Validity of the Conventional Indirect Methods Including Friedewald Method for Determining Serum Low-Density Lipoprotein Cholesterol Level: Comparison with the Direct Homogeneous Enzymatic Analysis

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Abstract: **Validity of the Conventional Indirect Methods Including Friedewald Method for Determining Serum Low-Density Lipoprotein Cholesterol Level: Comparison with the Direct Homogeneous Enzymatic Analysis: Noriyuki NAKANISHI, *et al.* Department of Social and Environmental Medicine, Course of Social Medicine, Osaka University Graduate School of Medicine F2**—Low-density lipoprotein cholesterol (LDLC) concentrations are most commonly estimated by the Friedewald formula [LDLC=total cholesterol (TC) - high-density lipoprotein cholesterol - triglycerides (TG)/5]. To assess the validity of the conventional indirect methods including Friedewald method for determining serum LDLC level, we analyzed 1953 serum samples from Japanese male office workers and compared the measured (the direct N-geneous assay) serum LDLC concentrations with calculated values, by using several terms for TG (i.e., TG/4, TG/4.5, TG/5, TG/5.5, TG/6, TG/7, and TG/8) in the Friedewald formula. Linear regression analyses showed the highest correlation for the estimated LDLC by the original Friedewald formula, with the term TG/5, with the measured LDLC ($r=0.958$). The mean LDLC estimated by means of the original Friedewald formula was 1.4 mg/dl higher than the mean measured LDLC ($p<0.001$), but the difference between the mean estimated LDLC and the mean measured LDLC was the smallest for estimation by means of the original

Friedewald formula. As for the accuracy of calculation methods, TG/5 agreed best with the direct assay for TG concentrations ≤ 99 mg/dl and TG/4.5 was best for TG concentrations of 100–249 mg/dl. For TG concentrations ≥ 250 mg/dl, TG/5 most closely matched the measured LDLC and gave the smallest mean percent errors, but with increasingly large estimation errors as TG increased. As for the accuracy of calculation methods according to lipidemic type, TG/5 was the best estimating term for normolipidemics (TC ≤ 219 mg/dl and TG ≤ 149 mg/dl) and hypertriglyceridemics (TC ≤ 219 mg/dl and TG ≥ 150 mg/dl), but the percentages of samples correctly estimated decreased with increasing TG concentrations. These results suggest that the original Friedewald formula, with the term TG/5, is reasonably well classified at TG concentrations ≤ 99 mg/dl or in normolipidemics, but the potential for significant estimation errors steadily increases with increasing TG concentrations. We conclude that direct LDLC assay such as the N-geneous method is a useful tool in the diagnosis and management of hypercholesterolemics, especially for those with increased TG.

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Low-density lipoprotein (LDL) cholesterol (LDLC) is a well-established risk factor in coronary heart disease (CHD)^{1–3}. Several studies have also shown that when high LDLC concentrations are decreased by means of diet and drugs, the subsequent incidence of CHD is diminished^{4–8}. Therefore, the Adult Treatment Panel of

the National Cholesterol Education Program (NCEP) has affirmed that decisions to treat high blood cholesterol must be primarily based on the concentration of LDLC⁽⁹⁾. When the total cholesterol (TC) concentration exceeds 240 mg/dl, or when it exceeds 200 mg/dl and two or more other CHD risk factors are present, NCEP has recommended further lipoprotein testing to determine the concentration of LDLC. LDLC then turns out to be the basis for definitive dietary and drug treatment.

In most clinical laboratories, LDLC is currently estimated from the Friedewald formula [$\text{LDLC} = \text{TC} - \text{high-density lipoprotein (HDL) cholesterol (HDL)} - \text{triglycerides (TG)}/5^{(10)}$], although alternative terms such as $\text{TG}/6^{(1)}$ instead of $\text{TG}/5$ have also been used to estimate the very-low-density lipoprotein (VLDL) cholesterol (VLDLC), and the estimated LDLC is practically used in many epidemiologic and clinical studies. Calculation methods assume a constant cholesterol/triglyceride ratio in the VLDL particles. The validity of calculated LDLC values therefore does not depend on the accuracy of a sole direct assay, but rather on the accuracy of three other assays (TC, HDLC, and TG) with a mathematical calculation formula that estimates the amount of cholesterol in VLDLs, but it has been reported that at higher triglyceride levels, this ratio tends to decrease, rendering the original formula inaccurate^(11, 12).

In the present paper, with the direct N-geneous LDLC assay⁽¹³⁾, we describe the results of a comparison between the direct method and the conventional clinical methods for determining serum LDLC in middle-aged Japanese men.

Material and Methods

A survey to evaluate calculated values for LDLC was conducted in 1999 for the employees of T Corporation, which is one of the biggest building contractors in Osaka, Japan. The participants in the 1999 survey consisted of 1953 Japanese male office workers aged 30–59 yr, and the participation rate was 99.9%.

Fasting blood samples were obtained for measurements of the TC, HDLC, TG, and LDLC levels. The participants were requested to fast for 12 h and to avoid smoking and heavy physical activity for more than 2 h before the examinations. Concentrations of TC, HDLC and TG were determined enzymatically in an Olympus AU-5200 auto analyzer (Olympus Japan Co., Ltd., Tokyo, Japan) by FALCO Biosystems Co., Ltd., Tokyo, Japan. Quality control of the laboratory was maintained by an internal method, and the coefficients of variation were all within 3% for TC, HDLC and TG. To measure the LDLC concentration directly, the direct N-geneous LDLC assay⁽¹³⁾, which has met the NCEP Laboratory Standardization Panel analytical performance criteria ($< \pm 4\%$ imprecision and $< \pm 4\%$ bias), was performed according to the manufacturer's specifications in an

Olympus AU-5200 auto analyzer. The assay was available from Equal Diagnostics, Exton, PA (cat. no. 7120). After Reagent 1 is mixed with the serum specimen, Detergent 1 specifically disrupts non-LDL lipoproteins and causes the release of cholesterol. The free cholesterol formed, a result of hydrolysis by cholesterol esterase, reacts with cholesterol oxidase, generating hydrogen peroxide. The latter is consumed by a peroxidase in the presence of 4-aminoantipyrine to generate a colorless product. On the addition of Reagent 2, Detergent 2 specifically releases cholesterol from LDL particles. A similar enzymatic reaction to that described above occurs, except for hydrogen peroxide, which reacts with *N,N'*-bis-(4-sulfobutyl)-*m*-toluidine disodium salt, to generate a colored product. The intensity of the color generated is proportional to the concentration of LDLC. The assay was calibrated daily with the calibrators provided (cat. no. 7272; LDLC concentrations of 0 and 113 mg/dl).

In addition, LDLC concentrations were estimated by seven different calculation formulas: (a) the method of Friedewald *et al.*⁽¹⁰⁾, where $\text{LDLC} = \text{TC} - \text{HDL} - \text{TG}/5$; (b) the method of DeLong *et al.*⁽¹⁾ with the same formula, except that $\text{TG}/6$ is used in place of $\text{TG}/5$; and by use of the same formula, but with (c) $\text{TG}/4$, (d) $\text{TG}/4.5$, (e) $\text{TG}/5.5$, (f) $\text{TG}/7$, or (g) $\text{TG}/8^{(12)}$. The percentage variation (V) between the estimated LDLC and the measured (direct N-geneous LDL cholesterol assay) LDLC (C_e and C_d , respectively) was calculated as $V = (C_e - C_d) \times 100 / C_d$.

Data analysis was performed with the SPSS/PC statistical package (Marija J. Norusis, SPSS Inc., Chicago, IL, USA). Analyses of the relationships between the various calculated estimations of LDLC and the concentrations determined by the direct N-geneous LDLC method were done by the least-squares linear regression method. Paired t-test analyses were used to compare the significance of differences between the estimated LDLC and the measured LDLC. All reported p-values are two-tailed, and the level of significance is $p < 0.05$.

Results

Table 1 shows the results for the estimated LDLC by the Friedewald formula compared with the measured LDLC by the homogeneous enzymatic method. Linear regression analyses showed a high correlation of each estimated LDLC with the measured LDLC ($r = 0.936$ – 0.958), and the estimated LDLC by the original Friedewald formula, with the term $\text{TG}/5$, had the highest correlation with the measured LDLC. The mean LDLC estimated by the original Friedewald formula was 1.4 mg/dl higher than the mean measured LDLC ($p < 0.001$), but the difference between the mean estimated LDLC and the mean measured LDLC was the smallest among these seven estimations. The comparison-of-methods plot for the homogeneous enzymatic method (x) versus the original Friedewald formula (y) demonstrated $y = 1.045x$

Table 1. Results for estimated LDL cholesterol obtained with the Friedewald formula (y) compared with measured LDL cholesterol obtained with the homogeneous enzymatic method (x)

Term used in estimation	Linear regression equation	r	LDL cholesterol (mg/dl) ^a		p
			Mean (SD)	$\bar{y} - \bar{x}$ (SD)	
TG/4	$y = 1.048x - 10.7$	0.947	114.1 (32.1)	-5.0 (10.4)	<0.001
TG/4.5	$y = 1.046x - 6.9$	0.954	117.7 (31.8)	-1.5 (9.7)	<0.001
TG/5	$y = 1.045x - 4.1$	0.958	120.5 (31.7)	1.4 (9.2)	<0.001
TG/5.5	$y = 1.042x - 1.3$	0.954	122.8 (31.7)	3.7 (9.5)	<0.001
TG/6	$y = 1.041x + 0.7$	0.952	124.7 (31.7)	5.6 (9.8)	<0.001
TG/7	$y = 1.039x + 4.0$	0.944	127.8 (31.9)	8.7 (10.6)	<0.001
TG/8	$y = 1.037x + 6.5$	0.936	130.1 (32.1)	10.9 (11.3)	<0.001

^a Mean LDL cholesterol obtained with the homogeneous enzymatic method was 119.1 (SD 29.0) mg/dl. LDL, low-density lipoprotein; TG, triglyceride.

Fig. 1. Comparison of measured LDL cholesterol with estimated LDL cholesterol, using the coefficient (1/5) of TG in the Friedewald formula.

- 4.1 of a least-squares regression equation (Fig. 1).

Table 2 shows the percentages of subjects of which LDLC by estimation differed by less than 10% from the measured concentration according to the TG level. The percentages of subjects with values within 10% of the measured values steadily decreased with increasing TG concentrations in each formula. When TG concentrations were ≤ 99 mg/dl, use of the term TG/5 (Friedewald) led

to the highest percentages of individuals having an estimated LDLC that fell within 10% of the measured LDLC (95% for TG ≤ 49 mg/dl and 90% for TG 50–99 mg/dl). Nevertheless, the differences in the LDLC estimated by means of the seven formulas were generally small, below the 99 mg/dl TG level. In the TG range 100–249 mg/dl, TG/4.5 gave the closest results (83% for TG 100–149 mg/dl, 79% for TG 150–199 mg/dl, and

Table 2. Percentage of samples for which estimated LDL cholesterol was within 10% of measured LDL cholesterol^a

TG (mg/dl)	n	Term used in estimation						
		TG/4	TG/4.5	TG/5	TG/5.5	TG/6	TG/7	TG/8
≤49	103	85	91	95*	93 ^b	92 ^b	92	92
50–99	793	85	89	90* ^b	89	87	84	80
100–149	587	77	83* ^b	82	80	76	66	57
150–199	217	70	79* ^b	76	70	67	56	42
200–249	109	63	70* ^b	69	61	50	36	25
250–299	59	36	49	59* ^b	58	51	36	25
300–349	30	37	53* ^b	47 ^b	40	40	27	13
350–399	15	13	40 ^b	60* ^b	60* ^b	40	27	13
≥400	40	13	15	30 ^b	38* ^b	33	30	8

^a Within each TG range, * indicates the estimation factor with the greatest number of samples with less than 10% difference between measured and estimated LDL cholesterol concentration. ^b Estimation factors where the LDL cholesterol concentration was not significantly different ($P \geq 0.05$) from the measured concentration in paired t-test analysis. LDL, low-density lipoprotein; TG, triglyceride.

Table 3. Mean ($\pm$ SD) percent deviation of estimated LDL cholesterol from measured LDL cholesterol^a

TG (mg/dl)	n	Term used in estimation						
		TG/4	TG/4.5	TG/5	TG/5.5	TG/6	TG/7	TG/8
≤49	103	-4 $\pm$ 6 ^b	-3 $\pm$ 6 ^b	-2 $\pm$ 6 ^b	-1 $\pm$ 6 ^b	0 $\pm$ 5* ^b	1 $\pm$ 5 ^b	2 $\pm$ 6 ^b
50–99	793	-3 $\pm$ 6 ^b	-1 $\pm$ 6 ^b	0 $\pm$ 6* ^b	1 $\pm$ 6 ^b	2 $\pm$ 6 ^b	4 $\pm$ 6 ^b	5 $\pm$ 6
100–149	587	-4 $\pm$ 8 ^b	-1 $\pm$ 8* ^b	2 $\pm$ 8 ^b	4 $\pm$ 7 ^b	5 $\pm$ 7	8 $\pm$ 7	9 $\pm$ 8
150–199	217	-4 $\pm$ 11	0 $\pm$ 11* ^b	3 $\pm$ 11 ^b	5 $\pm$ 12	7 $\pm$ 12	11 $\pm$ 12	13 $\pm$ 13
200–249	109	-6 $\pm$ 10	-1 $\pm$ 9* ^b	4 $\pm$ 9 ^b	7 $\pm$ 9	10 $\pm$ 10	15 $\pm$ 10	19 $\pm$ 10
250–299	59	-12 $\pm$ 15	-5 $\pm$ 15	0 $\pm$ 14* ^b	5 $\pm$ 14	9 $\pm$ 14	15 $\pm$ 14	19 $\pm$ 14
300–349	30	-10 $\pm$ 14	0 $\pm$ 16* ^b	8 $\pm$ 18	14 $\pm$ 21	19 $\pm$ 23	27 $\pm$ 27	33 $\pm$ 29
350–399	15	-20 $\pm$ 19	-10 $\pm$ 18	-1 $\pm$ 17* ^b	6 $\pm$ 17	12 $\pm$ 17	21 $\pm$ 18	28 $\pm$ 19
≥400	40	-40 $\pm$ 31	-21 $\pm$ 28	-6 $\pm$ 29*	7 $\pm$ 32 ^b	17 $\pm$ 35	33 $\pm$ 41	46 $\pm$ 47

^a Within each TG range, * indicates the estimation factor with the lowest percent error. ^b Estimation factors where the mean estimated LDL cholesterol and the mean measured LDL cholesterol concentration varied by < 5 mg/dl. LDL, low-density lipoprotein; TG, triglyceride.

70% for TG 200–249 mg/dl). In the TG range 250–299 mg/dl, the term TG/5 had the highest percentages of values within 10% of the measured values, but these encompassed only 59% of the samples with TG values in this range. At concentrations of 300–349 mg/dl, TG/4.5 gave the closest agreement (53%); at concentrations of 350–399 mg/dl, TG/5 and TG/5.5 were closest at 60%; and for 400 mg/dl and more of TG, TG/5.5 had the highest percentage of agreement (38%). And the values estimated from the formulas with the closest estimations were not significantly different from the measured values in the each TG range of 50 mg/dl and more on paired t-test

analysis.

Table 3 shows the mean percent deviations of the estimated LDLC from the measured LDLC. In the range 49 mg/dl or less of TG, the lowest percent error was noted when TG/6 was used. In the range 50–99 mg/dl of TG, the lowest percent error was found with TG/5. When TG values were 100–249 mg/dl and 300–349 mg/dl, LDLC had the least errors when TG/4.5 was used. When TG values were 250–299 mg/dl or 350 mg/dl and over, TG/5 gave the smallest mean percent errors. Standard deviations of mean percent deviations of the estimated LDLC from the measured LDLC increased with

Table 4. Percentage of samples for which estimated LDL cholesterol was within 10% of measured LDL cholesterol, grouped by lipidemic type^a

Classification	TG (mg/dl)	n	Term used in estimation						
			TG/4	TG/4.5	TG/5	TG/5.5	TG/6	TG/7	TG/8
Normolipidemics ^b	≤149	1164	80	87	88*	87	85	81	74
Hypercholesterolemics ^c	≤149	319	89*	88	84	81	76	67	63
Hypertriglyceridemics ^b	150–249	174	54	69	71*	67	60	47	33
	≥250	69	19	33	44*	42	36	29	17
Combined hyperlipidemics ^c	150–249	152	84*	84*	76	67	62	52	41
	≥250	75	35	45	53	55*	48	33	16

^a Within each lipid category, * indicates the estimation factor with the greatest number of samples with less than 10% difference between measured and estimated LDL cholesterol concentrations. ^b Total cholesterol concentrations ≤219 mg/dl.

^c Total cholesterol concentrations ≥220 mg/dl. LDL, low-density lipoprotein; TG, triglyceride.

increasing TG concentrations in each formula.

Table 4 shows the percentages of samples for which LDLC was within 10% of the measured LDLC according to lipidemic type. Samples were divided into six subgroups for TC and TG: normolipidemics (TC≤219 mg/dl, TG≤149 mg/dl), hypercholesterolemics (TC≥220 mg/dl, TG≤149 mg/dl), hypertriglyceridemics (TC≤219 mg/dl, TG 150–249 mg/dl and TC≤219 mg/dl, TG≥250 mg/dl), and “combined” hyperlipidemics (TC≥220 mg/dl, TG 150–249 mg/dl and TC≥220, TG≥250 mg/dl). The best estimating formula for normolipidemics was TG/5, with 88% having estimated LDLC values within 10% of the measured values. Hypercholesterolemics had an estimation accuracy rate of 89% with the term TG/4. Hypertriglyceridemics with TG 150–249 mg/dl had an estimation accuracy rate of 71% with the term TG/5. For hypertriglyceridemics with TG≥250 mg/dl, the best estimated formula was TG/5, with only 44% of subjects correctly estimated. Combined hyperlipidemics with TG values of 150–249 mg/dl were correctly estimated in 84% of cases with TG/4 and TG/4.5; for those with TG≥250 mg/dl, 55% were correctly estimated with the term TG/5.5.

Table 5 shows the percentages of values classified correctly for estimated vs measured LDLC concentrations in the three sub-classes according to TG concentrations. Regardless of TG concentrations, samples with the lowest (≤119 mg/dl) measured LDLC were best classified with a low estimating formula (i.e., use of TG/4) and, conversely, for those with the highest (≥160 mg/dl) measured values, best classification with a high estimating formula (i.e., use of TG/8). Subjects with measured LDLC values in the middle range of 120–159 mg/dl had the possibility of being misclassified bidirectionally.

Discussion

In this study, linear regression analyses showed a high correlation for each estimated LDLC with the measured

LDLC ($r=0.936-0.958$). As for the accuracy of calculation methods, the proportion of samples falling within the fairly broad 10% range decreased continuously with increasing TG concentrations, irrespective of the calculation factor used. Variability became noticeable at TG concentrations ≥250 mg/dl, and at least 41% of individuals had an estimated LDLC that deviated from the measured values by more than 10% at 250–299 mg/dl TG concentrations. This percentage increased to more than 61% of individuals at TG concentrations ≥400 mg/dl. Furthermore, the percent error and its standard deviation of the estimated LDLC from the measured LDLC steadily increased with increasing TG concentrations in each formula, so that the ability to correctly estimate the LDLC concentration varies inversely with the TG concentration.

There has been controversy as to the most accurate formula for estimating LDLC, and attempts have been made to evaluate and refine the original estimation calculations proposed by Friedewald *et al.*¹⁰ to decrease potential estimation errors^{1, 14–17}, but the most frequently used estimation method in clinical laboratories is that of Friedewald *et al.*¹⁰ In the present study, the LDLC estimated by the original Friedewald formula, with the term TG/5, had the highest correlation with the measured LDLC ($r=0.958$), and the difference between the mean LDLC estimated by the original Friedewald formula and the mean measured LDLC was the smallest. Furthermore, utilization of the original Friedewald formula appears to provide a reasonable alternative for individuals with TG of 99 mg/dl or less. For those with TG of 250 mg/dl or more, TG/5 is still the term that gives results most closely matching the measured LDLC, but the potential error is so great for any given individual that its use cannot be recommended.

As for the accuracy of classification of LDLC values according to lipidemic type, it was found that those with TG of 149 mg/dl or less were usually correctly estimated

Table 5. Percentage of values classified correctly for estimated vs measured LDL cholesterol concentrations according to TG levels

LDL cholesterol (mg/dl)			Estimation terms (% classified)						
Measurement	Estimation	n	TG/4	TG/4.5	TG/5	TG/5.5	TG/6	TG/7	TG/8
For TG≤149 mg/dl									
≤119	≤119	778	97*	96	94	92	89	87	87
	120–139		3	6	6	8	11	13	13
	140–159		0	0	0	0	0	1	1
	≥160		0	0	0	0	0	0	0
120–139	≤119	481	25	19	13	9	6	4	4
	120–139		58	57	59*	57	56	52	52
	140–159		17	24	29	34	36	41	41
	≥160		0	0	1	1	2	3	3
140–159	≤119	100	0	0	0	0	0	0	0
	120–139		8	4	2	1	1	0	0
	140–159		82*	75	70	64	51	43	43
	≥160		10	21	28	35	48	57	57
≥160	≤119	124	0	0	0	0	0	0	0
	120–139		0	0	0	0	0	0	0
	140–159		29	22	15	10	7	4	4
	≥160		71	78	86	90	93	96	96*
Overall		1483	81*	80	80	78	76	73	73
For TG 150–249 mg/dl									
≤119	≤119	121	91*	90	84	79	74	64	59
	120–139		5	9	15	19	22	30	32
	140–159		1	1	2	3	3	6	8
	≥160		0	0	0	0	0	1	1
120–139	≤119	99	35	22	12	6	4	3	1
	120–139		52	55*	53	53	44	32	26
	140–159		13	23	34	39	48	54	58
	≥160		0	0	1	2	4	11	15
140–159	≤119	52	4	0	0	0	0	0	0
	120–139		44	39	23	12	6	4	2
	140–159		16	48	54	64*	56	46	42
	≥160		6	14	23	25	39	50	56
≥160	≤119	54	0	0	0	0	0	0	0
	120–139		2	2	0	0	0	0	0
	140–159		26	22	19	15	9	2	2
	≥160		72	76	82	85	91	98*	98*
Overall		326	70*	70	69	69	65	57	53
For TG≥250 mg/dl									
≤119	≤119	94	98*	93	86	81	75	67	57
	120–139		2	5	12	17	19	18	22
	140–159		0	2	2	1	4	13	18
	≥160		0	0	0	1	2	2	2
120–139	≤119	24	75	46	25	13	0	0	0
	120–139		17	42	54	67*	63	25	17
	140–159		8	8	17	17	29	58	54
	≥160		0	4	4	4	8	17	29
140–159	≤119	19	26	5	0	0	0	0	0
	120–139		32	42	32	21	5	0	0
	140–159		42	26	37	42	53*	37	21
	≥160		0	26	32	37	42	63	79
≥160	≤119	7	0	0	0	0	0	0	0
	120–139		29	14	0	0	0	0	0
	140–159		43	29	29	29	14	0	0
	≥160		29	57	71	71	86	100*	100*
Overall		144	74*	74*	74*	73	70	58	48

^a Within each guideline's category, * indicates the estimation factor with the highest percent of properly classified samples. LDL, low-density lipoprotein; TG, triglyceride.

(89% with the term TG/5 for normolipidemics and 88% with the term TG/4 for hypercholesterolemics). But those with increased TG concentrations (≥ 250 mg/dl) were much less correctly estimated (43% with the terms TG/5 and TG/5.5 for hypertriglyceridemics and 55% with the term TG/5.5 for combined hyperlipidemics). As for the percentage of samples properly classified for LDLC assay according to TG levels, those with values far from the general reference value (i.e., very low or very high) were most accurately classified, irrespective of the TG level. This is in part due to misclassification errors in these ranges being unidirectional and in part to these categories containing more samples with concentrations that were farther from the reference values. Individuals with values close to the reference value, where accuracy is most critical, were naturally at the highest risk of misclassification. Accordingly, the individuals with estimates close to the reference value are recommended to be rechecked with the direct measurement of LDLC to minimize inappropriate counseling.

In the present study, fasting blood samples were obtained from Japanese men aged 30 to 59 yr, and the relatively wide range of age might have affected the results of this study. As for the lipid and lipoprotein levels according to the age group, HDLC did not significantly differ ($p=0.290$, ANOVA) among the three age groups: 30–39 yr ($n=593$), 40–49 yr ($n=519$) and 50–59 yr ($n=841$) [means (SD): 55.1 mg/dl (12.5 mg/dl), 54.1 mg/dl (13.5 mg/dl), and 54.0 mg/dl (13.7 mg/dl), respectively]. On the other hand, there were significant differences in TC ($p<0.001$), TG ($p<0.001$), and measured LDLC ($p<0.001$) among the three age groups of 30–39, 40–49, and 50–59 yr [189.9 mg/dl (32.2 mg/dl), 206.1 mg/dl (35.2 mg/dl), and 204.2 mg/dl (31.0 mg/dl) for TC, respectively; 105.5 mg/dl (71.6 mg/dl), 138.3 mg/dl (96.2 mg/dl), and 136.4 mg/dl (86.2 mg/dl) for TG, respectively; and 113.2 mg/dl (27.5 mg/dl), 122.8 mg/dl (31.8 mg/dl), and 121.1 mg/dl (27.6 mg/dl) for LDLC, respectively]. Nevertheless, the correlation coefficients between LDLC estimated by means of the original Friedewald formula and the measured LDLC were 0.958, 0.962, and 0.956 for the 30–39 yr, 40–49 yr and 50–59 yr age groups, respectively. The comparison-of-methods plots for the homogeneous enzymatic method (x) versus the original Friedewald formula (y) showed least-squares regression equations of $y=1.050x - 4.8$, $y=1.046x - 3.9$, and $y=1.043x - 4.1$ for the 30–39 yr, 40–49 yr and 50–59 yr age groups, respectively. These results suggest that the differences in the lipid and lipoprotein levels according to age did not greatly affect the results of this study.

Ultracentrifugation is generally regarded as the standard reference method for measuring lipoprotein levels directly. The ultracentrifugation technique depends on the accuracy of centrifugation, HDL precipitation and

the cholesterol assay.

Owing to the expense and lack of availability of ultracentrifugation, methods were developed to estimate the LDLC concentration, and the estimated LDLC is practically used for the diagnosis and management of hypercholesterolemic patients, especially both in the community and at the workplace. These estimation techniques must rely on the accuracy of the TC, HDLC and TG assays, and also on an additional mathematical term that is used in an attempt to estimate the VLDLC concentration. Furthermore, it should be noted that patients adhere to 12- to 14-h fasts, because those with higher TG concentrations are in double jeopardy of being misclassified: they are subject to a higher rate of inaccurate LDLC estimation due to the increase in TG and are generally more sensitive to changes in serum TG from dietary fat intake, often requiring a longer time to return to the baseline after a meal^[8, 19]. Considering that use of the Friedewald formula for estimating LDLC becomes less reliable with increasing TG concentrations and that the N-geneous LDLC assay is unaffected by increased TG and meets the established NCEP analytical performance goals, the N-geneous LDLC assay appears to be a potential screening test for hypercholesterolemia and possibly a useful tool in the management of hyperlipidemic patients.

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