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Significant Differences Between LDL-cholesterol Levels Obtained by Friedewald Formula and a Direct Method

Ashour S, Eljamil[1,*], Younes S. Elhenshiri[2], Ebtisam T. Etekbali[2] and Giuma S. Gimil[2]

[1]*Department of Biochemistry, Faculty of medicine, Tripoli University, Tripoli, Libya*

[2]*Laboratory Medicine Department, Biochemistry Unit, Tripoli Medical Centre, Tripoli, Libya*

Abstract: Estimation of low density lipoprotein cholesterol (LDL-C) level in serum is considered to be the basis of classification and management of hypercholesterolemia. In most clinical laboratories, LDL-C is usually estimated indirectly with the Friedewald's equation or more accurately with direct methods. The lack of agreement between the two methods has been reported in several clinical laboratories using different methods.

The present study is designed to compare LDL-cholesterol values obtained by Friedewald's formula and a direct method available in our laboratory (Dimension, RxL, SIEMENS). In the present study, we have found no significant differences between LDL-C obtained by Friedewald's formula (94.49 mg/dl ±28.81) and those determined by the direct method (93.98 mg/dl ±27.77) from samples with TG levels at $\leq$100 mg/dl ($p>0.4$) with correlation coefficient of 0.86. The LDL-C levels produced by Friedewald's formula were significantly lower than those obtained by the direct method when serum TG levels at 101-200 mg/dl ($p <0.001$) and 201-300 mg/dl ($p <0.01$) with correlation coefficient of 0.96 and 0.97 respectively. These differences are in agreement with those previously reported results in other laboratories. Therefore Friedewald's formula must be replaced by the direct method for better classification and management of hypercholesterolemia.

Keywords: Direct homogenous method, Friedewald's formula, High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Total cholesterol (TC), Triacylglycerol (TG).

INTRODUCTION

It is well established that high blood levels of low density lipoprotein cholesterol (LDL-C) is a major risk factor for developing coronary heart disease [1-5]. The recent National Cholesterol Education Program Adult Treatment Panel has identified LDL-C concentrations as the primary criterion of diagnosis and treatment of patients with hyperlipidemia [6]. Although the measurement of LDL-C is important, an easy, reliable, and suitable methodology for LDL-C has never existed in routine laboratories. β-Quantification has been considered the reference method [7], but it involves ultracentrifugation, requires large volumes of serum, and is a time-consuming and expensive technique. For those reasons, in most clinical laboratories LDL-C level has been estimated indirectly from measurements of total cholesterol, triacylglycerol (TG), and high density lipoprotein cholesterol (HDL-C) by the Friedewald' s equation [8].

$$[\text{LDL-C}] = [\text{total cholesterol}] - [\text{HDL-C}] - [\text{triacylglycerol}]/5$$

This equation is found to be valid when the triacylglycerol level is less than 400 mg/dl [9]. The potential advantages of directly measuring LDL-C include the ability to measure LDL-C even when the triacylglycerol are $\geq$400 mg/dl, the ability to measure LDL-C without the need to make the three measurements needed for the calculated result, and the potential reduction of imprecision by a single measurement instead calculating the value from three measured results [10]. Moreover Friedewald equation is invalid when samples are collected in the non-fasting state or in the presence of increased triacylglycerol levels [9, 11]. However, Friedewald equation has still considerable advantages including simplicity and lack of cost. Recently several direct homogenous methods for LDL-C estimation were developed and the kits are available for use by routine laboratories. There are several reports showing significant differences between the direct homogenous method and the Friedewald formula for LDL-C estimations [12-16]. The goal of the present study is to compare serum LDL-C levels obtained by the Friedewald formula and a direct homogenous (Dimension, RxL, SIEMENS) method available in our laboratory.

METHODS

Subjects and Blood Sampling

Fasting blood samples from 126 inpatient (52 men and 74 women), were collected in vacutainer tubes (Becton Dickinson), at Tripoli medical centre. The samples were allowed to clot at room temperature, and serum was isolated by centrifugation at 2000g for 5 minutes. All samples were analyzed within 2 hours from their arrival.

Statistical analysis

The statistical analyses of the present study were conducted by Microsoft office Excel (2007). Data are

*Address correspondence to this author at the Department of Biochemistry, Faculty of medicine, Tripoli University, Tripoli, Libya;
Tel: 00218913756793; Fax:00218215805085;
E-mail: ashoureljamil@yahoo.com

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Table 1. The Mean (± SD) Serum Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Triglycerides (TG) and a Comparison of Serum Low Density Lipoprotein Cholesterol (LDL-C), Obtained by Both Methods of all Subjects, (n = 126)

	mg\ dl Lipid ± SD	
C-LDL-C	95.10 ± 28.36	p < 0.0001
D-LDL-C	101.28 ± 27.32	r = 0.96
HDL-C	45.67 ± 13.59	n = 126
TC	166.42± 35.32	
TG	123.97 ± 62.50	

n= number of subjects, r = correlation coefficient, SD = standard deviation, p = paired t-test.

expressed as mean ± SD. The paired t-test and Pearson correlation analyses were used to assess the significance differences and correlation in LDL-C levels determined by Friedwald's formula and the direct method. Data were considered statistically significant at $p< 0.05$.

Lipid analysis

All lipid parameters were analyzed by using Dimension, RXL (SIEMENS, clinical chemistry system). The following parameters were measured with ready kits (Flex reagent cartridge) supplied by Dimension, RXL, SIEMENS, clinical chemistry system) and the procedure were carried out according to the manufacture. Triacylglycerol was carried out by enzymatic procedure using TGL, kit Cat. No. DF69A, Dimension, SIEMENS. Total cholesterol was estimated by cholesterol oxidase method, by using CHOL, Cat. No. DF27, Dimension, SIEMENS. HDL-C was measured directly without the need for any off-line pretreatment or centrifugation steps by using AHDL (automated HDL cholesterol) kit, Cat. No. DF48A, Dimension, SIEMENS. LDL-C was estimated by ALDL (automated LDL cholesterol) kit, Cat. No. DF131, Dimension, SIEMENS. The ALDL assay is a homogenous method for directly measuring LDL-C levels in serum or plasma, without the need for any off-line pretreatment or centrifugation steps. The method involves a detergent solubilization of only non-LDL particles, followed by consumption of the released cholesterol by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. The remaining LDL particles were solubillized by a second detergent and then LDL-C is oxidized by cholesterol esterase and cholesterol oxidase forming cholestenone and hydrogen peroxide (H_2O_2). The enzymatic action of peroxidase on H_2O_2 produces color in the presence of N, N-bis (4-sulfobutyl) - m-toluidine, disodium salt (DSBmT) and 4-aminoantipyrine (4-AA). The color formed is proportional to the amount of LDL-C present in the sample. LDL-C level was also determined by using the Friedewald equation [8].

Results

The serum lipid results obtained in the present study were expressed in mean ± S.D. Table **1** shows the mean serum TC, TG and LDL -C concentrations obtained by both methods of the 126 subjects. The mean serum HDL, TC and TG of the patients were found to be 45.67 mg \ dl ± 13.59 ,.42 166 ± 35.32 mg \ dl and 123.97 mg \ dl ± 62.50 respectively. The mean serum LDL-C values determined by the direct method (101.28 mg \ dl ± 27.32) were found to be significantly ($p<0.0001$) higher than those values obtained by the calculated formula (95.10 mg \ dl ± 28.36). There is a strong correlation between the 2 methods, r = 0.96. Table **2**, shows the comparison of serum LDL-C levels obtained by the calculated formula and the direct method, when TG concentrations are between 48 to 100 mg \ dl.

Table 2. Comparison of Serum Low Density Lipoprotein Cholesterol (LDL-C) Levels Obtained by the Calculated (C-LDL-C) and the Direct Method (D-LDL-C) when Triglycerides (TG) Concentrations are up to 100 mg / dl

	mg\ dl Lipid ± SD	
C-LDL-C	94.48 ± 28.81	P = 0.4
D-LDL-C	93.98 ± 27.77	r = 0.66
HDL-C	50.31 ± 14.13	n = 58
TC	159.78 ± 34.20	
TG	73.46 ± 15.47	

n= number of subjects, r = correlation coefficient, SD = standard deviation, p = paired t-test.

Table 3. Comparison of Serum Low Density Lipoprotein Cholesterol (LDL-C) Levels Obtained by the Calculated (C-LDL-C) and the Direct (D-LDL-C) Method when Triglycerides (TG) Concentrations are between 101-200 mg / dl.

	mg\ dl Lipid ± SD	
C-LDL-C	94.38 ± 27.85	P < 0.001
D-LDL-C	102.02 ± 25.16	r = 0.96
HDL-C	41.28 ± 12.57	n = 50
TC	163.36 ± 35.97	
TG	138.84 ± 28.43	

n= number of subjects, r = correlation coefficient, SD = standard deviation, p = paired t-test.

This group represents 46% of the present study subjects. The serum TC, HDL-C and TG contents were found to be (159.78 mg \ dl ± 34.20), (50.31 mg \ dl ± 14.13) and (73.46 mg \ dl ± 15.47) respectively. No significant differences (p= 0.4) were found in serum LDL-C levels obtained by the calculated formula (94.48 mg \ dl ± 28.81) and the direct method (93.98 mg \ dl ± 27.77), with a correlation coefficient of r = 0.86. Table **3**, shows the comparison of serum LDL-C levels obtained by the calculated formula and the direct method, when TG concentrations are between 101to 200 mg \ dl. This group represents 39.7% of the present study subjects.

The serum TC, HDL-C and TG contents were found to be (163.36 mg \ dl ± 35.97), (41.28 mg \ dl ± 12.57) and (138.84 mg \ dl ± 28.43) respectively. This group showed significantly higher ($P < 0.001$) serum LDL-C values obtained by the direct method (102.02 mg \ dl ± 25.16), than those estimated by the Friedwald formula (94.38 mg \ dl ± 27.85), with a correlation coefficient of r = 0.96. Table **4**, shows the comparison of serum LDL-C levels obtained by the calculated formula and the direct method, when TG concentrations are between 201to 300 mg \ dl. This group represents 14.3% of the present study subjects.

Table 4. Comparison of Serum Low Density Lipoprotein Cholesterol (LDL-C) Levels Obtained by the Calculated (C-LDL-C) and the Direct (D-LDL-C) Method when Triglycerides (TG) Concentrations are between 201-300 mg / dl.

	mg\ dl Lipid ± SD	
C-LDL-C	103.66 ± 28.71	p < 0.01
D-LDL-C	117.11 ± 22.53	r = 0.97
HDL-C	42.22 ± 9.40	n = 18
TC	195.00 ± 27.51	
TG	245.39 ± 28.29	

n= number of subjects, r = correlation coefficient, SD = standard deviation, p = paired t-test.

Table 5. Comparison of the Mean (± SD) Serum Low Density Lipoprotein Cholesterol (LDL-C) Values Obtained by the Direct Method (D-LDL-C) and the Calculated Formula (C-LDL-C) at Different TC Concentrations

	C-LDL-C	D-LDL-C	N	mg % TC
P < 0.0001	68.56 ±14.00	73.57±15.98	37	100-149
P < 0.00001	98.42±16.31	104.18± 14.48	67	150-199
P < 0.05	139.50±17.12	143.00±17.17	21	200-249

n= number of subjects, r = correlation coefficient, SD = standard deviation, p = paired t-test

The serum TC, HDL-C and TG contents were found to be (195.00 mg \ dl ± 27.51), (42.22 mg \ dl ± 9.40) and (245.39 mg \ dl ± 28.29) respectively. This group showed significantly higher (P < 0.01) serum LDL-C values obtained by the direct method (117.11 mg \ dl ± 22.53), than those estimated by the Friedwald formula (103.66 mg \ dl ± 28.71), with a strong correlation coefficient of r = 0.97.Table **5**. shows a comparison of LDL-C levels obtained by the direct method and the calculated formula when the samples were categorized into 3 groups, according to their TC content. A single sample result was omitted out of the 126 subjects due high TC content. The direct method showed significantly higher LDL-C levels (73.57 mg \ dl ± 15.98, 104.18 mg \ dl ± 14.48, & 143.00 mg \ dl ±17.17), than the calculated formula (68.56 mg \ dl ± 14.00, 98.42 mg \ dl ± 16.31, & 139.50 mg \ dl ± 17.12) when the samples TC content were at 100-149 mg \ dl, 150-199 mg \ dl and 200-249 mg/dl, with a p values of, 0.00001, 0.0001 and 0.05 respectively. There is a strong correlation coefficient between the C-LDL-C and D-LDL-C when the samples TC content were at 100-149 mg \ dl, 150-199 mg \ dl and 200-249 mg/dl, with r = 0.90, 0.84 and 0.90 respectively.

DISCUSSION

The national Cholesterol Education Programme' (NCEP) Adult Treatment Panel III (ATP III) indicated that LDL-C concentration was the primary basis for treatment and appropriate patients' classification in risk categories [6]. Therefore, LDL-C estimation must be accurate to establish a personal coronary heart disease (CHD) risk profile in order to initiate dietary adjustments, drug therapy and to monitor their effects [17].

The mean serum TC, HDL-C and TG contents reported in the present study were found to be (195.00 mg \ dl ± 27.51), (42.22 mg \ dl ± 9.40) and (245.39 mg \ dl ± 28.29) respectively, as shown in table1.The present study compared LDL-C levels estimated by Friedwald formula and a direct homogenous method in the serum of 126 randomly selected subjects. The mean serum LDL-C obtained by D-LDL-C = 101.28 mg \ dl ± 27.32 and C-LDL-C = 95.10 mg \ dl ± 28.36, were shown to be near optimal and optimal respectively according to NCEP, Adult Treatment Panel guidelines [6]. The direct LDL-C results were significantly higher (p < 0.0001) than the C-LDL-C results, with a correlation coefficient of, r = 0.96. When samples were categorized according to their TG content, no significant differences (p = 0.4) were found in serum LDL-C levels obtained by the calculated formula (94.48 mg \ dl ± 28.81), and the direct method (93.98 mg \ dl ± 27.77) for samples with TG up to 100 mg \ dl, with a correlation coefficient of r = 0.86. However, samples with serum TG levels between 101to 200 mg \ dl showed significantly higher (p < 0.001) serum LDL-C values obtained by the direct method (102.02 mg \ dl ± 25.16), than those estimated by the Friedwald formula (94.38 mg \ dl ± 27.85) , with a correlation coefficient of r = 0.96. Samples with TG serum levels between 201 and 300 mg \ dl showed also significantly higher (p < 0.01) serum LDL-C results for the direct method (117.11 mg \ dl ± 22.53), than those for the Friedwald formula (103.66 mg \ dl ± 28.71), with a correlation coefficient of r = 0.97.These results are in agreement with those reported by Kamal, *et al.* [18], and in disagreement with those reported by Mora, *et al.* [19]. We also compared LDL-C levels obtained by both methods when samples were divided according to their TC content into three categories. All categories showed that the direct LDL-C results (73.57 mg \ dl ±15.98, 104.18 mg \ dl ± 14.48 and 143.00 mg \ dl ±17.17) were significantly higher, (p < 0.0001, p < 0.00001 and p < 0.05) than the calculated LDL ones, (68.56 mg \ dl ±14.00, 98.42 mg \ dl ±16.31 and 139.50 mg \ dl ±17.12). However, there is a strong correlation coefficient between the C-LDL-C and D-LDL-C when the samples TC content were at 100-149, 150-199 and 200-249 mg/dl, with r = 0.90, 0.84 and 0.90 respectively. The Friedwald method is not recommended for use in non-fasting blood samples or in presence of hypertriglyceridemia (≥ 400 mg/dl) or type III hyperlipoproteinemia, for these reasons, an expert panel of the NCEP in 1995 has recommended the development of direct methods for the measurement of LDL-C [20, 21]. Finally, the significantly low LDL-C levels obtained by Friedwald's formula compared with the direct homogenous method reported in the present study, lead us to conclude that the calculated formula have to be replaced by the direct method.

LIST OF ABBREVIATIONS

Automated HDL cholesterol: AHDL, Automated LDL cholesterol: ALDL, Calculated Low density lipoprotein cholesterol: C-LDL-C, Direct Low density lipoprotein cholesterol: D-LDL-C, High density lipoprotein cholesterol: HDL-C, Low density lipoprotein cholesterol: LDL-C, National Cholesterol Education Program Adult Treatment Panel: NCEP ATP, Total cholesterol: TC, Triacylglycerol: TG, SD = Standard deviation.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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