Comparison of the Friedewald and Vujovic methods with the calculated LDL concentration in a biochemical auto-analyzer

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ABSTRACT

OBJECTIVE To compare the concentration of Low-Density Lipoprotein (LDL-c) obtained using the Friedewald formula with those obtained directly with the RAYTO CHEMRAY 120 autoanalyzer.

METHODS Cross-sectional study. We evaluated outpatients with a medical request for a lipid profile study (total cholesterol, triglycerides, LDL, and HDL). The analyses were carried out in a RAYTO CHEMRAY 120 autoanalyzer under the principle of spectrophotometry. We obtained LDL-c using the Friedewald and Vujovic formulas.

RESULTS We evaluated 199 individuals whose direct LDL concentration averages were measured by the RAYTO CHEMRAY 120 equipment. Those calculated by the Friedewald and Vujovic formulas were 129.97 ± 32.66, 119.28 ± 30.44, and 127.01 ± 32.01, respectively, and in all cases, significant differences ($P < 0.001$) were observed with the RAYTO analyzer. In both cases a low positive bias was found with the RAYTO analyzer.. The Passing-Bablok and Deming's regressions showed a linear correlation between both methods (Friedewald and Vujovic) with the LDL values obtained with the Rayto autoanalyzer.

CONCLUSIONS Our study found that the Friedewald and Vujovic methods are good predictors of LDL cholesterol levels and have a low level of bias. Therefore, they could be used as potential predictors.

KEYWORDS Low-Density Lipoprotein, Friedewald, Vujovic, Rayto Chemray 120 Analyzer

INTRODUCTION

Cardiovascular disease is one of the leading causes of death globally, and by 2019, an estimated 17.9 million deaths have been estimated, representing 32% of global mortality [1]. In developing countries, it continues to be one of the most critical public health concerns [2]. In Peru, it has been identified that the combination of two or more risk factors for cardiovascular disease with arterial hypertension and diabetes or overweight or obesity is associated with an increased risk of all-cause mortality [3]. In the last 40 years, one of the priorities has been to identify the people with the highest risk of cardiovascular disease and implement treatment and prevention strategies [4]. Surveillance activities and cardiovascular disease risk prediction tools estimate the probability of having a cardiovascular event within a defined time frame based on the presence of known risk factors [5].

For several decades, there has been sufficient evidence to establish that low-density lipoprotein (LDL) elevation is a significant risk factor for cardiovascular disease, especially in people over 40 years old [6]. LDL measurement using reliable methods is vital to achieving uniform clinical data interpretation, which is essential for cardiovascular disease prevention and treatment. LDL concentration is a good cardiovascular disease risk predictor and is the basis for accurate classification into risk categories. However, quantification by the reference method requires complex processes such as ultracentrifugation and large sample volumes, which is a slow and expensive technique [7]. Therefore, this method is unsuitable for routine laboratory tests [8]. Other recommended methods include direct

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MAIN MESSAGES

- Calculating cardiovascular risk is essential to identify and promptly prevent cardiovascular diseases.
- Measuring total cholesterol and low and high-density lipoproteins is critical for calculating different predictive models of cardiovascular risk.
- Many laboratories in low-income countries do not have the resources to measure parameters in an automated way.
- It is essential to guarantee the validity of lipoprotein measurement using formulas, especially in low-resource laboratories.

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homogeneous measurement. Direct methods require expensive automation and are only affordable for some laboratories in developing countries [9]. Due to these limitations, many clinical laboratories worldwide use a less expensive and more accessible approach to LDL calculation; among them is the Friedewald formula [10].

The National Cholesterol Education Program Adult Treatment Panel III guidelines recommend the use of LDL calculated by the Friedewald method for the prevention and treatment of cardiovascular disease [11]. However, this method has several areas for improvement, mainly underestimation of LDL cholesterol at high triglyceride levels and overestimation at low triglyceride levels [12].

Several authors point out that it is a reliable estimate [13]. However, other researchers state that the Friedewald formula may be biased because extreme HDL cholesterol concentrations may affect the calculation of LDL cholesterol in certain circumstances. One study compared 12 formulas for calculating LDL cholesterol in more than 100 000 people in Italy and found that the Sampson, Martin, and Vujovic methods were the most accurate [14]. The study of different formulas showed that the regression and the Bland-Altman plot disagreed with the four studied formulas, except for the formula proposed by Vujovic et al. [15]. It has been shown that the Friedewald formula modified by Vujovic provided higher accuracy with an acceptable degree of agreement compared to those derived from the original Friedewald formula or others. A study in India found that the Vujovic formula correlates well with LDL cholesterol levels measured by direct homogeneous methods [16]. The interference caused by hypertriglyceridemia decreased; thus, the Vujovic formula is more reliable than the others if triglyceride levels are less than 400 to 100 mg/dL.

Assays that use different physicochemical combinations of surfactants, polymer complexes, and especially molecular bonds and primarily measure cholesterol from LDL fractions improve analytical performance to meet the Adult Treatment Panel III recommendations [17]. However, these methodologies require automated equipment, continuous calibrations, and other logistic processes that guarantee the correct supply of goods and services. This situation makes its implementation unfeasible and complicated in clinical laboratories at the first level of care in developing countries. Therefore, measuring LDL with reliable and valid formulas is an essential alternative for its consideration.

Accordingly, our research aimed to compare the Friedewald and Vujovic methods with the LDL concentration obtained in a biochemical autoanalyzer since there are no studies of this type in Peru. It is essential to evaluate the accuracy of this method in different populations. Identifying the best option to calculate LDL is essential in areas lacking laboratory automation. This activity could strengthen epidemiological surveillance and improve primary prevention actions in populations at high risk of cardiovascular disease.

METHODS

Study area and participants

We evaluated patients attending an outpatient clinic at the Daniel Alcides Carrión Hospital in the City of Huancayo, Department of Junín,Peru, between January 2nd and May 30th, 2021. The number of participants was obtained using a calculation based on the correlation. We performed the post-hoc power calculation using an intraclass correlation model [18], assuming a significance level of 0.05 and a coefficient of 87.6% for the comparison between the Vujovic method and Biochemical Autoanalyzer as reported by Choi [19], and an expected coefficient of 0.99. We obtained a post-hoc power of 81%. We included patients of both sexes, between 18 and 70 years of age, who had a medical order for a lipid profile study (total cholesterol, HDL, LDL, and triglycerides). We excluded patients whith incomplete results and those who had not fasted for 12 hours before taking blood samples.

Techniques and procedures

Data collection form from the area's computer database where information was obtained on concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides (TG) of adult patients in the Biochemistry area of the Daniel Alcides Carrión Hospital of Huancayo.

Lipid profile: The methodology of determining total cholesterol, triglycerides, HDL, and LDL in the RAYTO CHEM-RAY 120 equipment was by homogeneous direct methods. LDL-c values were calculated using the Friedewald and Vujovic formulas.

For the calculation of LDL-c by the Friedewald method, the following formula is used [20]:

$$LDL(Friedewald) = Total colesterol - \left(HDL + \frac{TG}{5}\right)$$

Table 1. Summary of the statistical results of the comparison of the methods.

	N	Mean	Minimum	Maximum	Standard deviation	95% CI of two-tailed Paired Student's t-test	Standard Error	95% CI of Standard Error
LDL Rayto	199	129.97	59	254	32.66	Reference	Reference	Reference
LDL FF	199	119.28	53	232	30.44	-11.6 to 9.76	10.71	9.77 to 11.64
LDL VJ	199	127.01	58	246	32.01	-3.83 to 2.03	2.92	2.03 to 3.83

FF: Friedewald Formula. FV: Vujovic Formula. LDL : Low density lipoprotein.
Source: Prepared by the authors based on the results of the study.

Next, the formula developed by Vujovic [21]:

$$LDL(Vujovic) = Total colesterol - \left(\frac{TG}{6.85}\right) - HDL$$

The research was approved by the Ethics Committee from the Continental University.

Statistical analysis

We performed a descriptive statistical analysis of the variables. Subsequently, the comparison of means was evaluated with Student's t-test for paired samples after evaluating homoscedasticity with Levene's F test. Finally, we calculated the bias as the difference between the calculated and obtained values and evaluated it in the Bland-Altman plots. A Passing-Bablok and Deming regression analysis was performed. We made the calculations with the statistical program Stata version 16.0 (StataCorp College Station, TX, USA).

RESULTS

We evaluated 199 individuals whose direct LDL concentration averages measured by the RAYTO CHEMRAY 120 autoanalyzer, and those calculated by the Friedewald and Vujovic formulas were 129.97 ± 32.66, 119.28 ± 30.44 and 127.01 ± 32.01, respectively (Table 1).

We found a statistically significant difference (P < 0.001) between the means of LDL Friedewald's formula (119.28 ± 30.44) and LDL Rayto (129.97 ± 32.66) and the means of LDL Vujovic's formula (127.01 ± 32.01) and LDL Rayto (129.97 ± 32.66). Likewise, we evaluated the differences between the mean concentrations (error or bias) of LDL obtained by the Rayto team and the Friedewald and Vujovic method using the Bland-Altman method (Figure 1). The average error of the

Friedewald method was 10.710 mg/dL (95% CI: 9.777 to 11.642) ranging between -2.636 to 24.055; with the Vujovic method, the average error was 2.928 mg/dL (95% CI: 2.030 to 3.826) ranging between -9.919 to 15.774. So, both methods have a low positive bias regarding measured LDL with the Rayto analyzer.

We compared the Friedewald and Vujovic methods with the obtained LDL levels with the RAYTO equipment using two non-parametric tests (Passing-Bablok and Deming regression) (Table 2). We found that the Friedewald and Rayto methods differ statistically by at least a constant amount. This difference is because the confidence interval of the intercept does not include the zero value. Likewise, both methods present a statistically significant proportional difference since the confidence interval of the slope does not includes units. Similar results were found when comparing the Vujovic method with the results obtained with the Rayto; however, according to Deming regression, both methods do not differ statistically by at least a constant amount; according to Passing-Bablok, there is a statistical difference by at least a constant amount.

Figures 2 and 3 show the Passing-Bablok and Deming regressions, respectively. We can see a linear correlation between both methods (Friedewald and Vujovic) with the LDL values obtained with the Rayto autoanalyzer.

On the other hand, in Figures 4 and 5, we can observe the residual graphs of Passing-Bablok and Deming regression, where there is a pattern in the distribution of the residuals except for the residuals that escape the line (denoted as red dots) that could represent the values that escape the bisector line in Figure 2. This could indicate the presence of some type of non-linearity or heteroskedasticity in the data. That is, there may be a systematic trend in the errors, which could suggest that the model is not fully capturing the structure of the data.

Table 2. Passing-Bablok regression and Demming regression results.

|	Intercept	CI (95%)	Slope	Ci (95%)
Passing-Bablok regression: Friedewald Vs. Rayto	2.510	0.705 to 4.096	1.065	1.051 to 1.080
Deming regression: Friedewald Vs Rayto	2.039	0.092 to 3.986	1.073	1.056 to 1.090
Passing-Bablok regression: Vujovic Vs. Rayto	1.206	0.105 to 2.387	1.010	1.001 to 1.019
Deming regression: Vujovic Vs. Rayto	0.280	-1.418 to 1.978	1.020	1.007 to 1.035

Source: Prepared by the authors based on the results of the study.

Figure 1. Bland-Altman LDL dispersion mean difference obtained by the Rayto Chemray 120 autoanalyzer and the Friedewald (A) and Vujovic method (B).
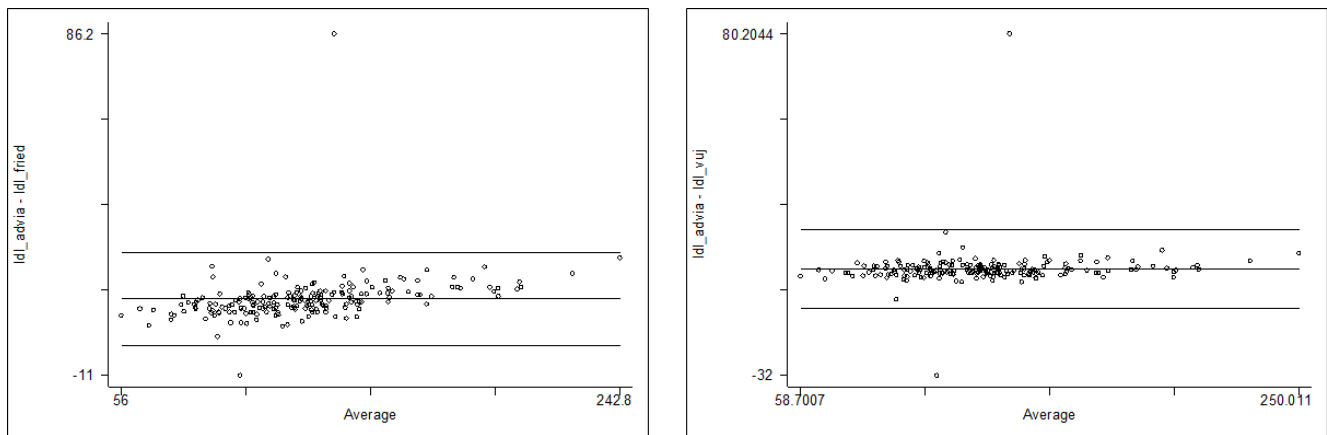
Source: Prepared by the authors based on the results of the study.

Figure 2. Passing-Bablok regression of LDL concentrations obtained by the Rayto Chemray 120 Equipment and the Friedewald (A) and Vujovic method (B).
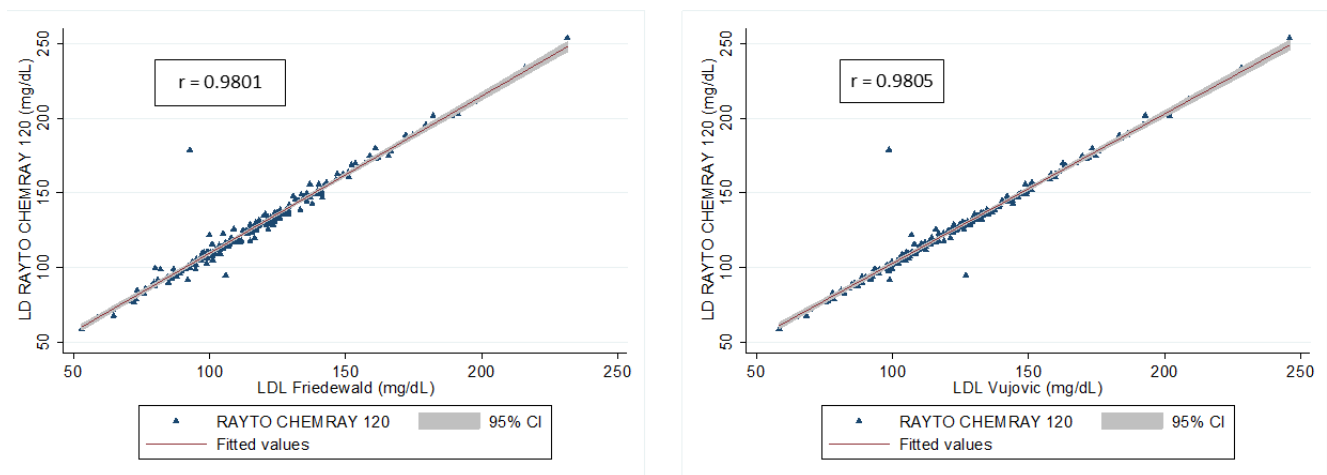
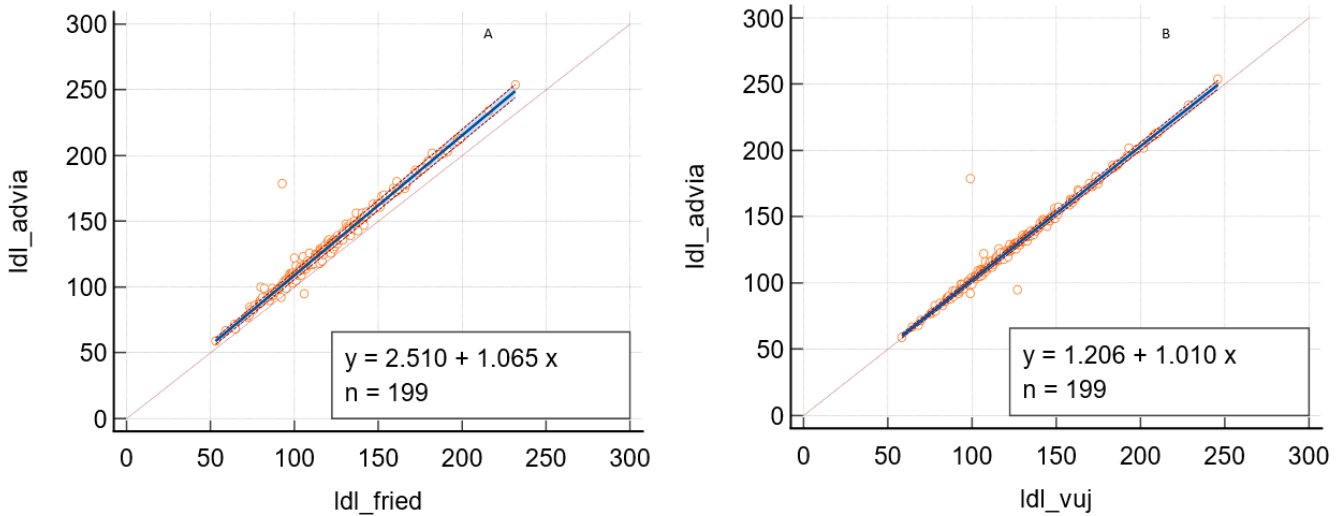Source: Prepared by the authors based on the results of the study.

DISCUSSION

There are various methods for calculating LDL cholesterol levels from triglyceride, total cholesterol and HDL values. However, many present a significant bias when triglyceride values are outside the normal range. Our study is one of the first to compare two methods (Friedewald and Vujovic) for estimating LDL cholesterol in a Peruvian population in the Andes. We found that both had an excellent positive linear correlation and a low-level bias. These findings are related to what was found in studies carried out in Italy and China [22].

We found a significant difference between the means of calculated LDL cholesterol levels (Friedewald and Vujovic methods) and the value obtained by the Rayto autoanalyzer. It could be explained by the low positive bias in these methods and because hypertriglyceridemic samples (trygliceride levels between 200 mg/dL and 400 mg/dL) tend to present more significant bias. In these cases, the utility of formulas such as Friedewald and Vujovic becomes null. It is recommendable to determine LDL cholesterol levels using the reference method [23].

Likewise, we found that the Vujovic method presented a lower average error (2.928 mg/dl) than the Friedewald method (10.70 mg/dL). Both methods present a positive bias, with the Vujovic method having a slightly smaller bias. Our results relate to what was found by Saldaña and Benites, 2017, where they reported a bias of 11.25 and 2.9 mg/dL for the Friedewald and Vujovic methods, respectively; in this study, they evaluated 4644 people from Lima, Peru [24]. Another study evaluated 4621 people from Lima and found that the Friedewald method presents a difference with the reference method of -11.94% when triglyceride levels exceed 200 mg/dL and a difference of -19.13% when triglyceride levels exceeded 400 mg/dL [25].

Figure 3. Deming regression of LDL concentrations obtained by the Rayto Chemray 120 Equipment and the Friedewald (A) and Vujovic method (B).

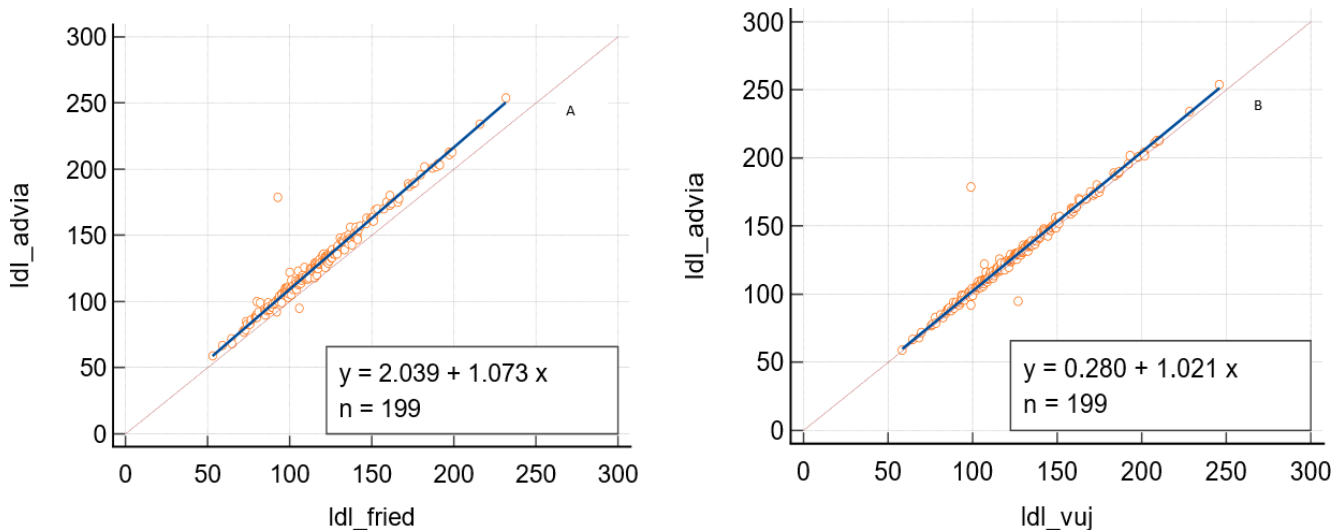Source: Prepared by the authors based on the results of the study.

Figure 4. Residual plots of the Passing-Bablok regression linear models of LDL concentrations obtained by the Rayto Chemray 120 Equipment and the Friedewald (A) and Vujovic method (B).
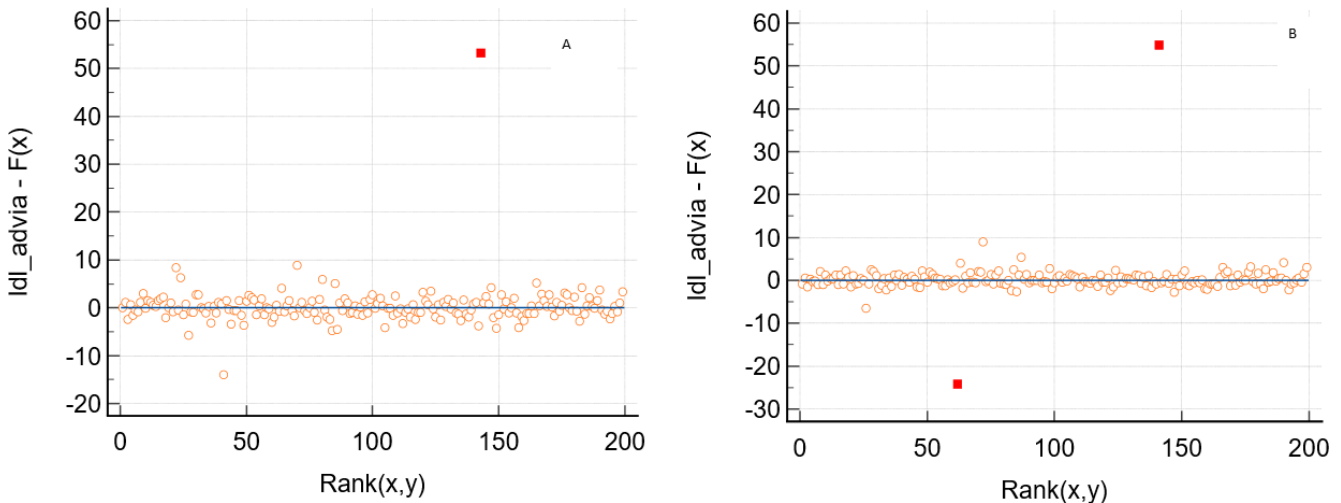
Source: Prepared by the authors based on the results of the study.

Finally, we evaluate both methods using Deming and Passing-Bablok regression. In the Passing-Bablok regression analysis, we found that both methods differ statistically by at least a constant amount with the results obtained by the Rayto team. On the other hand, the Deming regression results indicate that the Friedewald method and Rayto team's results differ statistically by at least a constant amount; in contrast, Vujovic's method does not statistically differ by at least a constant amount from Rayto team's results. This indicates that the Vujovic method would be more accurate in calculating LDL cholesterol levels. Our results are related to what was found by Vujovic et al., who, using using Passing-Bablok regression, did not find a statistically significant difference with the reference method [26].

We consider using formulas to estimate the LDL-c vital since it can be used in places where healthcare facilities and laboratories lack the infrastructure conditions to measure this directly. In this sense, we have shown that the formulas offer a simple, cost-free, and reliable way to obtain serum LDL results, but considering some assumptions, such as the distribution of values in normal concentration ranges. Therefore, applying these formulas would not be helpful within a hospital care context but could be useful within epidemiological surveillance of non-transmissible diseases. In this sense, this research contributes significantly to identifying laboratory indicators that are useful clinically and at an epidemiological level. That is why we are considering the development of alternative methods for the more accurate calculation of these parameters, which are important in diagnosing metabolic diseases. The main limitation

Figure 5. Residual plots of the linear Deming regression models of LDL concentrations obtained by the Rayto Chemray 120 Equipment and the Friedewald (A) and Vujovic (B) method.

Source: Prepared by the authors based on the results of the study.

of this study was the lack of access to other variables that could be influencing LDL cholesterol levels.

CONCLUSIONS

Our study found that the Friedewald and Vujovic methods are good predictors of LDL cholesterol levels and have a low level of bias. Therefore, they could be used as potential predictors.

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Comparación de los métodos de Friedewald y Vujovic con la concentración de LDL calculada en un autoanalizador bioquímico

RESUMEN

OBJETIVO Comparar las concentraciones de Lipoproteínas de Baja Densidad (LDL-c) obtenidas mediante la fórmula de Friedewald con las obtenidas directamente con el autoanalizador RAYTO CHEMRAY 120.

MÉTODOS Estudio transversal. Se evaluaron pacientes ambulatorios con solicitud médica de perfil lipídico (colesterol total, triglicéridos, LDL y HDL). Los análisis se realizaron con un autoanalizador RAYTO CHEMRAY 120 bajo el principio de espectrofotometría. Obtuvimos el LDL-c usando las fórmulas de Friedewald y Vujovic.

RESULTADOS Se evaluaron 199 individuos cuyos promedios directos de concentración de LDL fueron medidos con el equipo RAYTO CHEMRAY 120. Las concentraciones calculadas por las fórmulas de Friedewald y Vujovic fueron de 129,97 ± 32,66, 119,28 ± 30,44, y de 127,01 ± 32,01, respectivamente, y en todos los casos se observaron diferencias significativas (P < 0,001) con el analizador RAYTO. En ambos casos se encontró un sesgo positivo bajo en el analizador RAYTO. Las regresiones de Passing-Bablok y Deming mostraron una correlación lineal entre ambos métodos (Friedewald y Vujovic) con los valores de LDL obtenidos con el autoanalizador Rayto.

CONCLUSIÓN Nuestro estudio encontro que los métodos de Friedewald y Vujovic son buenos predictores de los niveles de colesterol LDL y presentan un nivel de sesgo bajo. Por lo que podrían usarse como potenciales predictores.