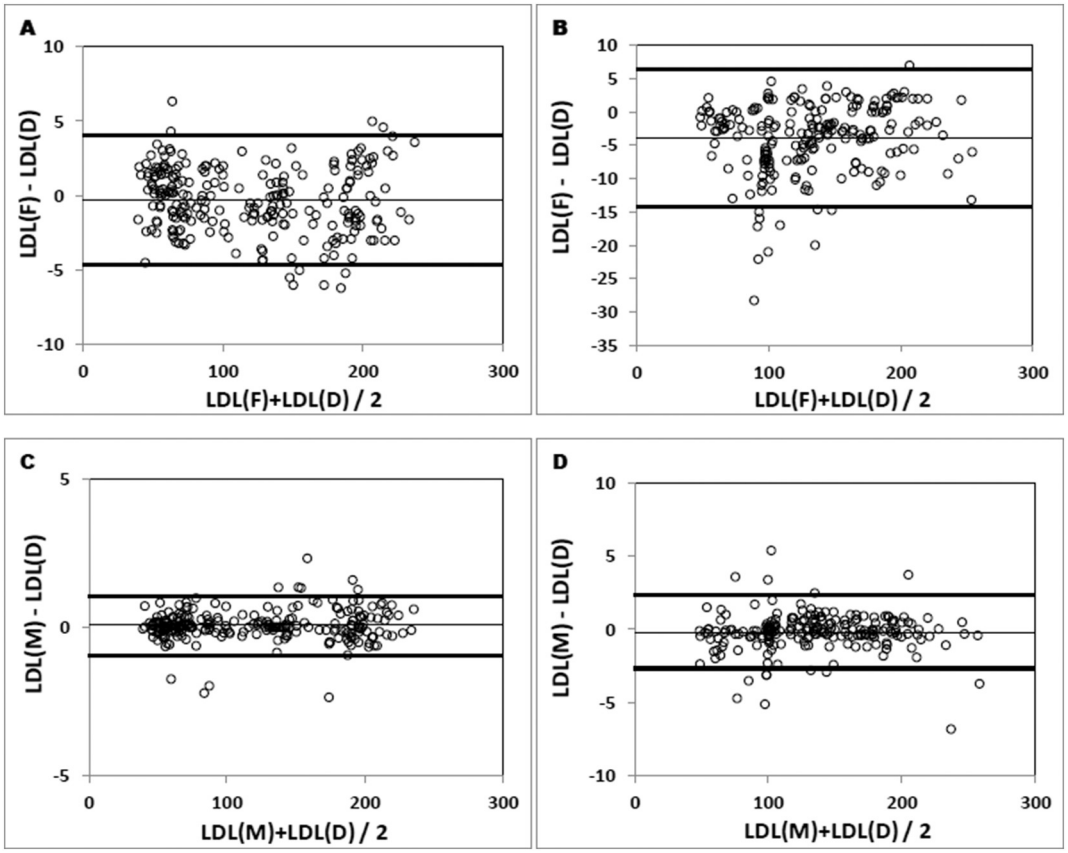

Fig. 1. Bland and Altmann plots of the average between measured and estimated LDL-C versus difference between them. (A) FE in non-diabetic patients (B) FE in diabetic patients (C) ME in non-diabetic patients (D) ME in diabetic patients.

4. Discussion

Coronary heart disease is a common chronic diabetes mellitus complication. DM had a 4-fold higher risk of mortality from heart disease [21]. Over decades, studies confirm the role of LDL-C as a risk of coronary heart disease [22–25]. The concentration of LDL-C helps in the assessment of cardiovascular risk and is confirmed as a target in the therapy decisions [26].

Although Beta-quantification by ultra-centrifugation is the golden standard reference method for measuring the LDL-C concentration, it has many limitations to be used in the routine laboratories. Beta-quantification is a laborious, time consuming and expensive technique [27]. So, LDL-C concentration is estimated through equations rather than direct measurements. FE is the most widely used equation for LDL-C concentration estimation in laboratory practice in spite of its limitations [28,29]. So, many researchers attempt to modify FE in order to have a more accurate formula [18,30,31]. Martin et al. [17] use adjustable novel factors instead of a fixed ratio of 5 in FE. This formula significantly improves the accuracy of LDL-C estimation [32]. The objective of this study is to evaluate the effectiveness of ME in Egyptian patients, especially those with type 2 diabetes.

In an unprecedented move, this study demonstrated the usefulness of ME for the Egyptian patients with and without diabetes. This study found that estimated LDL-C using FE mostly gives false lower LDL-C values in diabetic patients. This goes in agreement with previous studies which have detected the underestimation of LDL-C by FE in diabetic patients [13,33–35]. Rubies-Prat et al. [9] found that FE overestimate LDL-C levels in 39% of diabetic patients, while 13% of patients were underestimated. Differently, Whiting et al. [36] found that FE can accept accuracy in measurement of LDL-c in both diabetes mellitus types.

In this study, the researchers found that LDL-C estimated by ME was not significantly different from the one that was directly measured (in diabetic patients). Chaen et al. [37] showed a stronger correlation of LDL-C estimation by ME than that was shown by FE in diabetic patients.

Regarding HbA1c level, diabetic patients were rearranged into controlled and uncontrolled groups (HbA1c below and above 6.5% respectively). FE significantly underestimates LDL-C when compared with the direct method in uncontrolled patients. This confirms that diabetic patients with good control have a good correlation between FE estimated and directly measured LDL-C [34]. However, a non-significant difference in LDL-C between direct measured values and those estimated by ME in both uncontrolled patients and controlled patients was detected. So performing ME in all diabetic patients is acceptable.

This study showed that FE had lower sensitivity and specificity (80% and 88.9 respectively) in comparison with the ME (95.9% sensitivity, 95.6% specificity) at 100 mg/dL cutoff point. Razi et al. [38] found that FE at100 mg/dL level can misclassify diabetic patients and it is not recommended to not use FE for evaluation of risk in diabetic patients.

The Difference of LDL estimated by FE tended to increase significantly when triglyceride level increased. This limitation of FE was overcome by ME which was not influenced by triglyceride levels. In agreement with the previous studies, the researchers found LDL-C estimated by ME was less influenced by triglyceride than that was estimated by FE [16].

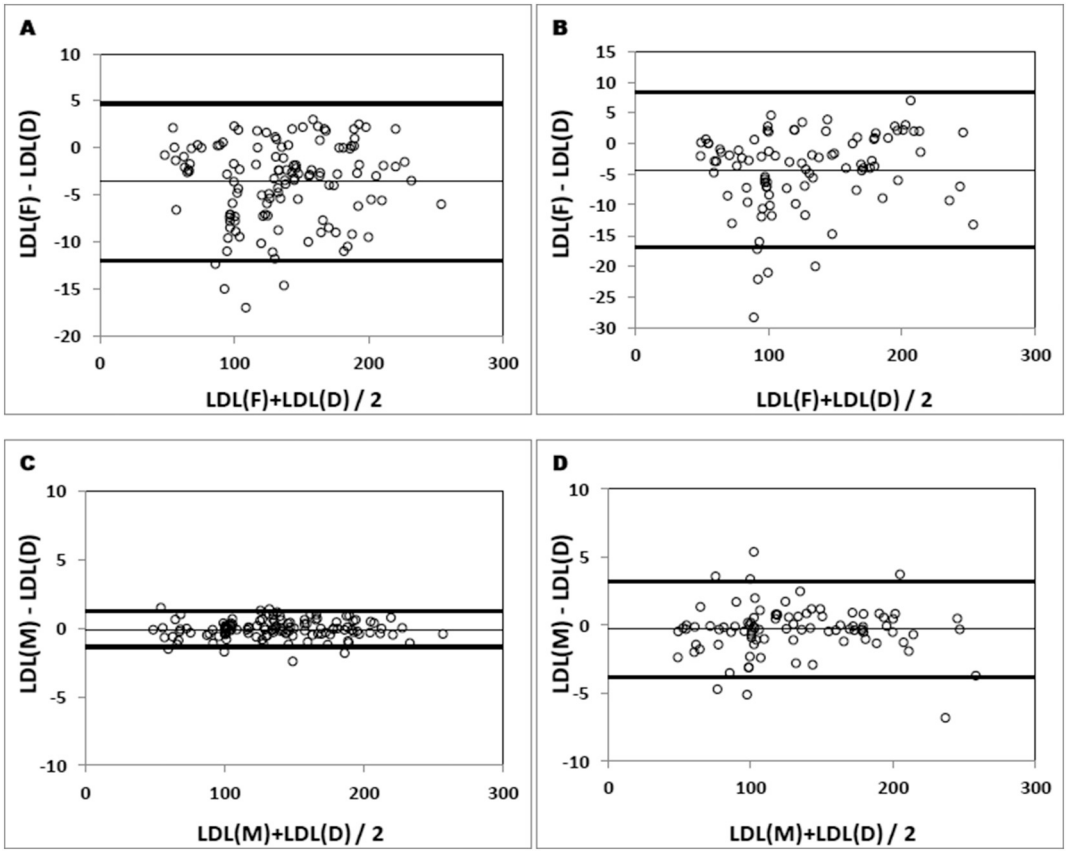

Fig. 2. Bland and Altmann plots of the average between measured and estimated LDL-C versus difference between them. (A) FE in controlled diabetic patients (B) FE in uncontrolled diabetic patients (C) ME in controlled diabetic patients (D) ME in uncontrolled diabetic patients.

This study limitation was the use of direct method (Roche) instead of beta quantification of LDL-C. However, Tanno et al. [39] recommended LDL-C measured by the direct assay in the epidemiological studies. Roche direct LDL-C measurement method is standardized against the reference method and Nauck et al. [40] reported that Roche assay meets the currently established analytical performance goals, so it is useful for both diagnosis and management decision in hyperlipidemic patients. Yamashita et al. [41] found that the selective solubilization method as used in Roche kit exhibited the highest correlation with the β-quantification method.

Further studies are recommended to evaluate a larger number of participants in multi-centers as ME can easily be incorporated in the laboratory data system with no additional cost. This also helps in the evaluation of ME validity in higher triglyceride levels (TG ≥ 400 mg/dl).

5. Conclusion

Compared to direct LDL-C assay, FE underestimates LDL-C levels in diabetic patients, especially in patients with high triglyceride levels and patients with uncontrolled diabetes. The ME improves concordance of calculated LDL-C with a direct LDL-C assay in Egyptian diabetic patients. So, ME is applicable in LDL-C estimation in order to assess the cardiovascular risk in type 2 diabetic patients.

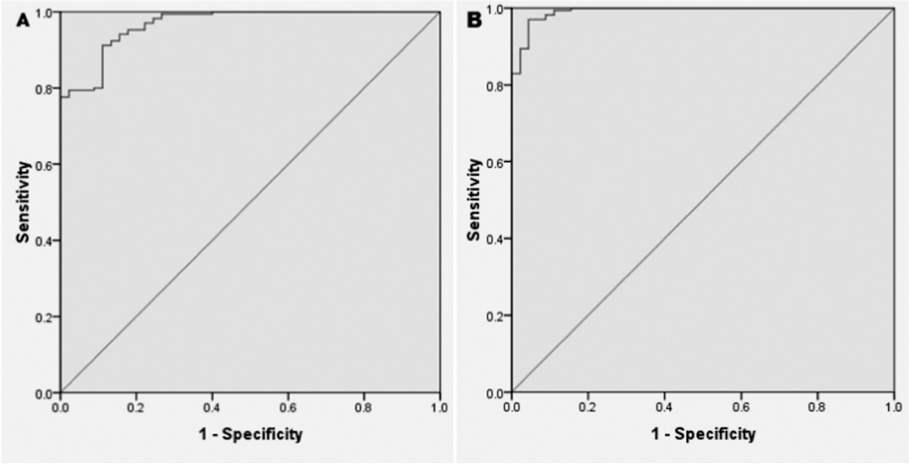

Fig. 3. ROC curves to validate estimated LDL-C from direct method in type 2 diabetes. (A) FE and (B) ME.

Table 2
Comparison of estimated LDL-C and directly-measured LDL-C by different triglyceride and HDL-C levels in diabetic patients.

	Triglyceride (mg/dL)			
	< 100 (No. = 44)	100–149 (No. = 49)	150–199 (No. = 55)	≥ 200 (No. = 67)
LDL (D) (mg/dL)	105.4 ± 46	129.3 ± 53.9	134.1 ± 30.6	157.6 ± 43.8
LDL (F) (mg/dL)	106.2 ± 47	128.1 ± 55.8	129.9 ± 32.9	148.8 ± 46.8
Difference*	1.5 ± 0.98	2.7 ± 2.14	4.4 ± 2.87	8.8 ± 5.37
LDL (M) (mg/dL)	105.1 ± 46	129.3 ± 54	134 ± 30.9	157.2 ± 43.5
Difference	0.6 ± 0.61	1 ± 1.24	0.7 ± 0.79	0.9 ± 1.17
	HDL-C (mg/dL)			
	< 40 (No. = 65)	40–49 (No. = 99)	50–59 (No. = 22)	≥60 (No. = 29)
LDL (D) (mg/dL)	146.2 ± 38.7	120.7 ± 45.8	130 ± 49.4	158 ± 55
LDL (F) (mg/dL)	141.7 ± 38.8	117.5 ± 46.3	124.5 ± 50.6	154.5 ± 58
Difference	4.84 ± 4.1	4.3 ± 4.1	5.8 ± 7.5	5.3 ± 3.6
LDL (M) (mg/dL)	146.2 ± 38.7	120.4 ± 45.8	129.6 ± 49.1	158.4 ± 54.9
Difference	0.66 ± 0.6	0.9 ± 1.2	0.7 ± 0.9	0.9 ± 1

No.: number of subjects; HDL-C: high-density lipoprotein cholesterol; LDL(D): directly measured LDL-C; LDL(F): LDL-C level estimated by Friedewald equation; LDL (M), LDL-C level estimated by the Martin equation.
Data are presented as mean ± SD; Difference expressed as absolute number.
Conversion for cholesterol, HDL-C and LDL-C from mg/dL to SI (in mmol/L): multiply by 0.0259. Conversion for triglyceride from mg/dL to SI units (in mmol/L): multiply by 0.0113.
* Significant using one-way ANOVA ($P < .0001$).

Declarations of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] B.V. Howard, Lipoprotein metabolism in diabetes mellitus, J. Lipid Res. 28 (6) (1987) 613–628 (PMID:3302085).
[2] S.M. Haffner, Management of dyslipidemia in adults with diabetes, Diabetes Care 21 (1998) 160–178 (PMID:9538988).
[3] M.K. Ali, K.M. Narayan, N. Tandon, Diabetes & coronary heart disease: current perspectives, Indian J. Med. Res. 132 (5) (2010) 584–597 (PMID: 21150011).
[4] W.C. Cromwell, J.D. Otvos, M.J. Keyes, M.J. Pencina, L. Sullivan, R.S. Vasan, P.W.F. Wilson, R.B. D'Agostino, LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study-Implications for LDL management, J. Clin. Lipidol. 1 (6) (2007) 583–592, https://doi.org/10.1016/j.jacl.2007.10.001.
[5] S.M. Boekholdt, G.K. Hovingh, S. Mora, B.J. Arsenault, P. Amarenco, T.R. Pedersen, J.C. Larosa, D.D. Waters, D.A. Demicco, R.J. Simes, A.C. Keech, D. Colquhoun, G.A. Hitman, D.J. Betteridge, M.B. Clearfield, J.R. Downs, H.M. Colhoun, A.M. Gotto, P.M. Ridker, S.M. Grundy, J.J.P. Kastelein, Very low levels of atherogenic lipoproteins and the risk for cardiovascular events: a meta-analysis of statin trials, J. Am. Coll. Cardiol. 64 (2014) 485–494, https://doi.org/10.1016/j.jacc.2014.02.615.
[6] P.B. Morris, C.M. Ballantyne, K.K. Birtcher, S.P. Dunn, E.M. Urbina, Review of clinical practice guidelines for the management of LDL-related risk, J. Am. Coll. Cardiol. 64 (2) (2014) 196–206, https://doi.org/10.1016/j.jacc.2014.05.015.
[7] N. Harris, E.J. Neufeld, J.W. Newburger, B. Ticho, A. Baker, G.S. Ginsburg, E. Rimm, N. Rifai, Analytical performance and clinical utility of a direct LDL-cholesterol assay in a hyperlipidemic pediatric population, Clin. Chem. 42 (8) (1996) 1182–1188 (PMID: 8697574).
[8] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972) 499–502 (PMID:4337382).
[9] J. Rubies-Prat, J.L. Reverter, M. Sentí, J. Pedro-Botet, I. Salinas, A. Lucas, X. Nogues, A. Sanmarti, Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus, Diabetes Care 16 (8) (1993) 1081–1086 (PMID:8375237).
[10] C. Matas, M. Cabre, A. La Ville, E. Prats, J. Joven, P.R. Turner, L. Masana, J. Camps, Limitations of the Friedewald formula for estimating low-density lipoprotein cholesterol in alcoholics with liver disease, Clin. Chem. 40 (1994) 404–406 (PMID: 8131276).
[11] R. Johnson, P. McNutt, S. MacMahon, R. Robson, Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis, Clin. Chem. 43 (11) (1997) 2183–2184 (PMID:9365406).
[12] I. Gazi, V. Tsimihodimos, T.D. Filippatos, V.G. Saougos, E.T. Bairaktari, A.D. Tselepis, M. Elisaf, LDL cholesterol estimation in patients with the metabolic syndrome, Lipids Health Dis. 5 (2006) 8, https://doi.org/10.1186/1476-511X-5-8.
[13] L. Sibal, R.D. Neely, A. Jones, P.D. Home, Friedewald equation underestimates low-density lipoprotein cholesterol at low concentrations in young people with and without Type 1 diabetes, Diabet. Med. 27 (1) (2010) 37–45, https://doi.org/10.1111/j.1464-5491.2009.02888.x.
[14] D. Sidhu, C. Naugler, Fasting time and lipid levels in a community-based population: a cross-sectional study, Arch. Intern. Med. 172 (1707) (2012) 1710, https://doi.org/10.1001/archinternmed.2012.3708.
[15] H. Scharnagl, M. Nauck, H. Wieland, W. Marz, The Friedewald formula underestimates LDL-C at low concentrations, Clin. Chem. Lab. Med. 39 (2001) 426–431, https://doi.org/10.1515/CCLM.2001.068.
[16] K.R. Jun, H.I. Park, S. Chun, H. Park, W.K. Min, Effects of total cholesterol and triglyceride on the percentage difference between the LDL-C concentration measured directly and calculated using the Friedewald formula, Clin. Chem. Lab. Med. 46 (2008) 371–375, https://doi.org/10.1515/CCLM.2008.064.
[17] M. Kang, J. Kim, S.Y. Lee, K. Kim, J. Yoon, H. Ki, Martin's equation as the most suitable method for estimation of low-density lipoprotein cholesterol levels in Korean adults, Korean J. Fam. Med. 38 (2017) 263–269, https://doi.org/10.4082/kjfm.2017.38.5.263.
[18] S.S. Martin, M.J. Blaha, M.B. Elshazly, P.P. Toth, P.O. Kwiterovich, R.S. Blumenthal, S.R. Jones, Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile, JAMA J. Am. Med. Assoc. 310 (2013) 2061–2068, https://doi.org/10.1001/jama.2013.280532.
[19] C.M.M. Cordova, M. Cordova, A new accurate, simple formula for LDL cholesterol estimation based on directly measured blood lipids from a large cohort, Ann. Clin. Biochem. 50 (1) (2013) 13–19, https://doi.org/10.1258/acb.2012.011259.
[20] J.W. Meeusen, A.J. Lueke, A.S. Jaffe, A.K. Saenger, Validation of a proposed novel equation for estimating LDL cholesterol, Clin. Chem. 60 (12) (2014) 1519–1523, https://doi.org/10.1373/clinchem.2014.227710.
[21] M.C. Bertoluci, V.Z. Rocha, Cardiovascular risk assessment in patients with diabetes, Diabetol. Metab. Syndr. 9 (2017) 25, https://doi.org/10.1186/s13098-017-0225-1.
[22] W.B. Kanne, W.P. Castelli, T. Gordon, P.M. Mcnamara, Serum cholesterol, lipoproteins, and the risk of coronary heart disease: the Framingham study, Ann. Intern. Med. 74 (1) (1971) 1–12, https://doi.org/10.7326/0003-4819-74-1-1.
[23] C.M. Ballantyne, Low-density lipoproteins and risk for coronary artery disease, Am. J. Cardiol. 82 (8) (1998) 3–12, https://doi.org/10.1016/S0002-9149(98)00769-3.
[24] H. Imano, H. Noda, A. Kitamura, S. Sato, M. Kiyama, T. Sankai, T. Ohira, M. Nakamura, K. Yamagishi, A. Ikeda, T. Shimamoto, H. Iso, Low-density lipoprotein cholesterol and risk of coronary heart disease among Japanese men and women: the Circulatory Risk in Communities Study (CIRCS), Prev. Med. 52 (5) (2011) 381–386, https://doi.org/10.1016/j.ypmed.2011.02.019.
[25] P. Ueda, P. Gulayin, G. Danaei, Long-term moderately elevated LDL-cholesterol and blood pressure and risk of coronary heart disease, PLoS One 13 (7) (2018) e0200017, , https://doi.org/10.1371/journal.pone.0200017.
[26] M.G. Silverman, B.A. Ference, K. Im, S.D. Wiviott, R.P. Giugliano, S.M. Grundy, E. Braunwald, M.S. Sabatine, Association between lowering LDL-C and

cardiovascular risk reduction among different therapeutic interventions: a systematic review and meta-analysis, JAMA J. Am. Med. Assoc. 316 (12) (2016) 1289–1297, https://doi.org/10.1001/jama.2016.13985.
[27] S. Gupta, M. Verma, K. Singh, Does LDL-C estimation using Anandaraja's formula give a better agreement with direct LDL-C estimation than the Friedewald's formula? Indian J. Clin. Biochem. 27 (2) (2012) 127–133, https://doi.org/10.1007/s12291-011-0186-3.
[28] M.J.A. Oliveira, H.E. van Deventer, L.M. Bachmann, G.R. Warnick, K. Nakajima, M. Nakamura, I. Sakurabayashi, M.M. Kimberly, R.D. Shamburek, W.J. Korzun, G.L. Myers, W.G. Miller, A.T. Remaley, Evaluation of four different equations for calculating LDL-C with eight different direct HDL-C assays, Clin. Chim. Acta 423 (2003) 135–140, https://doi.org/10.1016/j.cca.2013.04.009.
[29] J. Martins, S.A.S. Olorunju, L.M. Murray, T.S. Pillay, Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients, Clin. Chim. Acta 444 (2015) 137–142, https://doi.org/10.1016/j.cca.2015.01.037.
[30] S. Anandaraja, R. Narang, R. Godeswar, R. Laksmy, K. Talwar, Low-density lipoprotein cholesterol estimation by a new formula in Indian population, Int. J. Cardiol. 102 (1) (2005) 117–120, https://doi.org/10.1016/j.ijcard.2004.05.009.
[31] Y. Chen, X. Zhang, B. Pan, X. Jin, H. Yao, B. Chen, Y. Zou, J. Ge, H. Chen, A modified formula for calculating low-density lipoprotein cholesterol values, Lipids Health Dis. 9 (2010) 52, https://doi.org/10.1186/1476-511X-9-52.
[32] R. Mehta, E. Reyes-Rodríguez, O. Yaxmehen Bello-Chavolla, A.C. Guerrero-Díaz, A. Vargas-Vázquez, I. Cruz-Bautista, C.A. Aguilar-Salinas, Performance of LDL-C calculated with Martin's formula compared to the Friedewald equation in familial combined hyperlipidemia, Atherosclerosis (2018), https://doi.org/10.1016/j.atherosclerosis.2018.06.868.
[33] S. Hirany, D. Li, I. Jialal, A more valid measurement of low-density lipoprotein cholesterol in diabetic patients, Am. J. Med. 102 (1) (1997) 48–53, https://doi.org/10.1016/S0002-9343(96)00375-0.
[34] A.M. Wagner, J.L. Sanchez-Quesada, A. Perez, M. Rigla, M. Cortes, F. Blanco-Vaca, J. Ordonez-Llanos, Inaccuracy of calculated LDL-cholesterol in type 2 diabetes: consequences for patient risk classification and therapeutic decisions, Clin. Chem. 46 (11) (2000) 1830–1832 (PMID:11067819).
[35] P.L. Vieira, G.N. Araújo, G.H. Telo, L.F.S. Smidt, M.F. Jost, M.V. Furtado, E.H. Moriguchi, C.A. Polanczyk, Low-density lipoprotein values estimated by Friedewald equation are affected by diabetes control, Int. J. Cardiovasc. Sci. 29 (5) (2016) 348–354, https://doi.org/10.5935/2359-4802.20160045.
[36] M.J. Whiting, M.D. Shephard, G.A. Tallis, Measurement of plasma cholesterol LDL in patients with diabetes, Diabetes Care 20 (1) (1997) 12–14 (PMID: 9028686).
[37] H. Chaen, S. Kinchiku, M. Miyata, S. Kajiya, H. Uenomachi, T. Yuasa, K. Takasaki, M. Ohishi, Validity of a novel method for estimation of low-density lipoprotein cholesterol levels in diabetic patients, J. Atheroscler. Thromb. 23 (12) (2016) 1355–1364, https://doi.org/10.5551/jat.35972.
[38] F. Razi, K. Forouzanfar, F. Bandarian, E. Nasli-Esfahani, LDL-cholesterol measurement in diabetic type 2 patients: a comparison between direct assay and popular equations, J. Diab. Metabol. Disord. 16 (2017) 43, , https://doi.org/10.1186/s40200-017-0326-2.
[39] K. Tanno, T. Okamura, M. Ohsawa, T. Onoda, K. Itai, K. Sakata, M. Nakamura, A. Ogawa, K. Kawamura, A. Okayama, Comparison of low-density lipoprotein cholesterol concentrations measured by a direct homogeneous assay and by the Friedewald formula in a large community population, Clin. Chim. Acta 411 (21 – 22) (2010) 1774–1780, https://doi.org/10.1016/j.cca.2010.07.034.
[40] M. Nauck, M.S. Graziani, D. Bruton, C. Cobbaert, T.G. Cole, F. Lefevre, W. Riesen, P.S. Bachorik, N. Rifai, Analytical and clinical performance of a detergent-based homogeneous LDL-cholesterol assay: a Multicenter evaluation, Clin. Chem. 46 (4) (2010) 506–514 (PMID:10759474).
[41] S. Yamashita, R. Kawase, H. Nakaoka, K. Nakatani, M. Inagaki, M. Yuasa-Kawase, K. Tsubakio-Yamamoto, J.C. Sandoval, D. Masuda, T. Ohama, Y. Nakagawa-Toyama, A. Matsuyama, M. Nishida, M. Ishigami, Differential reactivities of four homogeneous assays for LDL-cholesterol in serum to intermediate-density lipoproteins and small dense LDL: comparisons with the Friedewald equation, Clin. Chim. Acta 410 (1–2) (2009) 31–38.