

Original Article

Martin's formula is best to calculate low-density lipoprotein cholesterol

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ABSTRACT

Objectives: Due to the cost-effectiveness, most of the laboratories in India estimate low-density lipoprotein cholesterol (LDL-C) levels with the Friedewald's formula. There were many shortcomings of the Friedewald's formula. Recently, Martins have derived a new formula for calculating LDL-C. The present study was undertaken to calculate LDL-C using various formulae (Friedewald's formula, Anandaraja's formula, and Martin's formula) and to compare directly measured LDL-C with calculated LDL-C at various ranges of triglyceride (TG) concentration.

Materials and Methods: The present study compared LDL-C measured by Martin's formula, Friedewald's formula, and Anandaraja's formula with directly measured LDL in 280 Outpatient fasting samples between the age group of 18 and 50 years. Depending on the TG values, study samples are divided into four groups. Group 1: <200 mg/dL, Group 2: 200–300 mg/dL, Group 3: 300–400 mg/dL, Group 4: >400 mg/dL.

Statistical Analysis: This was performed by the Statistical package for social sciences version 16. Paired *t* test and pearson correlation were performed to find the significant difference and correlation between direct LDL-C and calculated LDL-C by different formula.

Results: Martin's formula shows highest correlation with *r*-value of (0.9979) compared to Friedewald's (0.9857) and Anandaraja's (0.9683) *r*-values. The mean difference was least for Martin's formula (0.31 ± 3.53) compared to other formulae. Among all the groups, the percentage of error was least for Martin's formula (0.23%). Martin's LDL-C shows highest concordance (90.90%) compared to Friedewald's (79.60%) and Anandaraja's formulae (82.90%).

Conclusions: Among all the groups, Martin's formula shows highest correlation, least percentage of error, highest concordance, and least mean differences. At all TG levels, Martin's formula is the best formula compared to the Friedewald's formula and Anandaraja's formula.

Keywords: Low-density lipoprotein, Martin's formula, Anandaraja's formula, Friedewald's formula and direct low-density lipoprotein

INTRODUCTION

The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines suggest starting the drug therapy, if low-density lipoprotein cholesterol (LDL-C) levels >130 mg/dL. This makes accurate reporting of LDL-C crucial in the management of dyslipidemia patients.^[1] Ultracentrifugation and beta-quantitation are the gold standard methods for LDL-C

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measurement. The other methods include direct measurement of LDL-C using a homogenous assay. These methods are expensive, inconvenient, and not readily available in most of the routine laboratories.^[2] Due to these limitations, many clinical laboratories throughout the world use a less expensive and easy approach for the estimation of LDL-C, that is, Friedewald's formula.^[3] However, there are several shortcomings of this formula, mainly the underestimation of LDL-C at high triglyceride (TG) levels and overestimation at low TG levels.^[4] Many attempts have been made to evaluate and refine Friedewald's formula. Different modified formulae such as Anandaraja's formula^[5] and Martin's formula^[6] have been developed. Compared to Friedewald's formula, Anandaraja's formula^[5] uses only two analytes, TG and total cholesterol (TC) for calculation, which may decrease the total error when compared to the Friedewald's formula.

Friedewald's formula did not take account of inter individual variation as it uses fixed value 5 as divisor for TG, resulting in underestimation of risk and potentially under treatment.^[7] In contradictory, Martin's *et al.*^[7] provided a new formula, by introducing adjustable factor in the formula. The Martin's formula: (total cholesterol-high-density lipoprotein cholesterol [TC-HDL-C]) - (TGs/adjustable factor).^[7] Adjustable factor defined by levels of TG and non-HDL-C, as divisor for TG. This adjustable factor ranges from 3.1 to 11.9. This was derived from an analysis of TG-to-very low-density lipoprotein cholesterol (VLDL-C) ratios of more than 1.3 million people.^[7] There are few studies reporting the use of this formula in India.

Accurately determining LDL-C values is important in clinical laboratory practice because LDL-C is employed to manage patients having a high risk of coronary heart disease. Therefore, most alternative formulae have been developed to estimate LDL-C to be appropriate for ethnic- specific as well as other populations. The present study was undertaken with an aim, to determine, which of this calculated formula (Friedewald's, Anandaraja's, and Martin's formula) show maximum correlation with directly measured LDL-C at different serum TG levels.

MATERIALS AND METHODS

Study design

This study is an observational study. The study samples were collected from KLE centenary charitable hospital and medical research center Belagavi. Two hundred and eighty outpatient fasting complete lipid profile patients of 18–50 years of age were included in the study. Ethical clearance was obtained from the Institution Ethics Committee USM KLE International Medical Program Belagavi. Ethical approval number USM-KLE/IEC/04–2020. Written informed consent was taken from all participants.

Inclusion criteria

Two hundred and eighty outpatient fasting samples coming to laboratory for lipid profile and age group 18–50 years were included in the study.

Exclusion criteria

Patients with diabetes mellitus, hypothyroidism, cirrhosis, chronic hepatitis, chronic kidney disease, pancreatitis, and patients on active medication, such as steroids, statins, and omega-3 fatty acids, were excluded from the study.

Calculation of sample size

Direct method LDL mean = 118.02^[8]

Friedewald method mean = 107.22^[8]

Standard deviation in direct method = 35.45

Standard deviation in Friedewald method = 24.35

Effect size: 0.261538461538461

Power = 95%

Alpha error = 1%

Required sample size = 266 should be taken.

Z = 1.96 for 95% confidence

S1 = Standard deviation in Direct method = 35.45

S2 = Standard deviation in Friedewald method = 24.35

X2 = Direct method LDL mean = 118.02

X1 = Friedewald method mean = 110.22

$$n_{pairs} = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2} + \frac{Z_{1-\alpha/2}^2}{2}$$

$$\text{Where } \Delta = \frac{\bar{x}_2 - \bar{x}_1}{SD}, \quad SD = \frac{S_1 + S_2}{2}$$

Sample collection and lipoprotein analysis

As a routine procedure, the samples were collected after 10–12 h of overnight fasting by withdrawing 3 mL of venous blood in a plain vial. The samples were centrifuged at 3000 rpm for 15 min to obtain serum and were analyzed for lipid profile on the same day. The serum lipid profile parameters TC, TG, HDL-C, and LDL-C were analyzed on EM 360 clinical chemistry analyzer (TransAsia Bio-Medicals Ltd, Mumbai, Maharashtra, India). All the lipid parameters were estimated using kits purchased from Erba Mannheim XL system packs. The linearity (intra-assay coefficients) of TC, TG, HDL-C, and LDL-C assays were 4.2–695 mg/dL (0.98–1.21%), 9.74–1062 mg/dL (0.48–0.86%), 1.90–193 mg/dL (1.32–1.95%), and 2.60–263 mg/dL (1.74–2.16%), respectively. The intra-

assay coefficients observed in our analysis were in concurrence with the manufacturer's measurements. All quality controls were performed to ensure the accuracy of the analytical testing (internal and external controls). The internal control is routinely processed every 24 h on two levels (normal and pathological) by liquichek lipids control from Bio-Rad laboratories, Inc. The results are analyzed daily and periodically for the evaluation of the Levey Jennings graph. The laboratory's external quality control is performed every three months. All the lipid parameters assays meet the National Institutes of Health NCEP goals for acceptable performance (LDL – CV < 4%, Bias < 4% and Total Error of < 12%, for HDL – CV < 4%, Bias $\leq \pm 5\%$ and total error $\leq 13\%$, for TC – CV < 3%, Bias $\leq \pm 3\%$ and total error $\leq 8.9\%$, for TG – CV < 5%, and Bias $\leq \pm 5\%$ and total error $\leq 15\%$).

LDL-C was calculated by following formulae:

- Friedewald's formula^[4] (F-LDL) = TC – (TG/5 + HDL-C)
- Anandaraja's formula^[5] (A-LDL) = (0.9 × TC) – (0.9 × TG/5) – 28
- Martin's formula^[6] (M-LDL) = (TC – HDL-C) – (TGs/adjustable factor*)

*Adjustable factor: The adjustable factor based on TG and non-HDL-C concentrations. Mart's method matches each person with one of 180 different factors to estimate VLDL cholesterol from TGs. The Martin's LDL-C was calculated using an LDL-C calculator (<http://www.ldlcalculator.com>). Copy the values for TC, HDL-C, and TGs from the research database into the Excel file. Non-HDL-C, the adjustable factor, and LDL-C by Martin's formula, will be automatically calculated.

Depending on the TG values, study samples were divided in to four groups.

1. Group 1: <200 mg/dL
2. Group 2: 200–300 mg/dL
3. Group 3: 300–400 mg/dL
4. Group 4: >400 mg/dL.

Statistical analysis

The data obtained were entered into Microsoft Excel sheet, and statistical analysis was performed with the Statistical Package for the Social Sciences version 16.0. Paired *t*-test and Pearson correlation were performed to find the significant difference

and correlation between direct LDL-C and calculated LDL by a different formula. Scatter plot was used to represent the correlation between the two methods. The mean percentage of error was calculated using the formula: (Calculated LDL-C – D-LDL-C) / D-LDL-C × 100. *P* < 0.05 is considered as significant.

RESULTS

The study consists of a total 280 samples. Depending on the TG values (66–533 mg/dL), the study population is divided into four groups. There were 124 participants in Group 1, 91 participants in Group 2, 36 participants in Group 3, and 29 participants in Group 4.

Table 1 shows the baseline characteristics of the study population gender and sex. Comparison of gender and age between groups is statistically not significant. There was no significant difference in age and gender in the study population between groups [Table 1].

LDL-C was calculated according to three different formulae and compared with directly measured LDL-C [Table 2]. correlation coefficient *r* was calculated with each equation by correlation analysis of the data. The best formula was chosen in terms of the highest correlation and the lowest mean difference and standard deviation. LDL-C by Martin's formula showed a highest correlation of *r* = 0.9979, compared to other formulae *r*-value Friedewald's formula 0.9857 and Anandaraja's formula 0.9683 [Table 2 and Figure 1].

Comparison of mean of directly measured LDL-C with calculated LDL-C [Table 3] by Friedewald's formula and Anandaraja's formula shows that it is underestimated at all levels of TG, and it is statistically significant. Among the total sample, mean difference of the direct and calculated formula was the least for Martin's formula 0.31 ± 3.53 compared to other formulae. In Group 1, mean difference was least for Anandaraja's formula 1.08 ± 8.35 compared to other formulae. In Groups 2, 3, and 4, mean difference was least for Martin's formula with values 0.65 ± 5.17 , 0.00 ± 2.47 , and 0.77 ± 5.13 , respectively, compared to other formulae.

Percentage of error from direct LDL-C to calculated LDL-C was the least for Martin's formula [Table 4 and Figure 1] in total study sample and in all groups compared to other formulae.

Table 1: Comparison of four groups by age and gender.

	Group 1	Group 2	Group 3	Group 4	Total	P-value
Gender						
Male	64 (52)	37 (41)	20 (55)	9 (31)	130	0.089
Female	60 (48)	54 (59)	16 (44)	20 (69)	150	
Age						
Mean±SD	40.9±8.0	38.8±9.2	39.1±10.0	39.8±8.2	39.9±8.7	0.337
Total	124	91	36	29	280	

SD: Standard deviation. *P*<0.05 is statically significant

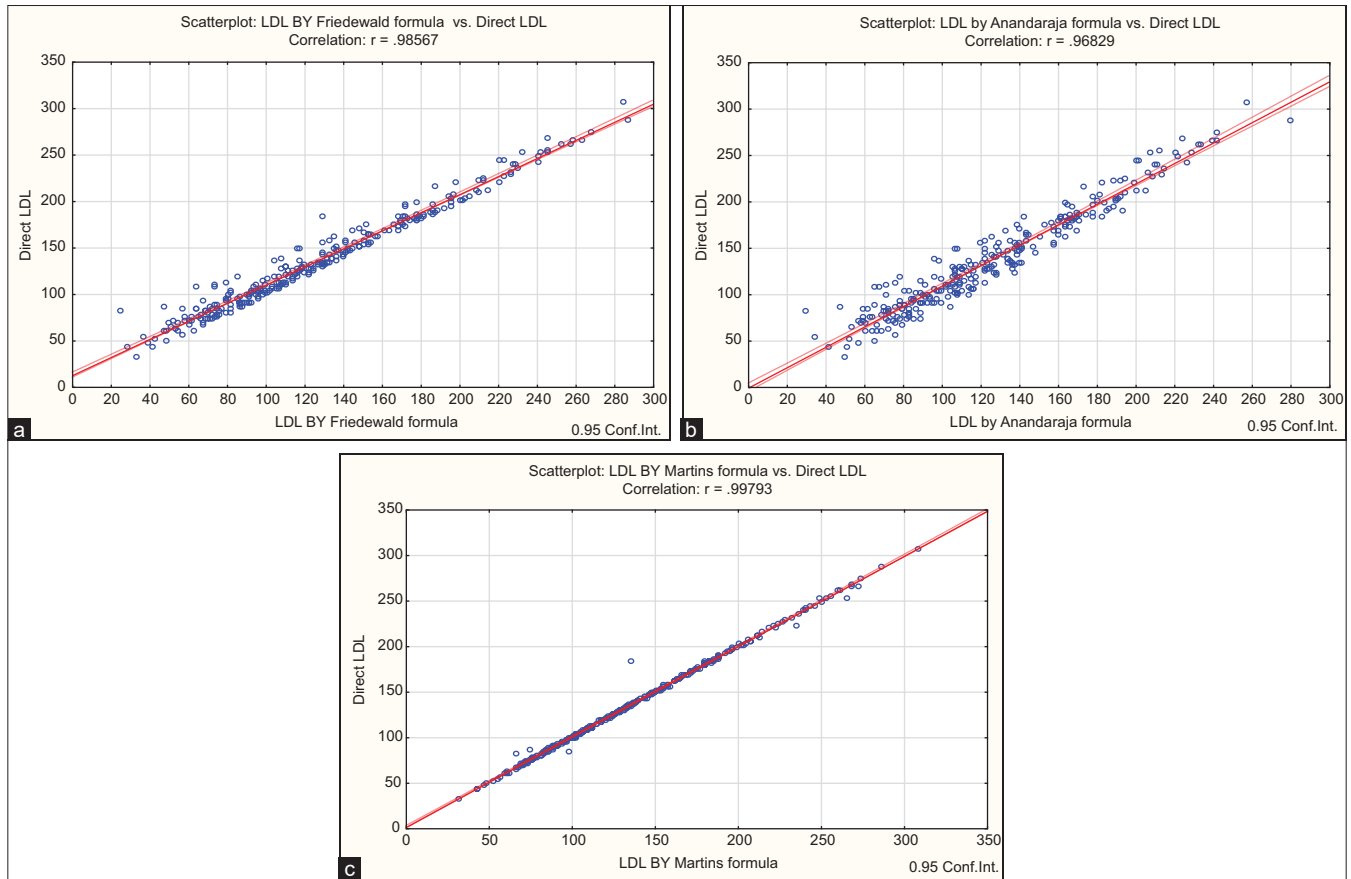


Figure 1: Correlation between direct low-density lipoprotein cholesterol (LDL-C) and calculated LDL-C. Blue dots indicate values of LDL by direct low density lipoprotein cholesterol (LDL-C) and calculated LDL-C by Friedewald formula, red line indicates positive correlation between them. (a) Correlation between direct LDL-C and Friedewald formula LDL-C. (b) Correlation between direct LDL-C and Anandaraja formula LDL-C. Blue dots indicate values of LDL by direct low density lipoprotein cholesterol (LDL-C) and calculated LDL-C, red line indicates positive correlation between them. (c) Correlation between direct LDL-C and Martins formula LDL-C. Blue dots indicate values of LDL by direct low density lipoprotein cholesterol(LDL-C) and calculated LDL-C by Martins formula, red line indicates positive correlation between them.

Table 2: Correlation between direct LDL-C with calculated LDL-C by different formula by Karl Pearson’s correlation method.

Samples	Variables	r-value	P-value
Total	Friedewald formula	0.9857	<0.001*
	Anandaraja formula	0.9683	<0.001*
	Martins formula	0.9979	<0.001*
Group 1	Friedewald formula	0.9983	<0.001*
	Anandaraja formula	0.9864	<0.001*
	Martins formula	0.9998	<0.001*
Group 2	Friedewald formula	0.9944	<0.001*
	Anandaraja formula	0.9884	<0.001*
	Martins formula	0.9953	<0.001*
Group 3	Friedewald formula	0.9976	<0.001*
	Anandaraja formula	0.9908	<0.001*
	Martins formula	0.9991	<0.001*
Group 4	Friedewald formula	0.9958	<0.001*
	Anandaraja formula	0.9967	<0.001*
	Martins formula	0.9967	<0.001*

LDL-C: Low-density lipoprotein cholesterol, r: Correlation coefficient, *P<0.05 is statically significant

The present study compared the concordance of the directly measured LDL-C with the estimated LDL-C when classifying LDL-C values by NCEP-ATP III. We labeled the result as being “concordant” if the two values were in the same classification, as an “overestimation simultaneously”, the estimated value was greater than the direct measurement, or as an “underestimation” if the estimated value was less than the direct measurement.

Martin’s formula (90.90%) resulted in the best concordance with the direct measurement compared to Friedewald’s formula (79.60%) and Anandaraja’s formula (82.90%). Overestimation and underestimation rates produced by Martin’s formula are less than those produced by Friedewald’s and Anandaraja’s formula.

DISCUSSION

The underestimation of LDL-C will lead to delay in the initiation of treatment of patients who are at high risk of dyslipidemia. In a mean, overestimation also leads to

Table 3: Comparison of mean value of direct LDL-C and calculated LDL-C by different formulas.

Total sample			
Method	Mean±SD	Mean difference (mg/dL)	P-value
Direct	137.42±54.51		
Friedewald formula	128.06±55.18	9.37	<0.001*
Anandaraja formula	125.56±47.96	11.86	<0.001*
Martins formula	137.11±54.82	0.31	0.1443
Group 1			
Direct	118.61±44.55		
Friedewald formula	115.60±45.27	3.01	<0.001*
Anandaraja formula	117.53±39.93	1.08	0.1536
Martins formula	118.21±44.47	0.40	<0.001
Group 2			
Direct	134.85±53.09		
Friedewald formula	124.33±56.93	10.52	<0.001*
Anandaraja formula	121.54±50.16	13.31	<0.001*
Martins formula	134.20±52.76	0.65	0.2343
Group 3			
Direct	177.48±50.13		
Friedewald formula	163.74±55.03	13.74	<0.001*
Anandaraja formula	153.87±48.31	23.61	<0.001*
Martins formula	177.47±51.33	0.00	0.9941
Group 4			
Direct	176.21±58.99		
Friedewald formula	148.70±65.78	27.52	<0.001*
Anandaraja formula	137.39±58.33	38.83	<0.001*
Martins formula	176.98±60.58	-0.77	0.4288

LDL-C: Low-density lipoprotein cholesterol, SD: Standard deviation.
Mean difference=Direct LDL-C-Formula calculated LDL-C. *P<0.05 is statically significant

Table 4: Comparison of percentage of error from direct LDL-C to calculated LDL-C by different formulas.

	LDL by Friedewald	LDL by Anandaraja	LDL by Martins
Total	6.82	8.63	0.23
Group 1	2.54	0.91	0.34
Group 2	7.80	9.87	0.48
Group 3	7.74	13.30	0.00
Group 4	15.62	22.04	0.44

LDL-C: Low-density lipoprotein cholesterol. Percentage of error=(Calculated LDL-C-Direct LDL-C)/Direct LDL-C×100.

exposure of patients to unnecessary drug therapy. Hence, there is a need to find an accurate equation for the estimation of LDL-C with the best performance comparable to the directly measured LDL-C. Since Friedewald's formula has limitations, many attempts have been made to derive a more accurate formula for LDL-C calculation. The present study was undertaken with the aim of determining which of these calculated formulae (Friedewald's, Anandaraja's, and Martin's

formula) show maximum correlation with directly measured LDL-C at different serum TG levels.

The previous studies such as Sahu *et al.*^[9] and Molavi *et al.*^[10] have shown that Friedewald's equation performs better for certain groups of populations. However, in the study, we found that calculated LDL-C is underestimated in all the groups. Among all the formulas, the mean difference and percentage of error produced by Friedewald's equation are high in the total sample and in Groups 2, 3, and 4. The results are consistent with the results previously reported by authors^[11-13] that show that Friedewald's formula underestimates LDL-C at higher TG ranges. It may be because the performance of Friedewald's equation steadily decreases with increasing TG and is not recommended for hypertriglyceridemia (<400 mg/dL) ranges. In contradictory, studies done by various author^[14,15] have reported overestimation of LDL-C by Friedewald's formula as compared to directly measured LDL-C.

The present study shows, underestimation by Anandaraja's formula compared to the directly measured LDL-C. The previous studies conducted by Kapoor *et al.*,^[8] Kamal *et al.*,^[11] and other authors^[16-18] also reported underestimation by Anandaraja's formula. In Group 1, mean difference between Anandaraja's formula and directly measured LDL -C is least compared to other formulas. The results are consistent with Krishnaveni and Gowda.^[19] Krishnaveni and Gowda^[19] showed that for subjects with serum levels of TG <100 mg/dL, Anandaraja's Formula was the most accurate equation.

Kamal *et al.*,^[11] and author's^[20,21] have showed that as TG levels increase, there is an increase in mean difference between direct and formula calculated LDL-C. The present study results support this finding, with an increase in TG concentrations, the difference between D LDL-C and Friedewald's, Anandaraja's calculated LDL-C increased. Gupta *et al.*^[16] and Lee *et al.*^[22] observed that LDL-C cholesterol concentrations had no relation with TG concentrations. Martin-LDL-C values were closer to direct LDL-C in all the groups.

Martin's formula (90.90%) resulted in the best concordance with the direct measurement compared to Friedewald's formula (79.60%) and Anandaraja's formula (82.90%). The results are consistent with studies done by Kang *et al.*,^[6] Martin *et al.*,^[7] and by Lee *et al.*^[22] Overestimation and underestimation rates produced by Martin's formula are less than those produced by Friedewald's and Anandaraja's formula; the difference is particularly pronounced in the underestimation rate. This is of particular importance because underestimation is generally considered riskier than overestimation. Primarily, underestimation can cause delays in the initiation of treatment, while screening the general population.

The present study shows the tendency of Friedewald's formula to underestimate LDL. It is in these clinical conditions that the Martin's formula may be more useful. In all the groups, Pearson

correlation coefficient r value was high for Martin's formula compared to the Friedewald's formula. It was suggested that the Martin's formula may prevent under-treatment due to the underestimation of LDL using the Friedewald's formula. Our results thereby confirmed that the study by author's^[6,7,22] stated that Martin's formula offers a significant improvement in LDL-C estimation when compared with Friedewald's formula. Martin's formula can be used instead of routine Friedewald's formula as Martin's formula is more accurate.

In developing countries like India with a burdening population of high TG, there is a need to adopt the novel equation. Martin's 180-cell approach could be coded into an online calculator, smartphone application, or automated laboratory reporting system.

Limitation of study

This present study has a few limitations. Firstly, the results may not be generalizable to the overall population, as there may be differences in baseline characteristics between our subjects and the general population. We had access only to the lipid profiles of the subjects. Moreover, clinical characteristics or clinical outcomes of patients in our sample were unknown. Second, instead of calculating an adjustable factor for the Martin's equation, we used the calculator that was suggested by the authors. However, there is a possibility that the adjustable factor for the Indian population is different from what Martin's reported.

CONCLUSIONS

In the present study, Martin's formula showed high correlation, lower mean difference. Additionally it shows the highest concordance and low percentage of errors in all the groups. This is mainly in comparison to Friedewald's formula and Anandaraja's formula. At all TG levels, Martin's formula is best compared to Friedewald's formula and Anandaraja's formula.

Ethical approval

Ethical clearance was obtained from the Institution Ethics Committee USM KLE International Medical Program Belagavi. Ethical approval number USM-KLE/IEC/04-2020.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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