ORIGINAL ARTICLE

Friedewald's Formula is Applicable up to Serum Triacylglycerol to Total Cholesterol Ratio of Two in Bangladeshi Population

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ABSTRACT

Friedewald's formula is the most frequently used formula for the calculation of serum low-density lipoprotein cholesterol from serum total cholesterol, serum triacylglycerol and serum high-density lipoprotein cholesterol. Most laboratories use serum triacylglycerol concentration of 400 mg/dl as upper cut-off limit for the calculation of LDL cholesterol, but a combination of serum triacylglycerol to total cholesterol ratio and serum triacylglycerol may have more advantages than serum triacylglycerol concentration alone to use Friedewald's formula effectively. The aim of this study was to determine the upper cut-off limit of serum triacylglycerol concentration and serum triacylglycerol to total cholesterol ratio to calculate LDL cholesterol using Friedewald's formula in Bangladeshi population. Serum total cholesterol, serum triacylglycerol, serum high-density lipoprotein cholesterol and serum low-density lipoprotein cholesterol were measured by direct method on 644 sera obtained from adult Bangladeshi study subjects after 12 hours of fasting. Serum low-density lipoprotein cholesterol was also calculated by using Friedewald formula. Low-density lipoprotein cholesterol obtained by Friedewald's formula in this study was compared with that obtained by direct method in different level of triacylglycerol and also in different triacylglycerol to total cholesterol ratio. Friedewald's formula underestimates low-density lipoprotein cholesterol when serum triacylglycerol concentration >300 mg/dL. But when direct serum low-density lipoprotein cholesterol was compared with low-density lipoprotein cholesterol calculated using Friedewald's formula up to serum triacylglycerol to total cholesterol ratio of 2, underestimation subsides, and the serum triacylglycerol level up to 700 mg/dl could be confidently included for the calculation of low-density lipoprotein cholesterol by Friedewald's formula. Friedewald's calculation formula can be confidently used up to serum triacylglycerol concentration of 700 mg/dl in Bangladeshi population, provided the serum triacylglycerol to total cholesterol ratio is two or less.

Key Words: Friedewald Formula, Low-Density Lipoprotein Cholesterol

Introduction

Serum low-density lipoprotein cholesterol is considered as an independent risk factor for coronary artery disease (CAD).[1] The reference method for the measurement of serum low-density lipoprotein cholesterol (LDLC) is the ß-Quantification that is costly, labor intensive and requires expensive ultracentrifuges, rotors, and

tubes.[2] It is time consuming and only a few number of samples can be investigated a day. Hence, its use in routine clinical laboratories is limited. Several direct methods for the estimation of low-density lipoprotein cholesterol are available now but all are expensive. As a result Friedewald's formula[3] or other calculation formulas developed by DeLong et al[4], Hattori et al[5], Rao et al[6] are used for the estimation of serum low-density lipoprotein cholesterol from three other lipid parameters. Friedewald's formula is most widely used. One of the most important limitations of the Friedewald formula is that it is invalid at serum triacylglycerol (TG) concentration > 400 mg/dL.[3] The equation is considerably inaccurate even at TG concentration of 200 – 400 mg/dL[7,8]. One study did not recommend the use of Friedewald formula when serum TG concentration> 265.5 mg/dL.[9] Friedewald formula frequently underestimates low-density lipoprotein cholesterol even when serum TG ≤ 400 mg/dL.[10] But Tremblay[11] showed that Friedewald's formula can be used at TG levels up to 786.5 mg/dL (9 mmol/L) but should be used with caution. TC/TG ratio was proposed as additional criteria to discern the validity limit.[12] As lipid parameters are influenced by genetic, environmental, socioeconomic status of the study population variation is observed in different studies.[13-17] So there might have different upper limit of serum triacylglycerol in our population for using the Friedewald's formula and the value can be obtained by correlating the serum triacylglycerol to total cholesterol ratio with direct low-density lipoprotein cholesterol.[7] Hence, it is necessary to set the upper cut-off limit of Friedewald's equation in our population.

Most of the laboratories in our country use 400 mg/dL or 450 mg/dL of serum TG concentration for the calculation of low-density lipoprotein cholesterol as upper cut-off limit. As combination of serum triacylglycerol concentration and serum triacylglycerol to total cholesterol ratio is stronger criteria than serum triacylglycerol concentration alone, we aimed to determine the upper cut-off limit of Friedewald's formula in terms of serum triacylglycerol concentration and serum triacylglycerol to total cholesterol ratio in our population.

Methods

Study subjects: A total of 644 adult subjects were selected purposively from the outpatient department of Bangladesh Institute of Health Sciences & Hospital. The mean age of the study subjects was 45±9 years. Of the total subjects, 52% were male and 48% were female. Blood specimens were collected after 12 hours of fasting and serum was separated for lipid profile analysis.

Biochemical analysis

Serum total cholesterol (TC), serum triacylglycerol (TG), serum high-density lipoprotein cholesterol (HDLC) and serum low-density lipoprotein cholesterol (LDLC) were measured on 644 sera obtained from adult study subjects after 12 hours of fasting. Lipid profiles were done on the same day using Dimension® RxL max auto-analyzer (Siemens Healthcare Diagnostics Ltd. UK). The TG estimation method is based on an enzymatic procedure in which a combination of enzymes are employed for the measurement of serum TG. In brief, incubation of sample with lipoprotein lipase enzyme reagent converts TG into free glycerol and fatty acids. Glycerol kinase catalyzes the phosphorylation of glycerol to glycerol-3-phosphate which is oxidized to dihidroxyacetone phosphate and hydrogen peroxide by the action of glycerol-3-phosphate oxidase. The catalytic action of peroxidase forms quinoneimine from hydrogen peroxide, aminoantipyrine and 4-chlorophenol. The change in absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and was measure using a bichromatic (510, 700 nm) endpoint technique. Serum TC was measured by an enzymatic method. In brief, cholesterol esterase catalyzes the hydrolysis of cholesterol esters to produce free cholesterol which, along with preexisting free cholesterol, is oxidized in a reaction catalyzed by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase, the hydrogen peroxide thus formed is used to oxidize N,N diethylaniline-HCl/4-aminoantopyrine to produce a chromophore that absorbs at 540 nm. The

absorbance due to oxidized diethylaniline-HCl/4-aminoantopyrine is directly proportional to the TC concentration and was measured using a polychromatic (452, 540, 700 nm) endpoint technique. Serum HDLC was measured by direct method using Dimension® RxL max. The HDLC assay measures HDLC concentration without sample pretreatment or specialized centrifugation steps, using a two reagent format. In the first reaction, chylomicrons, VLDL and LDL form water soluble complexes with dextran sulfate in the presence of magnesium sulfate. These complexes are resistant to the polyethylene glycol-modified cholesterol esterase and cholesterol oxidase that react with HDLC. In the presence of oxygen, HDLC is oxidized to Δ4-cholestenone and hydrogen peroxide. The hydrogen peroxide thus formed then reacts with 4-aminoantipyrine to form color complex that is measured by a bichromatic (600/700 nm) endpoint technique. The LDLC were measured by direct method using Dimension® RxL max. The LDLC assay is a homogenous method for directly measuring LDLC levels in human serum or plasma, without the need for any off-line pretreatment or centrifugation steps. The method is in a two reagent format and depends on the properties of detergent 1 which solubilizes only non-LDL particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. Detergent 2 solubilizes the remaining LDL particles. The soluble LDLC is then oxidized by the reaction of cholesterol esterase and cholesterol oxidase forming cholestenone and hydrogen peroxide. 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) that is measured using a bichromatic (540, 700 nm) endpoint technique. The color produced is directly proportional to the amount of LDLC present in the sample. This method has been certified using the Low Density Lipoprotein (LDL) Cholesterol Method Evaluation Protocol by the Cholesterol Reference Method Laboratory Network (CRMLN) at Northwest Lipid Research Laboratories, University of Washington, Seattle, Washington, 98103. Serum low-density was also calculated by using Friedewald formula. All tests kits, calibrators, quality control material were from Siemens Healthcare Diagnostics Ltd., Sir William Siemens Sq., Frimley, Camberly, UK GU16 8QD.

Statistical analysis

Data were expressed as mean±SD. Linear regression (using *STATISTICA* 6.0) analysis was used for the correlation between variables and two tailed paired t test analysis (using GraphPad Prism 5.0) was performed to assess significant differences in LDLC concentrations obtained by calculation and direct measurement.

Results

Mean±SD of lipid parameters are shown in table I and mean ± SD of direct and calculated LDLC is presented in table II in different serum TG groups. Table II shows that Friedewald's formula underestimates LDLC (bias±SD: −13.28±11.83) when serum TG concentration >300 mg/dL and when TG >400 mg/dL (bias±SD >−15.68±13.24) and negative bias increased in the subsequent TG groups. Comparison of the calculated LDLC with the direct LDLC is presented in table III according to different TG:TC groups. The difference between calculated and direct LDLC is out of limit of agreement when serum TG:TC >2 (table 3).

Table I: mean±SD of lipid parameters in the study group (644 subjects)

	Mean±SD
Serum TC (mg/dL)	218.78±52.34
Serum TG (mg/dL)	383.59±197.02
Serum HDL-C (mg/dL)	36.11±7.52
Serum LDL-C (mg/dL)	120.01±40.18

TC, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table II: Comparison of direct LDL-C and calculated LDL-C (644 subjects) in different TG groups

	TG (mg/dL)	dLDLC(mg/dL)	fLDLC(mg/dL)	Mean difference
Up to 100	75.43±16.85	101.65±33.04	102.06±34.87	0.41±7.18
101-200	153.62±27.46	130.73±41.81	127.38±46.43***	-3.35±8.64
201-300	244.48±29.45	147.18±54.27	142.68±62.19**	-4.50±13.13
301-400	353.87±29.71	120.82±36.82	107.54±45.56***	-13.28±11.83
401-500	450.95±28.18	120.02±33.44	104.33±38.80***	-15.68±13.24
501-600	543.98±25.09	110.82±30.39	87.67±36.45***	-23.14±14.00
601-700	646.34±27.55	102.54±35.99	75.04±46.02***	-27.50±18.89
701-800	750.78±28.59	101.17±34.86	63.87±45.85***	-37.29±21.38

TG, serum triacylglycerol; dLDLC, direct low-density lipoprotein cholesterol; fLDLC, low-density lipoprotein cholesterol calculated by Friedewald's formula; all concentrations are expressed as mg/dL; **P*<0.05, ***P*<0.01, ****P*<0.001

Table III: Comparison of direct and calculated LDLC (644 subjects) in different TG:TC groups

TG:TC	dLDLC(mg/dL)	fLDLC(mg/dL)	Mean difference
Up to 1.0	138.93±49.25	138.40±53.19	-0.53±9.67
>1 to ≤2	133.00±32.52	123.13±34.76***	-9.87±11.57
>2 to ≤3	103.26±23.19	81.03±24.65***	-22.22±12.62
>3 to ≤4	73.74±17.59	30.96±17.57***	-42.78±15.27

Table IV: Comparison of dLDLC and fLDLC (379 subjects) excluding TG:TC>2

	TG(mg/dL)	dLDLC (mg/dL)	fLDLC(mg/dL)	Mean difference
Up to 100	75.43±16.85	101.65±33.04	102.06±34.87	0.41±7.18
101-200	153.62±27.46	130.73±41.81	127.38±46.43***	- 3.35±8.64
201-300	243.89±29.27	148.51±53.59	144.36±61.15*	-4.15±12.91
301-400	347.91±30.44	137.09±28.21	127.11±35.92***	-9.99±11.37
401-500	444.11±25.42	144.75±25.92	134.12±28.57***	-10.64±13.61
501-600	535.76±32.84	156.06±25.05	147.84±25.53*	-8.22±13.92
601-700	652.00±39.15	194.4±35.5	190.27±17.27	-4.13±21.99
701-800	Nil	Nil	Nil	-

TG, serum triacylglycerol; dLDLC, direct low-density lipoprotein cholesterol; fLDLC, low-density lipoprotein cholesterol calculated by Friedewald's formula; all concentrations are expressed as mg/dL; **P*<0.05, ***P*<0.01, ****P*<0.001

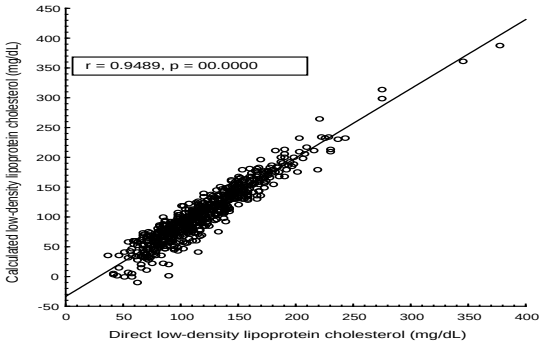

Figure 1: correlation of calculated low-density lipoprotein cholesterol with directly measured low-density lipoprotein cholesterol (all subjects)

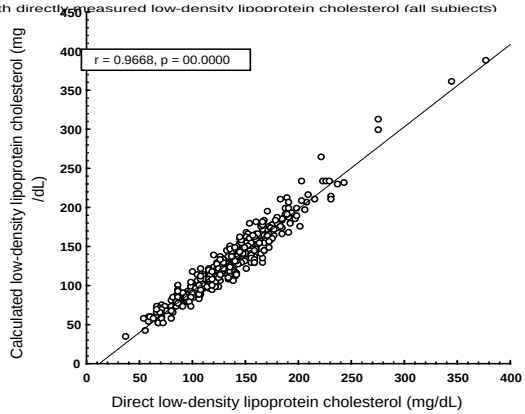

Figure 2: correlation of calculated low-density lipoprotein cholesterol with directly measured low-density lipoprotein cholesterol (excluding data having TG:TC >2)

Discussion

Serum LDLC calculated by Friedewald's formula correlated well with directly measured LDLC (r = 0.9668, *P* <0.0001) up to serum TG:TC ratio of 2 but mean value differ significantly (135.8±41.24 mg/dL and 130.3±44.93 mg/dL; *P* <0.0001). Difference between calculated and direct LDLC was – 5.5±11.68 mg/dL which is within acceptable limit. Moreover, this difference does not create any discrepancy regarding clinical aspects.

Friedewald formula underestimates LDLC when serum TG:TC>2. After exclusion of cases having TG:TC ratio>2, the difference of the calculated and direct low-density lipoprotein cholesterol was decreased (table 4) and the correlation coefficient was increased from 0.9489 to 0.9668. Though some cases were excluded (due to TG:TC> 2), some additional cases were included and most of

those had serum TG > 400 mg/dL but TG:TC<2 . When TG:TC ratio of 2 is considered as a primary criterion for the upper cut-off limit, Friedewald's formula can be used confidently up to serum TG concentration of 700 mg/dl in the population studied. Above TG: TC>2 and serum TG >700 mg/dl, serum LDLC should be measured by direct method.

Conclusion

Friedewald's calculation formula can be confidently used to estimate serum low-density lipoprotein cholesterol up to serum triacylglycerol concentration of 700 mg/dl with serum TG to TC ratio 2 in our population.

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