Effect of low serum triglyceride on LDL-cholestrol estimation by friedewald formula

Efecto de los triglicéridos séricos bajos en la estimación del colesterol LDL por la fórmula de Friedewald

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Abstract

The aim of the current study was to investigate the impact of low serum triglyceride (TG) on LDL-cholestrol estimation by Friedewald formula. **Methods:** Serum samples were taken from 540 patients and divided into 4 groups based on the level of the TG 100>, 100-150, 150-250 and 250-400 mg/dl. The total cholestrol (TC), TG, high density lipoprotein (HDL) and low density lipoprotein (LDL) were measured. Then, the LDL of patients whose serum levels of TG>400 was measured with the direct method and calculated as follows: Friedewald method= LDL – C (mg/dl) = TC–TG/5 – HDL. The results of Friedewald formula compared with the direct measurement results. **Findings:** According to the results, a significant difference observed between Cholesterol (LDL-C) measured by the direct method than Friedewald equation in patients with TG>400 levels (P<0.001). No significant differences were observed in patients with TG 250-400 levels (P>0.05). A correlation reported between LDL-C measured by the direct and Friedewald Equation methods (P<0.001). **Conclusion:** These results suggested there was higher difference of LDL-C in low TG levels (100> and 100-150 mg/dl) measured by direct method and Friedewald Equation formula. Negligible differences observed in high TG levels (150-250 and 250-400 mg/dl) using direct method and Friedewald Equation formula. With increased TG levels (up to 400mg/dl), the overestimation of the LDL-C level is decreased by Friedewald formula. So, in high TG levels, Friedewald Equation formula is reliable.

Keywords: LDL-C, Triglyceride, Friedewald Equation

Resumen

El objetivo del presente estudio fue investigar el impacto del bajo nivel de triglicéridos en suero (TG) en la estimación de colesterol LDL por la fórmula de Friedewald. **Métodos:** se tomaron muestras de suero de 540 pacientes y se dividieron en 4 grupos según el nivel de TG 100>, 100-150, 150-250 y 250-400 mg / dl. Se midieron el colesterol total (TC), TG, lipoproteína de alta densidad (HDL) y lipoproteína de baja densidad (LDL). Luego, la LDL de pacientes cuyos niveles séricos de TG> 400 se midieron con el método indirecto y se calculó de la siguiente manera: método de Friedewald = LDL - C (mg / dl) = TC – TG / 5 - HDL. Los resultados de la fórmula de Friedewald se compararon con los resultados del cálculo directo. **Resultados:** Según los resultados, se observó una diferencia significativa entre el colesterol (LDL-C) medido por la ecuación directa de Friedewald (P <0.001). No se observaron diferencias significativas en pacientes con niveles de TG 250-400 (P> 0.05). Una correlación reportada entre LDL-C medida por los métodos de ecuación directa y de Friedewald (P <0.001). **Conclusión:** Estos resultados sugirieron que hubo una mayor diferencia en los niveles bajos de TG (100> y 100-150 mg / dl) medidos por la fórmula directa y la fórmula de la ecuación de Friedewald. Se observaron diferencias insignificantes en los niveles altos de TG (150-250 y 250-400 mg / dl) usando la fórmula de ecuación directa y de Friedewald. Con el aumento de los niveles de TG, la fórmula de Friedewald disminuye la sobreestimación del nivel de LDL-C. Entonces, en niveles altos de TG, la fórmula de la ecuación de Friedewald es confiable.

Palabras clave: LDL-C, triglicéridos, ecuación de Friedewald

Introduction

Hyperlipidemia leads to atherosclerosis and an increased risk of coronary heart disease (CHD). Blood lipids, such as total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and especially low-density lipoprotein cholesterol (LDL-C), become increasingly important in the diagnosis and management of CHD and related diseases. Epidemiological, autopsy, and animal studies have firmly established that a high LDL-C level is atherogenic[1]. The LDL-C known as a main risk factor in the cardiovascular disease and is the chief basis for diagnosis, risk classification and treatment in patients with hyperlipidemia[2]. This makes it very essential to determine serum LDL-C with high precision and accuracy[3].The direct homogenous assay and the indirect calculation methods are commonly used for LDL-C estimation[4]. Direct assays are reasonably specific and free from endogenous interference. However, there are disadvantages with direct methods such as over/under estimation of LDL-C, high cost and failure to meet the NCEP total error goals especially in diseased individuals[5]. The indirect LDL estimation method is by calculation using the landmark equation proposed by Friedewald et al.[6] using other lipid parameters viz. TC, HDL-C and TG. This Friedewald equation is LDL-C (mg/dl) = TC–TG/5 – HDL. However, this formula was not applicable to plasma samples containing chylomicrons, patients with Type III hyperlipoproteinemia and plasma TG>400 mg/dl[6]. This equation requires fasting serum sample and due to its simplicity, reliability and cost effectiveness this formula is the method of choice for routine quantification of LDL-C by most of the clinical laboratories[7]. 893 subjects' serum LDL-C was determined by direct and FF method and significantly higher LDL-f values found compared to LDL-d at TG <200 and TC>150 mg/dl. In this study, LDL-f classified 23.5% of patients at higher cardiac risk whereas by direct assays it was 17.58%[8]. In other study, no difference between LDL-f and d LDL-C at TG<150 mg/dl but at other levels of TG the d LDL-C was significantly higher than F LDL-C in 260 subjects[9]. Likewise, Warade et al.[10] also found FF LDL-C was lower than d LDL-C in 1768 Indian subjects. So, the aim of the current study was to investigate the impact of low serum TG on LDL-cholestrol estimation by Friedewald formula in Iranian population.

Materials and methods

Serum samples were taken from 540 patients referred to the laboratory centers under the Ahvaz University of Medical Sciences, Ahvaz, Iran during 2017. The patients fasted 12 hours before the sampling then TC, TG, HDL and LDL were measured using BT-1500 autoanalyzer and Pars diagnostic test kit. All patients informed about the study and signed the agreement form. Then samples divided into 4 groups based on the TG level 100>, 100-150, 150-250 and 250-400 mg/dl. Then, the LDL of patients whose serum levels of TG> 400 mg/dl measured with the indirect method and calculated as follows: Friedewald method= LDL – C (mg/dl) = TC–TG/5 – HDL. The results of Friedewald formula compared with the direct calculation results.

Statistical analysis

The obtained results Friedewald formula compared with the direct calculation results analyzed using one way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). For treatment showing a main effect by ANOVA, means compared by Tukey–Kramer test. $P<0.05$ was considered as significant differences between treatments.

Results

The frequency of the patients based on TG levels is presented in table 1. According to the results, there was no significant differences on patients frequency based on their TG level ($P>0.05$).

Table 1. The frequency of the patients based on TG level

Groups	Sex	N
TG <100	Male	53
	Female	91
TG 100-150	Male	60
	Female	84
TG 150-250	Male	65
	Female	77
TG 250-400	Male	60
	Female	50
Total		540

TG: triglycerides

The LDL, HDL, TC and TG levels of the patients based on their TG level is presented in table 2.

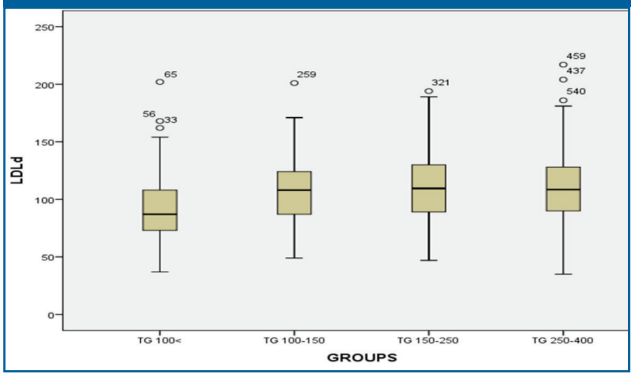
Table 2. The lipid profile of the patients based on the TG levels

TG Levels	Age (years)	LDL F (mg/dl)	LDL d (mg/dl)	TC (mg/dl)	HDL (mg/dl)
<100	37.67±16.809	104.134±31.9806	91.45±26.693	166.85±36.550	48.17±9.551
100-150	44.67±14.328	116.232±33.6095	106.35±27.744	190.44±40.496	49.60±11.300
150-250	43.23±13.169	116.918±33.0843	110.97±27.783	199.30±37.732	48.51±12.85
250-400	46.09±12.752	113.135±43.0730	109.70±30.273	214.21±48.313	41.45±10.471

TC: total cholesterol, TG: triglycerides , HDL: high density lipoprotein, LDL: low density lipoprotein

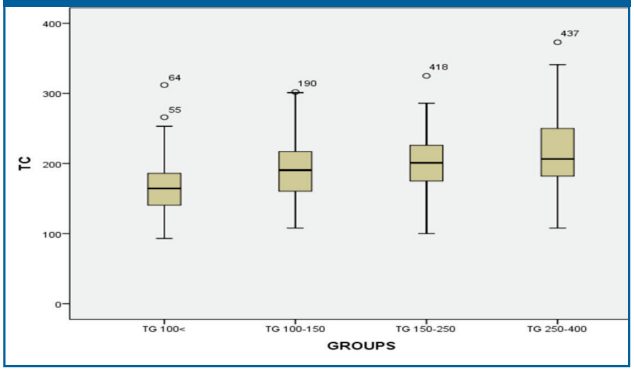
As seen in figure 1, The LDL d levels increased by elevation of the TG in patients.

Figure 1. The frequency of the serum LDL based on the TG levels. TG: triglycerides, LDL: low density lipoprotein.



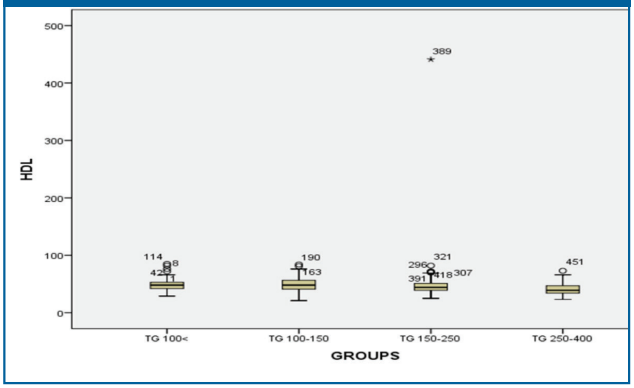
As seen in figure 2, by increase on TG levels, the TC level increased in patients.

Figure 2. the frequency of the serum TC based on the TG levels. TG: triglycerides, TC: total cholesterol



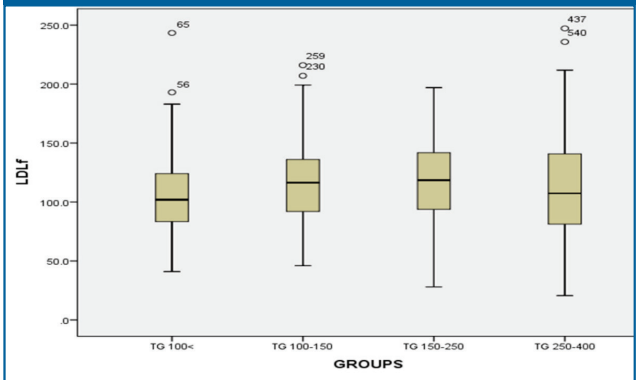
As seen, HDL levels diminished in patients by increase on TG level (figure 3).

Figure 3. the frequency of the serum HDL based on the TG levels. TG: triglycerides, HDL: high density lipoprotein



According to the results, the LDL f levels significantly increased on patients based on TG level (figure 4).

Figure 4. the frequency of the serum LDL-f based on the TG levels. TG: triglycerides, LDL: low density lipoprotein



According to the Table 3, a significant difference observed between LDL-C measured by the direct and by Friedewald equation (P<0.001). No significant differences were observed in patients with TG 250-400 levels (P>0.05). A correlation was found between between LDL-C measured by the direct and Friedwald Equation methods (P<0.001).

Table 3. The correlation between TG and LDL levels among patients

Groups	Mean (mg/dl)		P-value	Pair correlation
TG < 100	**LDL d**	91.45	<0.001	0.950
	LDL f	104.134		
TG 100-150	**LDL d**	106.35	<0.001	[0.867
	LDL f	116.232		
TG 150-250	**LDL d**	110.97	<0.001	0.905
	LDL f	116.918		
TG 250-400	**LDL d**	109.70	0.05	0.916
	LDL f	113.135		

TG: triglycerides, LDL: low density lipoprotein

Aggressive LDL-C lowering strategies are recommended for prevention of cardiovascular events in high-risk populations. Guidelines recommend a 30% to 50% reduction in at-risk patients even when LDL-C concentrations are between 70 and 130 mg/dl (1.8-3.4 mmol/L)[11]. Elevation of the serum LDL-C constitutes a major risk factor for the development of atherosclerosis and coronary heart disease[12]. Based on the serum LDL levels the National Cholesterol Education Program (NCEP) suggests different criteria for decision-making in treatment of hypercholesterolemic patients who have coronary heart disease or other risk factors[12]. The reference procedure for lipoprotein separation and measurement is analytical ultracentrifuge; however, this method is not readily available in the routine laboratory evaluation and its use is confined to research and specialized laboratories[13].

According to the results of the present study, a significant difference observed between LDL-C measured by the direct and by Friedewald equation (P<0.001). No significant differences were observed in patients with TG 250-400 levels (P>0.05). A correlation was found between LDL-C measured by the direct and Friedwald Equation methods (P<0.001). In a study, Ahmadi et al.[14] studied the impact of low serum triglyceride on LDL-C estimation among 230 Iranian population and revealed when TG is <100 mg/dL, calculated LDL-C is significantly overestimated (mean 12.17 mg/dL) whereas when TG is between 150 and 300 mg/dL no significant difference between calculated and measured LDL-C is observed. In patients with low serum TG and undesirably high TC levels, Friedewald equation may overestimate LDL-C concentration and it should be either directly assayed or be calculated by a modified Friedewald equation[14]. Sridevi et al.[15] studied compared Friedewald's and Anandaraja's formula with direct estimation of the LDL-C in Shivamogga population and revealed LDL-C levels were D-LDLC (117.78 ± 13.797 mg/dl), FF-LDL-C (115.51 ± 12.854 mg/dl) and AR-LDL-C (112.93 ± 11.671 mg/dl). There was underestimation of LDL-C by 2.27 mg/dl and 4.85 mg/dl by Friedewald's and Anandaraja's formulas respectively. It is reported FF is better in agreement with D-LDL-C than Anandaraja's formula for estimation of LDL-C by calculation though both lead to its underestimation[15]. In the past few decades attempts have been made to derive more accurate formulas for LDL-C calculation than the widely used Friedewald's formula[16]. Although the newer formulas offered few advantages over the Friedewald's, they have performed only marginally better, possibly due to diversity in terms of study populations and/or pathologies[17]. Kamazeki et al.[18] reported an underestimation of 5.9 mg/dl by FF compared to the directly measured LDL-C[17]. Vujovic et al.[1] have also reported higher values for D-LDL-C. They have found a percentage difference of −6.9% for FF-LDL-C and −3.9% for AR-LDL-C. Jun et al.[19] revealed that F-LDL-C differed significantly from D-LDL-C over the concentration ranges of both TC and TG. They found that the LDL was -9.1% and assumed that this difference was critical for the evaluation of patients with hyperlipidemia. Their study demonstrated that higher TG resulted in a greater F-LDL-C and increased TC was associated with decreased F-LDL-C, which was also confirmed in our current study[19]. In a study, Eljamil et al.[20] no significant differences between LDL-C obtained by Friedewald's formula (94.49 mg/dl) and the direct method (93.98 mg/dl) from samples with TG levels at <100 mg/dl with correlation coefficient of 0.86. The LDL-C levels produced by Friedewald's formula were significantly lower than those obtained by the direct method when serum TG levels at 101-200 and 201-300 mg/dl with correlation coefficient of 0.96 and 0.97 respectively. The 95% confidence intervals for a calculated LDL-C of 70 mg/dl (1.8 mmol/L) and 30 mg/dl (0.8 mmol/L) were 60 to 86 mg/dl (1.6 to 2.2 mmol/L) and 24 to 60 mg/dl (0.6 to 1.6 mmol/L), respectively. Previous recommendations have emphasized the requirement for a fasting sample with triglycerides <400 mg/dl (4.5 mmol/L) to calculate LDL-C by the Friedewald equation. However, no recommendations have addressed the appropriate lower reportable limit for calculated LDL-C[20]. Additionally, Choi et al.[21] revealed the Friedewald formula had the highest accuracy of all the triglyceride ranges, while the Vujovic formula had the highest accuracy in people with triglycerides≥300 mg/dL. The mean difference was the lowest for the Friedewald formula (0.5 mg/dL) and the percentage error was the lowest for the Vujovic formula (30.2%). However, underestimation of the LDL-C formulas increased with triglyceride concentrations. The accuracy of the LDL-C formulas varied considerably with differences in triglyceride concentrations. The Friedewald formula outperformed other formulas for estimating LDL-C against a direct measurement and the Vujovic formula was suitable for hypertriglyceridemic samples; it could be used as an alternative cost-effective tool to measure LDL-C when the direct measurement cannot be afforded[1,21].

In conclusion, these results suggested there was higher difference in low triglyceride levels (100> and 100-150 mg/dl) measured by direct and Friedewald Equation formula. Negligible differences observed in high TG levels (150-250 and 250-400 mg/dl) using direct and Friedewald Equation formula. With increased TG levels, the overestimation of the LDL-C level is decreased by Fried Wald formula. So, in high TG levels, Friedewald Equation formula is reliable.

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