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Original article

Evaluation of Different Formulas for Calculation of LDL Cholesterol in Northern Indian Population

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ABSTRACT

Background

LDL-cholesterol (LDLC) is implicated as one of major risk factor in the development of coronary heart disease and basis for diagnosis, treatment and risk classification. Estimation of LDLC with accuracy and precision is of paramount importance. LDLC is calculated by different formulas in different population like Friedewald, de Cordova and de Cordova, Ahmadi et al., Hattori et al., Anandaraja et al., Vujovic et al., Puavilai and Chen et al., Teerakanchana et al., Delong et al, Rao et al noting each formulas limitation. The present study was designed to evaluate the comparison of LDL cholesterol with different reported formula and its correlation with FF commonly used.

Material and Methods

This was a retrospective study which included subject's male and female adults aged 18–50 years. Dyslipidemic, obese, hypothyroid, nephrotic syndrome, diabetes mellitus, pregnant women were excluded. Spearman test was used to see correlation, Bland–Altman plots were used to demonstrate bias. The level of statistical significance was established at $p < 0.05$.

Results

The study included 830 subjects. Study showed positive correlations between dLDLC and all calculated LDLC, but Teerkanchana formula showed the best correlation $r = 0.92$, $p< 0.001$ for all TG levels. Ahmadi's equation had the least correlation $r = 0.49$, $p< 0.001$ for TG 150-300mg/dL, but when applied to samples with TG < 150 mg/dL, the correlation showed a strong, positive relationship, $r = 0.92$, $p < 0.001$ and rest equations were comparable.

For TG values <150mg/dL on Bland Altmann analysis Delong, Rao, Ahmadi, Vujovic showed negative bias least with Delong and positive bias with Teerakanchana, Puavilai, AF, Chen, Cordova, Hattori, FF least with Teerakanchana. For TG values 150-300 mg/dL all formulas showed positive bias least with teerakanchana whereas Ahmadi et al showed very high bias.

Conclusion

Teerakanchana's LDLC showed good correlation with minimal bias. No reliable calculation of LDL cholesterol and varied results in different population. The LDL -C formulas should be devised according to the population and used with other indices like total/HDL cholesterol ratio along with clinical findings to provide better patient care without affecting diagnosis and management.

INTRODUCTION

LDL-cholesterol (LDLc) is implicated as one of major risk factor in the development of coronary heart disease. It is the primary basis for diagnosis, treatment and risk classification of patients with hyperlipidaemia [1,2]. Estimation of LDLc with accuracy and precision is of paramount importance in coronary heart disease. Reference method for estimation of serum LDLc is by β-quantitation procedure (BQ) [3] by ultracentrifugation technique. However, the procedure is time consuming, expensive, requires large volume of serum and is not available in routine laboratories. The two commonly used methods used in clinical laboratories for quantification of LDLc by Friedewald's formula and by direct homogeneous assays for LDLc measurement.

LDL-c estimation was developed by Friedewald and colleagues in 1972 using data from 448 individuals suffering from known inaccuracies at extremes of triglyceride (TG)

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and total cholesterol (TC) values.[4] Cordova formula [LDL-c = 3/4 (TC - HDL-c)] was recommended for use in non-fasting specimen which was validated in large number (10664) of Brazilian individuals with wide range of TC, HDLc and TG levels. However, it did not perform better than Friedewald's formula in healthy south African population and warranted validation in other populations [5]. Similarly, Chen formula was validated in 2180 Chinese subjects, which correlated well with directly measured LDLc even in TG concentration greater than 400mg/dl overcoming disadvantage of FF. The concentrations of LDL-C were measured or estimated by formula LDL-C (mg/dl) = Non-HDL-C x 90% - TG x 10%.[6] Further Vujovic et al formulated LDLc=TC-TG/6.85-HDLc which was Validated in 1043 serbian patients proving better than Friedewald's and Anandaraja's formulas. But it needed to be validated in other population as well as in specimens with TG>400mg/dl [7]. None the less, Ahmadi et al provided LDLc=TC/1.19+TG/1.9-HDLc/1.1. which was validated in Iranian population, which performed well with low TG specimens [8]. However, Onyenekwu et al. [9] found that Friedewald formula performed better than this formula at very low TG levels in healthy South African population. Anandaraja et al. [10] devised LDLc = 0.9TC -0.9 TG/5 -28 in this formula only TC and TG were used. However sera having TG > 350mg/dl was excluded. HDLc value not needed (economical), validated in 1008 Indian subjects, also validated in Brazilian and Greek population, low total error due to omission of HDLc. Not worked better than Friedewald's equation in another Indian study [11]. However, Shalini et al. [11] reported that Friedewald's formula was better in agreement with measured LDLc (Direct homogeneous method) than Anandaraja's formula in Indian subjects. Interestingly, this new formula was found to be working well in Brazilian [12] and Greek population [13]. But most recently Cordova and Cordova [16] found that their formula (LDLc = ¾ (TC – HDLc) out performed Anandaraja's formula in Brazilian population. Puavilai et al. [14] validated formula of LDLc = TC – HDLc – TG/6(mg/dl) in 1079 fasting samples, which worked better than Friedewald's formula when TG was > 200 mg/dl (200-499) and needed validated in other populations. However, authors suggested to do direct LDLc in patients with hypertriglyceridemia in the treatment of LDLc in high-risk cardiovascular disease.

Hattori et al. [15] tried the formula LDLc = 0.94TC - 0.94HDLc - 0.19TG (mg/dl) seemed better correlated with Ultracentrifugation data from 2179 Japanese subject but needs to be validated in other population. Delong et al [16]LDL-C = TC – HDL-C – (0.16 × TG) prepared data from over 10000 men, women, and children who participated in the Lipid Research Clinics prevalence studies. The proposed formula was more precise for plasmas or sera with a TG concentration within the normal range. Teerakanchana et al [17] LDL-C = (0.91 × TC) – (0.634 × HDL-C) – (0.111 × TG) – 6.755 performed in 1,016 Thai patients. Patients' ages ranged 8-89 years, 573 (56.4%) were females. Upon comparing the two methods, at TG levels of 151-200 mg/dl, bias was 18.3 mg/dl; and for TG levels of 201-300 mg/dl, bias was lower at 11.4 mg/dl; for TG levels of 301-400 mg/dl, bias increased to 20.9 mg/dl. Rao et al [18] [(4.7 × TC) – (4.364 × HDL-C) –TG]/4.487 Low-density lipoprotein cholesterol was calculated with a formula that utilizes the triglyceride/cholesterol ratios in the different lipoprotein fractions, and also with different variations of the Friedewald formula. Results of the former calculation correlated well with the ultracentrifugation-derived values and performed better than the other calculations at different lipid concentrations [19-21].

The present study was designed to evaluate the comparison of LDL cholesterol with different reported formula and its correlation with direct LDL cholesterol.

MATERIAL AND METHODS

Study design

This was a retrospective study of the serum lipid profile results of patient attending OPDs for 1 year.

Study population

This study was conducted at the outpatient clinic of tertiary health institution in the New Delhi. Subjects included were male and female adults aged 18–60 years. Children and patients with secondary causes of dyslipidaemia, for example, obesity, hypothyroidism, nephrotic syndrome, Type II diabetes mellitus, pregnant women, and nursing mothers were excluded from the study. Confidentiality of the patient information was maintained.

Data collection

The medical records of the subjects were reviewed to obtain their lipid profile results, demographics, clinical, and medication history. As per the standard operating procedure of the laboratory, serum samples for lipid profile had been obtained from the subjects after an overnight 10–12 h fast. The samples were allowed to clot and retract before centrifuging at 4000 rpm for 10 min to obtain the serum. Sera were analyzed daily using enzymatic methods for TC, HDL-C, and TG, while LDLC was determined by a direct, homogeneous assay,[18] on a ADVIA 2400 Autoanalyzer.

Data were entered onto a Microsoft office Excel. The lipid results were calculated in mg/dl and LDLC was calculated using each of the formulas stated by Friedewald,[7] de Cordova and de Cordova,[19] Ahmadi et al.,[20] Hattori et al.,[21] et al.,[22] Anandaraja et al.,[23] Vujovic et al.,[24] Puavilai and Chen et al.,[26] and Teerakanchana et al.[27], Delonge etal, Rao etal noting each formulas limitation.

Statistical analysis

Statistical analyses were performed using the Graph pad prism v7. Kolmogorov– Smirnov test was used to check normality of the data and descriptive statistics were presented as mean and standard deviation (SD). Student's t-test was used to compare the means of dLDLC and each calculated LDLC. Pearson's correlation was used to determine the relationship, while Bland–Altman plots were used to demonstrate bias graphically. The level of statistical significance was established at $P < 0.05$.

RESULTS

The study population comprised of 830 subjects 530 males and 300 females with mean ± SD age of 41.6 ± 8.1 years and 44.1 ± 11.5 years, respectively. 595 subjects had TG<150mg/dL, TG(150-300mg/dL) in 235 subjects. The mean levels of LDL derived by standra formulas are defined in Table 1a(TG<150mg/dL), Table1b(TG150-300 mg/dL).

Table 1a: Summary statistics of LDL with different formula in TG<150mg/dL

Sno	N=595	Mean	Median	SD	25 - 75 P
1	LDL	93.64	93.0	32.6164	70.0 to 115.0
2	Friedewald_LDL	89.9	90.5	31.5878	68.10to 110.0
3	Ananadaraja	90.3	89.7	30.7363	68.2 to 109.2
4	Vujovic	94.4	93.46	29.8345	73.1 to 113.2
5	Chen	88.6	87.0	27.2756	69.5 to 105.4
6	Cordova	82.1	81.1	23.8814	65.0 to 97.7
7	Hattori	84.2	83.8	27.5510	64.2 to 101.7
8	Teerkanchana	93.6	92.68	34.3	72.2 to 110.7
9	Puavilai	93.2	92	37.68	70.4 to 111.8
10	Rao	96.06	95.3	39.00	73.3 to 115.1
11	Ahmadi	102.6	74.87	40.89	103.5 to 128
12	De long	93.86	92.64	37.73	70.9 to 112.5

Table 1b: Summary statistics of LDL with different formula in TG>150mg/dL

		Mean	Median	SD	25 - 75 P
Sno	N=235				
1	LDL	115.1	112.9	42.6	81.1 to 141.5
2	Friedewald	102.7	99.6	43.3	74.5 to 125.0
3	Anandaraja	99.5	95.3	50.3	68.21to 122.5
4	Vujovic	112.4	109.2	41.8	85.9 to 133.0
5	Chen	109.8	106.5	37.1	86.0 to 127.3
6	Cordova	112.3	108.0	38.1	90.6 to 127.4
7	Hattori	96.9	92.9	47.9	69.2 to 114.9
8	Teerkanchana	113.5	87.27	41.3	109.2 to 134.6
9	Puavilai	110.4	82.48	44.3	106.8 to 132.7
10	Rao	107.3	76.12	47.6	103.5 to 132.6
11	Ahmadi	206.7	165.9	65.1	194.9 to 232.5
12	De long	112.0	84.32	44.2	108.0 to 134

Our study showed strong, positive correlations between dLDLC and all calculated LDLC, but Teerkanchana formula showed the best correlation r = 0.92, 0.95, P < 0.001 for all TG levels <150 and TG150-300mg/dL [Table2a, 2b]. Ahmadi's equation had the least correlation r = 0.49, P < 0.001for TG 150-300mg/dL, but when applied to samples with TG < 150 mg/dL, the correlation showed a strong, positive relationship, r = 0.92, P < 0.001, whereas rest of the equations were comparable at both levels of TG [Table2a, 2b].

Table 2a: Spearman Correlation data of LDL with different formula in TG<150mg/dL

N=595	Friedewald	Anandaraja	Vujovic	Chen	Cordova	Hattori
LDL Correlation coefficient	0.91	0.91	0.935	0.90	0.89	0.90
Significance Level P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2b: Spearman Correlation data of LDL with different formula in TG 150-300 mg/dL

N=595	Teerakanchana	Puavilai	Rao	Ahmadi	De long
LDL Correlation coefficient	0.956	0.88	0.89	0.83	0.88
Significance Level P	<0.05	<0.05	<0.05	<0.05	<0.05

N=235	FFLDL	AF	Vujovic	Chen	Cordova	Hattori
LDL Correlation coefficient	0.91	0.915	0.939	0.923	0.902	0.91
Significance Level P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

For TG values <150mg/dL on Bland Altmann analysis Delong, Rao, Ahmadi, Vujovic showed negative bias least

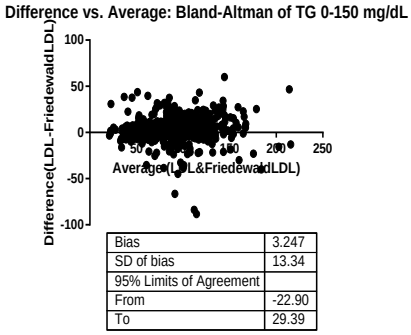
N=235	Teerakanchana	Puavilai	Rao	Ahmadi	De long
LDL Correlation coefficient	0.95	0.94	0.94	0.49	0.942
Significance Level P	<0.05	<0.05	<0.05	<0.05	<0.05

with Delong (-0.1) and positive bias with Teerakanchana, Puavilai, Ananadaraja, Chen, Cordova, Hattori, FF least with Teerakanchana(0.128). [Table 3a & Figure 3a].

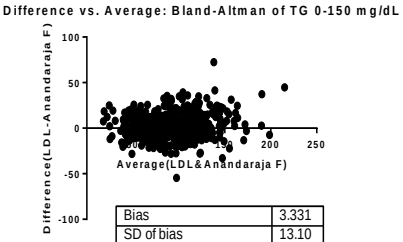
Table 3a Bland Altman analysis of LDL direct and different formulas at TG <150mg/dL

1	Friedewald_LDL	5	Cordova	9	Rao
2	Ananadaraja	6	Hattori	10	Ahmadi
3	Vujovic	7	Teerkanchana	11	De long
4	Chen	8	Puavilai		

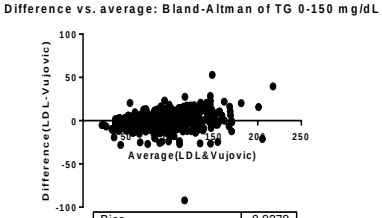
Figure 3a: Difference vs average: Bland-Altman of TG <150 mg/dl for different formulae.



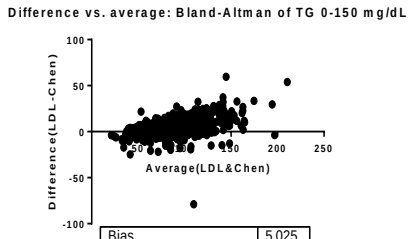
Bias	3.247
SD of bias	13.34
95% Limits of Agreement	
From	-22.90
To	29.39



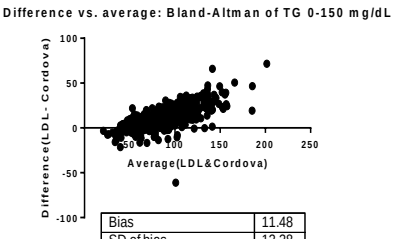
Bias	3.331
SD of bias	13.10
95% Limits of Agreement	
From	-22.35
To	29.02



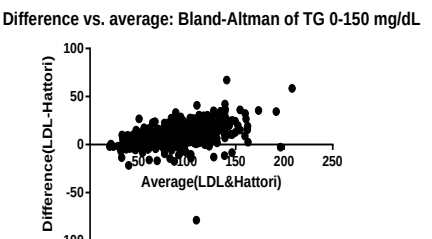
Bias	-0.8278
SD of bias	9.814
95% Limits of Agreement	
From	-20.06
To	18.41



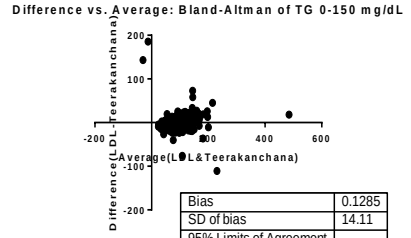
Bias	5.025
SD of bias	10.39
95% Limits of Agreement	
From	-15.34
To	25.39



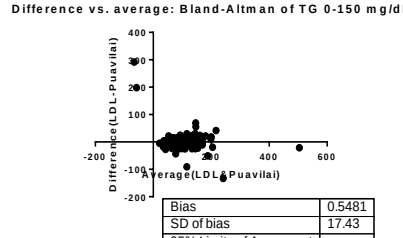
Bias	11.48
SD of bias	12.28
95% Limits of Agreement	
From	-12.60
To	35.55



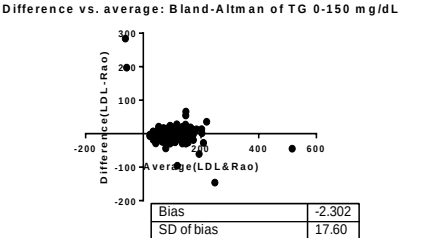
Bias	9.448
SD of bias	10.61
95% Limits of Agreement	
From	-11.36
To	30.25



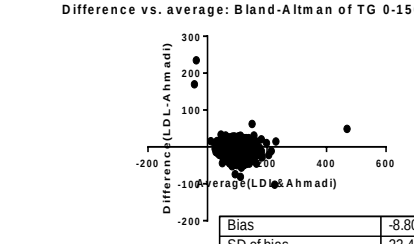
Bias	0.1285
SD of bias	14.11
95% Limits of Agreement	
From	-27.52
To	27.78



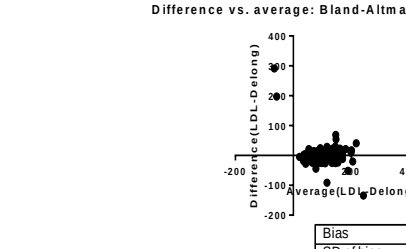
Bias	0.5481
SD of bias	17.43
95% Limits of Agreement	
From	-33.61
To	34.71



Bias	-2.302
SD of bias	17.60
95% Limits of Agreement	
From	-36.80
To	32.19



Bias	-8.809
SD of bias	22.45
95% Limits of Agreement	
From	-52.81
To	35.19



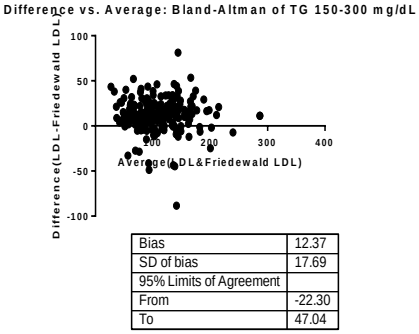
Bias	-0.1034
SD of bias	17.40
95% Limits of Agreement	
From	-34.21
To	34.01

For TG values 150-300 mg/dL on Bland Altmann analysis all formulas showed positive bias least with Teerakanchana (1.9) whereas Ahmadi et al showed very high bias(45.5) .[Table 3b & Figure 3b].

Table 3b: Bland Altman analysis of LDL direct and different formulas at TG 150-300mg/dL

1	Friedewald_LDL	5	Cordova	9	Rao
2	Ananadaraja	6	Hattori	10	Ahmadi
3	Vujovic	7	Teerkanchana	11	De long
4	Chen	8	Puavilai		

Figure 3b: Difference vs average: Bland-Altman of TG levels of 150 -300 mg/dL for different formulae.



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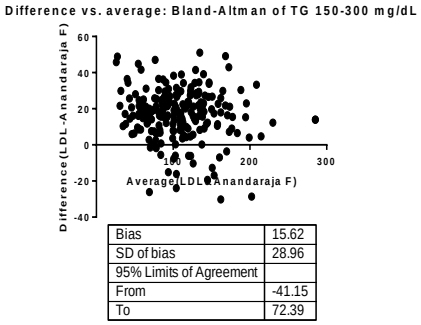

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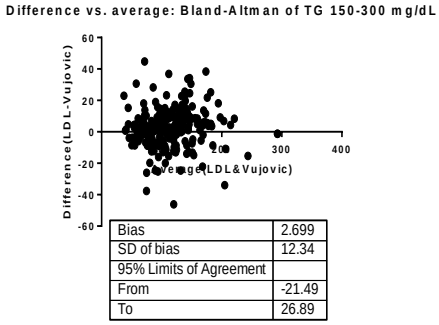

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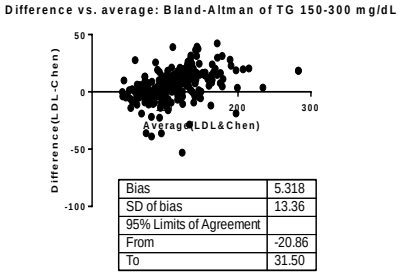

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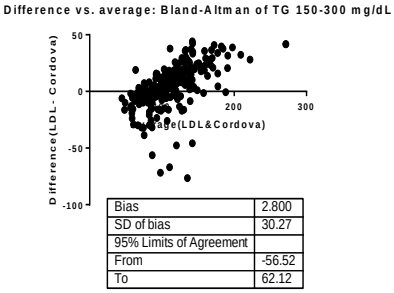

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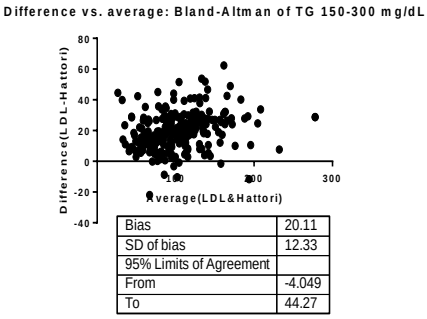

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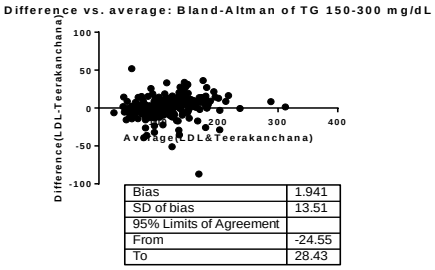

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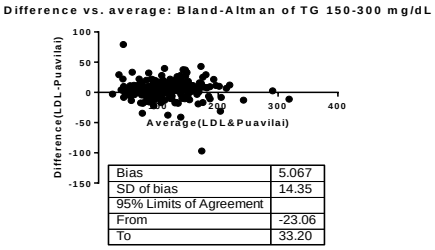

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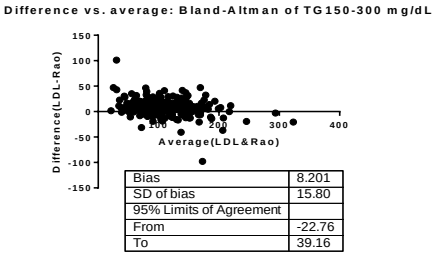

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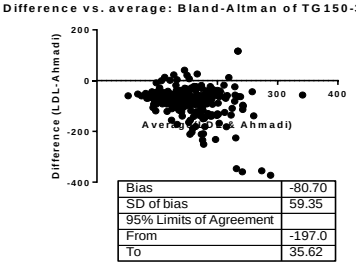

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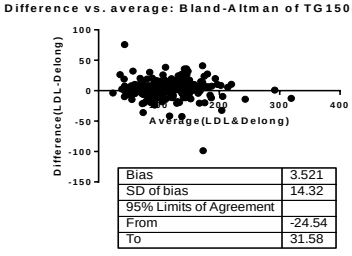

11

DISCUSSION

Homogeneous assays for the direct measurement of LDLC have been critically assessed against to succeeded in attaining the NCEP requirements for LDLC testing which include: Imprecision less than 4%, inaccuracy less than 4% and total allowable error not exceeding ±12%.[22,23]

Friedewald's equation is the most widely used formula in estimating LDLC, but it cannot be applied when TG >400 mg/dl, in Type II diabetics,[25] hepatic failure,[26] and end-stage renal disease patients,[27] as well as HIV-positive because they are characterized by hypertriglyceridemia. [28,29] Further different formulas are available for LDL c calculation. The present study the association of different formulas with direct LDL-C calculation was observed.

LDL-C was overestimated by Vujovic, Ahmadi, Rao, Delong in TG <150 mg/dL whereas in TG>150mg/dL Ahmadi equation overestimated LDL concentrations. Our study showed strong, positive correlations between dLDLC and all calculated LDLC, but Teerkanchana formula showed the best correlation. Ahmadi's equation had the least correlation for TG 150-300mg/dL, but when applied to samples with TG < 150 mg/dL, the correlation showed a strong, positive relationship, whereas rest of the equations were comparable at both levels of TG.

For TG values <150mg/dL on Bland Altmann analysis Delong, Rao, Ahmadi, Vujovic showed negative bias least with Delong and positive bias with Teerakanchana, Puavilai, Ananadaraja, Chen, Cordova, Hattori, FF least with Teerakanchana.

For TG values 150-300 mg/dL on Bland Altmann analysis all formulas showed positive bias least with Teerakanchana whereas Ahmadi et al showed very high bias.

A number of studies have studied the impact of TG on the FF. These studies suggest LDL may be underestimated by the FF at low LDL levels and higher TG levels. [30,31,32] Limited study results from India have reached discordant conclusions on this topic. A study by Sahu et al.,[33] noted that the mean LDL calculated by FF was significantly higher than the direct LDL measurement at TG between 1 and 300 mg/dl. However, the study by Gupta et al.,[7] reported underestimation of LDL by FF at all levels of TG (ranging from 45 to 635 mg/dl). LDL was measured using direct homogenous assay in both the above studies. Anandaraja et al.,[10] noted that FF overestimated LDL in subjects with TG <350 mg/dl.

LDL by FF may underestimate or overestimate depending on the TG levels [34]. Vujovic formula appeared to be more accurate than any other formula when applied to Indian population.[35] It was also noted that Chen's equation had the best prediction and Ahmadi's equation performed the poorest.[36]. Some studies showed Frieldwald formula as the best one, some others showed that other formulas are more accurate. An important part of discrepancies in results of multiple studies accounted by probable systematic and random errors in measuring TC, TG, HDL-C, and LDL C concentrations or mode of judgement and interpretation of results of statistical analysis. [37,38]

CONCLUSION

Teerakanchana's LDLC showed good correlation with minimal bias, and the linear regression showed no difference between the two methods. Only when TG <300 mg/dL is Ahmadi's equation recommended.

This study had limitations by the retrospective study design as it was difficult to assess patients' preparation, specimen collection, and analysis. However standard operating procedures utilized by the laboratory and quality control records of their analytical system was maintained.

The total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the LDL/HDL cholesterol ratio are also important indicators of vascular risk, the predictive value of which is greater than the isolated parameters [37].

Morever, it is clear that when there was no reliable calculation of LDL cholesterol particularly when TG concentration greater than 150 mg/dL and varied results in different population. The LDL -C formulas should be devised according to the population and other indices along with clinical findings may be synchronously used to provide the best management for patient care. It is preferable to use ratios such as total/HDL cholesterol ratio alongwith LDL-C and direct method in critical setting to provide better patient care without affecting diagnosis and management.

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