Comparison of Low Density Lipoprotein Cholesterol Concentrations by Direct Measurement and Friedewald Formula in Diabetic Patients with and without Hemoglobin E Disorders

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Background: *Low density lipoprotein cholesterol (LDL) levels were significantly lower in diabetic patients with homozygous hemoglobin E (HbEE) measured by a homogeneous assay.*
Objective: *Comparison of direct measurement of LDL (dLDL) determined by a homogeneous assay and calculated LDL (cLDL) determined by the Friedewald formula in diabetic patients with and without hemoglobin E disorders.*
Material and Method: *The hemoglobin E (HbE) screening test and hemoglobin (Hb) typing were conducted in diabetic patients at Surin Hospital. In 2,973 cases with triglyceride (TG) levels under 400 mg/dL, classification was determined into three groups, negative screening (NS), HbE trait (HbEA), and HbEE. The measurements of total cholesterol (TC) and TG were performed using enzymatic methods. The direct measurements of high density lipoprotein cholesterol (HDL) and LDL were performed using homogeneous methods.*
Results: *The prevalence of HbEE and HbEA were 7.6% and 35.7% respectively. The means of TG, CHOL, dLDL, cLDL, and non-HDL cholesterol (non-HDL-C) were significantly lower in HbEE ($p = 0.009$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$ respectively). The mean of cLDL in each group was significantly lower than the mean of dLDL ($p<0.001$ at all). By the Passing-Bablok regression, the interception with 95% confidence interval (95% CI) of NS, HbEA, and HbEE were 4.322 (3.082 to 5.485), 6.625 (5.094 to 7.981), and 6.60 (3.347 to 10.356) respectively. The slope with 95% CI were 1.017 (1.007 to 1.027), 1.002 (0.991 to 1.016), and 1.0 (0.963 to 1.033) respectively. Using the Bland-Altman method, the mean with standard deviation of the difference between dLDL and cLDL in NS, HbEA, and HbEE were 6.758 (7.856) mg/dL, 7.350 (8.212) mg/dL, and 7.225 (7.129) mg/dL respectively. The 95% limits of agreement between the dLDL and cLDL in NS, HbEA, and HbEE were -8.640 to 22.156 mg/dL, -8.746 to 23.446 mg/dL, and -6.748 to 21.197 mg/dL respectively. The statistically significant difference of having more patients with cLDL <100 mg/dL than dLDL <100 mg/dL in each group were observed in most of the subgroups of TG levels at 100 mg/dL to <200 mg/dL and higher. HbEE had more patients of dLDL <100 mg/dL and cLDL <100 mg/dL than NS. The adjusted odds ratio and 95% CI were 1.383 (1.022 to 1.871) with $p = 0.036$ and 1.838 (1.375 to 2.456) with $p<0.001$ respectively.*
Conclusion: *The direct homogeneous method showed a higher LDL concentration than the Friedewald formula indicated in diabetes and diabetes with HbE disorders. The percentage of higher LDL levels by direct method than Friedewald formula significantly increased along the subgroups of higher TG levels. The dissociation occurred at TG levels of 100 mg/dL and higher. Systematic biases between both methods were found in all groups but the proportional difference between both methods was only observed in diabetes without HbE disorders.*

Keywords: *Low density lipoprotein cholesterol, LDL, Direct measurement of LDL, Friedewald formula, Hemoglobin E disorders, HbEE, HbE trait, Diabetes mellitus, DM, Surin Hospital*

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Studies across different populations in the third report of the National Cholesterol Education Program (NCEP III) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) revealed that the elevation of low density lipoprotein cholesterol (LDL) serum concentration is one of the major risk factors for atherosclerosis and coronary heart disease (CHD)[1]. Diabetic patients carry a risk for CHD similar to that of people with established CHD and should have LDL levels less the 100 mg/dL. The results

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from those studies are mainly based on LDL concentrations calculated by the Friedewald formula (cLDL)[2]. However, different limitations of cLDL especially at higher levels of serum triglycerides (TG) lead to the reporting of erroneous results[3,4]. Homogeneous assays have been developed for the direct measurement of LDL (dLDL) concentrations, which have become popular for determining LDL. They have the advantages of being completely automated, performing without any manipulation, showing better precision, reproducibility and meeting the NCEP analytical goals[5,6].

The author had reported that dLDL by homogeneous assay was significantly lower in diabetic patients with homozygous hemoglobin E (HbEE) than in those patients with the hemoglobin E trait (HbEA) and the negative screening group[7]. Despite all of the information published related to cLDL and its limitations, there is no data about cLDL in diabetes with hemoglobin E (HbE) disorders. The objective of the present study was to compare the dLDL determined by a homogeneous assay and the cLDL determined by the Friedewald formula in diabetic patients with and without HbE disorders.

Material and Method

The present study was carried out by a simple random sampling method in the diabetes clinic at Surin Hospital between June 2009 and May 2010. The present study protocol was approved by the Ethics Committee of Surin Hospital. The demographic data were recorded on the day that the laboratory tests were performed after an overnight fasting for 12 hours. The fasting plasma glucose (FPG), Hemoglobin A1c (HbA1c), lipid profile, complete blood count including hemoglobin (Hb) concentration, BUN, and creatinine were tested on the same day.

The combination of the dichlorophenol-indolephenol (DCIP) test and low mean corpuscular volume (MCV) level were used as a HbE screening test[8]. The DCIP test utilized KKU-DCIP Clear reagent[9]. The Hb typing was performed in cases of a positive HbE screening test by the Hb Gold analyzer (Drew Scientific Ltd., England) using low-pressure liquid chromatography (LPLC). The interpretation of HbE from Hb Gold chromatogram was based on hematologic data in various HbE syndromes[10]. The cut-off point of anemia for each sex was classified by WHO standards[11]. The glomerular filtration rates (GFR) were calculated by using the modification of diet in renal disease (MDRD) study equation[12]. HbA1c was measured by turbidimetric inhibition immunoassay and the reagent was Tina-Quant Hemoglobin A1c II Cobas. The lipid profile consisted of the total cholesterol level (CHOL), TG, dLDL and high density lipoprotein cholesterol level (HDL). The CHOL and TG were measured by enzymatic colorimetric assay. The reagents were Cholesterol CHOD-PAP Cobas and Triglyceride GPO-PAP Cobas respectively. HDL and dLDL were measured by homogeneous enzymatic colorimetric assay. The reagents were HDL-C plus third generation Cobas and LDL-C plus second generation Cobas respectively. Both the HbA1c and lipid profile were analyzed by a Roche/Hitachi 917 automatic analyzer. The non-HDL cholesterol (non-HDL-C) level was calculated as CHOL minus HDL. The cLDL was determined indirectly by using the Friedewald formula[2] as follows: cLDL = CHOL-HDL-TG/5.

The participants with following characteristics were excluded, TG level of 400 mg/dL or higher, present of chylomicron in the sera, having history of chronic liver disease, and present of jaundice. The rest of participants were then classified into three groups, HbEA, HbEE, and negative screening (NS).

Statistical analysis

The data were presented as numbers and percentages for categorical variables, as means and standard deviations (SD) for continuous variables. The Pearson Chi-square and McNemar test were used to compare the differences between the categorical variables. Two-tailed tests were used to determine the statistical significance at a p-value of less than 0.05. The normality of distribution for each group was checked using the Kolmogorov-Smirnov test. The differences in mean values were compared using the Kruskal-Wallis test or Wilcoxon signed ranks test. The cLDL results obtained by Friedewald formula were compared to dLDL determined by homogeneous method using Passing-Bablok regression[13] with cumulative sum linearity test and the Bland-Altman method[14]. The logistic regression analysis by backward method was used to calculate odds ratio and 95% confidence interval (95% CI). These statistical analyses were performed using the MedCalc version 12.

Results

The present study included 2,973 of the 3,128 diabetic patients screened. Four hundred and forty two patients (14.9%) lived in the municipal area, 1,368 patients (46.0%) were older than 60 years,

2,587 patients (87.0%) had been diabetics for more than one year, and 1,560 patients (52.5%) had received statin therapy. The prevalence of HbEE and HbEA were 7.6% and 35.7% respectively. The quantities and the biochemical characteristics between NS, HbEA, and HbEE are shown in Table 1. The means of Hb and HbA1c were significantly lower in HbEE ($p<0.001$ both).

Table 1. Demographic data between diabetic patients with and without hemoglobin E disorders

	NS (n = 1,685) mean (SD) range	HbEA (n = 1,061) mean (SD) range	HbEE (n = 227) mean (SD) range	p-value*
Female (%)	70.7	72.3	72.7	0.621**
Age (year)	59.1 (10.8) 15.0 to 93.0	59.0 (10.6) 18.0 to 88.0	59.7 (10.4) 36.0 to 91.0	0.829
BMI (kg/m^2)	23.8 (3.98) 14.2 to 41.3	23.7 (4.16) 14.3 to 42.7	23.3 (3.86) 15.0 to 35.8	0.300
Hb (g/dL)	12.3 (1.71) 6.3 to 19.1	12.0 (1.60) 6.0 to 17.3	10.8 (1.44) 6.9 to 14.2	<0.001
FPG (mg/dL)	140.3 (45.6) 48.0 to 468.0	142.2 (48.1) 49.0 to 554.0	141.3 (43.9) 83.0 to 318.0	0.732
HbA1c (%)	7.63 (1.84) 4.5 to 17.6	7.42 (1.78) 4.0 to 16.3	6.45 (1.43) 4.1 to 14.2	<0.001
GFR (ml/min/1.73 m^2)	66.2 (22.2) 10.7 to 150.8	67.1 (22.9) 9.7 to 195.9	66.2 (23.2) 22.1 to 135.3	0.793

* Kruskal-Wallis test, ** Pearson Chi-square test
NS = negative screening; HbEA = hemoglobin E trait; HbEE = homozygous hemoglobin E; SD = standard deviation; BMI = body mass index; Hb = hemoglobin concentration; HbA1c = hemoglobin A1c; GFR = glomerular filtration rate

Table 2. Lipid profiles between diabetic patients with and without hemoglobin E disorders

	NS (n = 1,685) mean (SD) range	HbEA (n = 1,061) mean (SD) range	HbEE (n = 227) mean (SD) range	p-value*
TG (mg/dL)	159.7 (71.5) 40.0 to 398.0	163.4 (71.7) 33.0 to 397.0	148.5 (67.3) 43.0 to 399.0	0.009
CHOL (mg/dL)	198.7 (41.6) 87.0 to 362.0	198.9 (42.8) 92.0 to 362.0	180.7 (32.7) 102.0 to 309.0	<0.001
HDL (mg/dL)	50.2 (12.5) 21.0 to 111.0	50.0 (12.3) 24.0 to 121.0	49.2 (13.2) 21.0 to 130.0	0.358
dLDL (mg/dL)	123.3 (12.5) 34.0 to 263.0	123.6 (38.3) 35.0 to 271.0	109.0 (29.2) 38.0 to 210.0	<0.001
cLDL (mg/dL)	116.5 (36.7) 24.0 to 263.0	116.3 (38.2) 32.4 to 249.8	101.8 (29.2) 13.6 to 205.6	<0.001
p-value**	<0.001	<0.001	<0.001	
dLDL-cLDL (mg/dL)	6.76 (7.86) -33.2 to 34.6	7.35 (8.21) -46.2 to 56.0	7.23 (7.13) -16.6 to 24.4	0.115
Non-HDL-C (mg/dL)	148.4 (41.0) 46.0 to 315.0	148.9 (41.9) 57.0 to 297.0	131.5 (31.9) 65.0 to 271.0	<0.001

* Kruskal-Wallis test, ** Wilcoxon signed ranks test
NS = negative screening; HbEA = hemoglobin E trait; HbEE = homozygous hemoglobin E; SD = standard deviation; TG = triglycerides; CHOL = cholesterol; HDL = high density lipoprotein cholesterol; dLDL = direct measurement of low density lipoprotein cholesterol; cLDL = calculated low density lipoprotein cholesterol; Non-HDL-C = non-high density lipoprotein cholesterol

The means of TG, CHOL, dLDL, cLDL, and non-HDL-C were significantly lower in HbEE ($p = 0.009$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$ respectively); and the mean of cLDL in each group was significantly lower than the mean of dLDL ($p<0.001$ at all) as shown in Table 2.

The comparisons between dLDL and cLDL using Passing-Bablok regression and Bland-Altman method of all groups are shown in Table 3 and Fig. 1. By Passing-Bablok regression the interception with 95% CI of NS, HbEA, and HbEE were 4.322 (3.082 to 5.485), 6.625 (5.094 to 7.981), and 6.60

Table 3. Correlations between low density lipoprotein cholesterol levels determined by homogeneous assay and Friedewald formula of each group

	NS	HbEA	HbEE
Passing-Bablok regression			
Regression equation	y = 4.32+1.02x	y = 6.63+1.0x	y = 6.60+1.0x
Interception (95% CI)	4.322 (3.082 to 5.485)	6.625 (5.094 to 7.981)	6.600 (3.347 to 10.356)
Slope (95% CI)	1.017 (1.007 to1.027)	1.002 (0.991 to 1.016)	1.000 (0.963 to 1.033)
RSD (95% CI)	5.556 (-10.889 to 10.889)	5.819 (-11.404 to 11.404)	5.072 (-9.940 to 9.940)
Cumulative sum linearity test	p = 0.27	p = 0.80	p = 0.21
Bland-Altman method			
Mean of difference (SD)	6.758 (7.856)	7.350 (8.212)	7.225 (7.129)
95% CI of mean (mg/dL)	6.383 to 7.133	6.856 to 7.845	6.292 to 8.157
Lower limit (at -1.96 SD)	-8.640	-8.746	-6.748
95% CI of lower limit (mg/dL)	-9.281 to -7.998	-9.592 to -7.900	-8.344 to -5.152
Upper limit (at 1.96 SD)	22.156	23.446	21.197
95% CI of upper limit (mg/dL)	21.514 to 22.797	22.601 to 24.292	19.601 to 22.793

NS = negative screening; HbEA = hemoglobin E trait; HbEE = homozygous hemoglobin E; RSD = residual standard deviation; SD = standard deviation; 95% CI = 95% confidence interval

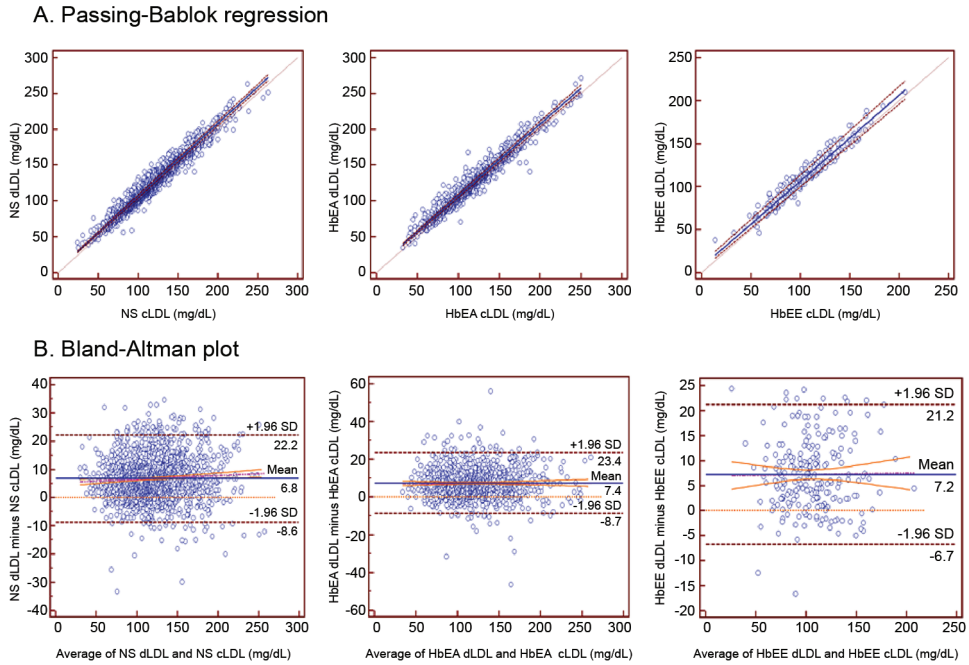

Fig. 1 Illustration of Passing-Bablok regressions between direct LDL vs. calculated LDL with 95% CI of each group; and Bland-Altman plots between means difference of dLDL and cLDL vs. average of dLDL and cLDL of each group.

(3.347 to 10.356) respectively; and the slope with 95% CI of NS, HbEA and HbEE were 1.017 (1.007 to 1.027), 1.002 (0.991 to 1.016), and 1.0 (0.963 to 1.033) respectively. No significant deviation from linearity was demonstrated by cumulative sum linearity test in each comparison analysis. By the Bland-Altman method the mean (SD) of the difference between dLDL and cLDL in NS was 6.758 (7.856) mg/dL, in HbEA was 7.350 (8.212) mg/dL and in HbEE was 7.225 (7.129) mg/dL. The 95% limits of agreement between dLDL and cLDL in NS was -8.640 to 22.156 mg/dL, in HbEA was -8.746 to 23.446 mg/dL and in HbEE was -6.748 to 21.197 mg/dL.

All groups were classified by TG levels into four subgroups, TG <100 mg/dL, TG 100 to <200 mg/dL, TG 200 to <300 mg/dL, and TG 300 to <400 mg/dL respectively. The quantities and percentages of dLDL <100 mg/dL and cLDL <100 mg/dL in each group are shown in Table 4. The statistically significant difference of having more patients of cLDL <100 mg/dL than dLDL <100 mg/dL in each group were observed in most of the subgroups of TG levels at 100 mg/dL to <200 mg/dL and higher.

The percentage of dLDL > cLDL significantly increased step-by-step along the subgroups of higher TG levels, whereas the percentage of (dLDL minus cLDL) >-10 to <10 mg/dL significantly decreased along the subgroups of higher TG levels as shown in Table 5.

The HbEE significantly had more patients of dLDL <100 mg/dL and cLDL <100 mg/dL than NS; after being adjusted with sex, age over 60 years, living in the municipal area, having length of diabetes more than one year, BMI, statin therapy, and anemia, the odds ratio and 95% CI were 1.383 (1.022 to 1.871) with p = 0.036 and 1.838 (1.375 to 2.456) with p<0.001 respectively. The HbEA had more patients of dLDL <100 mg/dL and cLDL <100 mg/dL than NS insignificantly, the adjusted odds ratio and 95% CI were 1.057 (0.89 to 1.257) with p = 0.526 and 1.055 (0.897 to 1.241) with p = 0.519 respectively. Moreover, 10.6% of dLDL 100 mg/dL and higher in NS had cLDL <100 mg/dL, 10.9% of dLDL 100 mg/dL and higher in HbEA had cLDL <100 mg/dL, and 23.1% of dLDL 100 mg/dL and higher in HbEE had cLDL <100 mg/dL.

Discussion

The prevalence of hemoglobin E disorders was very high in the diabetic clinic at Surin Hospital.

Table 4. Comparisons between dLDL <100 mg/dL and cLDL <100 mg/dL in subgroups of different TG levels

	NS (%)	HbEA (%)	HbEE (%)	p-value*
TG <100 mg/dL				
dLDL <100 mg/dL	137 (40.3)	80 (41.0)	24 (42.1)	0.962
cLDL <100 mg/dL	143 (42.1)	89 (45.6)	29 (50.9)	0.402
p-value**	0.263	0.078	0.125	
TG 100-<200 mg/dL				
dLDL <100 mg/dL	258 (27.4)	167 (24.8)	44 (35.5)	0.180
cLDL <100 mg/dL	318 (33.9)	202 (34.4)	62 (50.0)	0.002
p-value**	<0.001	<0.001	<0.001	
TG 200-<300 mg/dL				
dLDL <100 mg/dL	58 (18.3)	41 (19.2)	11 (29.7)	0.249
cLDL <100 mg/dL	95 (30.0)	62 (29.0)	19 (51.4)	0.021
p-value**	<0.001	<0.001	0.008	
TG 300-<400 mg/dL				
dLDL <100 mg/dL	22 (24.4)	19 (29.7)	5 (55.6)	0.134
cLDL <100 mg/dL	35 (38.9)	29 (45.3)	6 (66.7)	0.244
p-value**	<0.001	0.002	1.000	
Total				
dLDL <100 mg/dL	475 (28.2)	307 (28.9)	84 (37.0)	0.023
cLDL <100 mg/dL	591 (35.1)	382 (36.0)	116 (51.1)	<0.001
p-value**	<0.001	<0.001	<0.001	

* Pearson Chi-square, ** McNemar test

NS = negative screening; HbEA = hemoglobin E trait; HbEE = homozygous hemoglobin E; TG = triglycerides; dLDL = direct measurement of low density lipoprotein cholesterol; cLDL = calculated low density lipoprotein cholesterol

Table 5. Comparisons of dLDL >cLDL and (dLDL-cLDL) >-10 to <10 mg/dL between subgroups of different TG levels

	TG <100 mg/dL n = 592	TG 100-<200 mg/dL n = 1,650	TG 200-<300 mg/dL n = 568	TG 300-<400 mg/dL n = 163	p-value*
NS (%)					
dLDL > cLDL	203 (59.7)	783 (83.5)	310 (97.8)	90 (100)	<0.001
(dLDL-cLDL) >-10 to <10 mg/dL	315 (92.6)	720 (76.8)	117 (36.9)	10 (11.1)	<0.001
HbEA (%)					
dLDL > cLDL	120 (61.5)	498 (84.7)	207 (96.7)	64 (100)	<0.001
(dLDL-cLDL) >-10 to <10 mg/dL	174 (89.2)	445 (75.7)	81 (37.9)	7 (10.9)	<0.001
HbEE (%)					
dLDL > cLDL	41 (71.9)	109 (87.9)	35 (94.6)	9 (100)	0.005
(dLDL-cLDL) >-10 to <10 mg/dL	52 (91.2)	84 (67.7)	19 (51.4)	1 (11.1)	<0.001
Total (%)					
dLDL > cLDL	364 (61.5)	1,390 (84.2)	552 (97.2)	163 (100)	<0.001
(dLDL-cLDL) >-10 to <10 mg/dL	541 (91.4)	1,249 (75.7)	217 (38.2)	18 (11.0)	<0.001

* Pearson Chi-square

NS = negative screening; HbEA = hemoglobin E trait; HbEE = homozygous hemoglobin E; TG = triglycerides; dLDL = direct measurement of low density lipoprotein cholesterol; cLDL = calculated low density lipoprotein cholesterol

The means of Hb and HbA1c were significantly lower in diabetic patients with HbEE which results were similar to a previous study[7]. The CHOL, TG, dLDL, cLDL, and non-HDL-C were significantly lower in diabetic patients with HbEE whereas the HDL had no statistical difference between each group. There was no scientific data about these findings at this time. Because the different pattern of lipid profiles were found in diabetes with HbEE and TG levels also related to cLDL, the author aimed to clarify the correlation of dLDL and cLDL in each group. The dLDL levels were usually higher than the cLDL levels[15] as shown in Table 2, therefore dLDL levels could not replace cLDL levels if one wants to use the LDL cut-off values recommended in NCEP III as a guide for management of patients with dyslipidemia. Moreover, when TG levels were 200 mg/dL and higher, cLDL declined in accuracy with dLDL within a -10 to 10 mg/dL difference[16]. The recommendations from many studies in NCEP III are mainly based on cLDL, which was calculated from various levels of TG in each study[1,2]. The present study showed the effects of TG on the recommended cut-off points of LDL in diabetes determined by Friedewald formula compared with dLDL. In all groups, the dissociations between cLDL <100 mg/dL and dLDL <100 mg/dL significantly occurred at TG 100 mg/dL or higher as shown in Table 4. This finding raises the question on the definition of misinterpretation of LDL levels. Whether dLDL levels lead to over diagnosis of dyslipidemia[15] especially in diabetes as described, or cLDL levels in diabetes are more unreliable than dLDL at higher TG levels[16] at least in this population. However, the author had also clarified the difference between dLDL and cLDL levels and the acceptable range of the difference within -10 mg/dL to 10 mg/dL as shown in Table 5. The percentage of higher dLDL levels than cLDL levels significantly increased step-by-step along the higher TG levels in all groups whereas the percentage of the acceptable range of difference had reverse characteristics.

The Kolmogorov-Smirnov test was performed to study normality, and the distribution was not normal for both cLDL and dLDL in all groups. Neither the LDL from the Friedewald formula nor the LDL from the homogeneous method is free of random error because each method is not the gold standard. Furthermore, the methods were compared over a wide concentration range of the analyses that cover the values of normal and abnormal. Because of these conditions, the combination of Passing-Bablok regression and Bland-Altman method instead of simple linear regression were chosen for the comparisons as shown in Table 3 and Fig. 1. The linear model fit the data of each group, and systematic errors were demonstrated in all groups by Passing-Bablok regression since 95% CI of the interceptions did not cover zero. Only the group of NS had proportional difference for 95% CI of the slope did not cover one. The Bland-Altman method also demonstrated substantial means of difference between LDL levels of both methods that had no significant statistical

difference among all groups as shown in Table 2 and 3. Nevertheless, the 95% limits of agreement in all groups covered wide ranges (roughly around 30 mg/dL in all groups) which were clinically important. Thus, both methods may not be interchangeably used in clinical practice. The magnitude of LDL levels had an influence on the association of both methods in NS as upward slope of the trend line by Bland-Altman plot was observed in Fig. 1.

Although most of trials on risk factors for atherosclerosis and CHD have been performed with cLDL, recent recommendations of ESC/EAS guidelines for the management of dyslipidemias suggested that direct methods for determining LDL should be used whenever available[17]. The direct methods have good reproducibility and specificity, and have the advantage that the analysis is made in one-step and they are not sensitive to variations in TG levels to the same extent. The author preferred dLDL rather than cLDL because the interferences of TG on cLDL were obviously found at lower TG level than the previous studies[4,16]. The author also suggested that even though there were good correlations between both methods, the regression equations should not be used to transform between cLDL and dLDL because there were wide ranges of 95% limits of agreement in all groups.

Conclusion

The direct homogeneous method showed higher LDL concentration than the Friedewald formula indicated in diabetes and diabetes with HbE disorders. The percentage of higher LDL levels by direct methods than Friedewald formula significantly increased along the subgroups of higher TG levels, the dissociation occurred at TG levels of 100 mg/dL and higher. Systematic biases between both methods were found in all groups but the proportional difference between both methods was only observed in diabetes without HbE disorders. Because the wide ranges of 95% limits of agreement are clinically important, both methods could not be used interchangeably by regression equations in all groups.

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Potential conflicts of interest

None.

References

1. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 106: 3143-421.
2. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
3. Jun KR, Park HI, Chun S, Park H, Min WK. Effects of total cholesterol and triglyceride on the percentage difference between the low-density lipoprotein cholesterol concentration measured directly and calculated using the Friedewald formula. Clin Chem Lab Med 2008; 46: 371-5.
4. Charuruks N, Milintagas A. Evaluation of calculated low-density lipoprotein against a direct assay. J Med Assoc Thai 2005; 88 (Suppl 4): S274-9.
5. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clin Chem 2002; 48: 236-54.
6. Esteban-Salan M, Aguilar-Doreste JA, Arranz-Pena ML, Juve-Cuxart S, Gich-Salarich I, Zapico-Muniz E, et al. Multicentric evaluation of the homogeneous LDL-cholesterol Plus assay: comparison with beta-quantification and Friedewald formula. Clin Biochem 2008; 41: 1402-9.
7. Srisurin W. Prevalence and effect of hemoglobin E disorders on HbA1c and lipid profile of diabetic patients at Surin Hospital. J Med Assoc Thai 2011; 94: 36-42.
8. Prayongratana K, Polprasert C, Raungrongmorakot K, Tatone K, Santiwatanakul S. Low cost combination of DCIP and MCV was better than that of DCIP and OF in the screening for hemoglobin E. J Med Assoc Thai 2008; 91: 1499-504.
9. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. Bull World Health Organ 2004; 82: 364-72.
10. Vichinsky E. Hemoglobin e syndromes. Hematology Am Soc Hematol Educ Program 2007; (1): 79-83.

11. World Health Organization. Methods of assessing iron status. In: Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. WHO/NHD/01.3. Geneva: WHO; 2001: 33-45.
12. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007; 53: 766-72.
13. Passing H, Bablok. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem 1983; 21: 709-20.
14. Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet 1995; 346: 1085-7.
15. Wongtiraporn W, Wattanamongkonsil L, Kiartivich S, Mingvivat N, Thanakhumtorn S, Opartkiattikul N, et al. Utilization of calculated low density lipoprotein cholesterol and measured low density lipoprotein cholesterol in Siriraj Hospital. J Med Assoc Thai 2006; 89 (Suppl 5): S156-63.
16. Puavilai W, Laoragpongse D. Is calculated LDL-C by using the new modified Friedewald equation better than the standard Friedewald equation? J Med Assoc Thai 2004; 87: 589-93.
17. Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Eur Heart J 2011; 32: 1769-818.

เปรียบเทียบค่าของไขมันชนิดแอลดีแอลระหว่างวิธีวัดโดยตรงกับวิธีคำนวณโดยใช้สูตรของ Friedewald ในผู้ป่วยเบาหวานที่เป็นและไม่เป็นฮีโมโกลบิน อี

วสันต์ ศรีสุรินทร์

วัตถุประสงค์: *ศึกษาเปรียบเทียบค่าของไขมันชนิดแอลดีแอลระหว่างวิธีวัดโดยตรงกับวิธีคำนวณโดยใช้สูตรของ Friedewald ในผู้ป่วยเบาหวานที่เป็นและไม่เป็นฮีโมโกลบิน อี*

วัสดุและวิธีการ: *ทำการคัดกรองและตรวจหาชนิดของฮีโมโกลบิน อี ในผู้ป่วยเบาหวานที่มีค่าไตรกรีเซอไรด์ต่ำกว่า 400 มิลลิกรัมต่อเดซิลิตร ในโรงพยาบาลสุรินทร์ จำนวน 2,973 ราย แบ่งผู้ป่วยออกเป็น 3 กลุ่ม คือ โรคโลหิตจางธาลัสซีเมียชนิดอี, ฮีโมโกลบินอีแฝง และการคัดกรองให้ผลลบ วัดค่าไขมันชนิดแอลดีแอลโดยตรงด้วยวิธี homogeneous เปรียบเทียบกับวิธีคำนวณโดยใช้สูตรของ Friedewald*

ผลการศึกษา: *พบผู้ป่วยเบาหวานที่มีโรคโลหิตจางธาลัสซีเมียชนิดอีร้อยละ 7.6 มีฮีโมโกลบินอีแฝงร้อยละ 35.7 พบค่าเฉลี่ยของไตรกรีเซอไรด์ คอเลสเตอรอล ไขมันแอลดีแอลโดยวิธีวัดตรง ไขมันแอลดีแอลโดยการคำนวณ และค่าคอเลสเตอรอลที่หักค่าไขมันเอชดีแอลออกแล้วในกลุ่มโรคโลหิตจางธาลัสซีเมียชนิดอีต่ำกว่าอย่างมีนัยสำคัญทางสถิติ ค่าเฉลี่ยของแอลดีแอลวัดโดยการคำนวณต่ำกว่าค่าเฉลี่ยโดยวิธีวัดตรงอย่างมีนัยสำคัญทางสถิติในทุกกลุ่ม ใช้วิธี Passing-Bablok regression เปรียบเทียบ พบค่า Interception และค่าความเชื่อมั่นร้อยละ 95 ของกลุ่มการคัดกรองให้ผลลบ, ฮีโมโกลบินอีแฝงและโรคโลหิตจางธาลัสซีเมียชนิดอี คือ 4.322 (3.082 ถึง 5.485), 6.625 (5.094 ถึง 7.981) และ 6.60 (3.347 ถึง 10.356) ตามลำดับ พบค่า Slope และค่าความเชื่อมั่นร้อยละ 95 ของกลุ่มการคัดกรองให้ผลลบ, ฮีโมโกลบินอีแฝง และโรคโลหิตจางธาลัสซีเมียชนิดอี คือ 1.017 (1.007 ถึง 1.027), 1.002 (0.991 ถึง 1.016) และ1.0 (0.963 ถึง 1.033) ตามลำดับ ใช้วิธี Bland-Altman พบค่าเฉลี่ยและค่าเบี่ยงเบนมาตรฐานของผลต่างระหว่างไขมันแอลดีแอลวัดโดยวิธีวัดตรงกับวัดโดยการคำนวณ ในกลุ่มการคัดกรองให้ผลลบ, ฮีโมโกลบินอีแฝง และโรคโลหิตจางธาลัสซีเมียชนิดอี คือ 6.758 (7.856), 7.350 (8.212) และ 7.225 (7.129) มิลลิกรัมต่อเดซิลิตร ตามลำดับ พบค่า 95% limits of agreement ระหว่างไขมันแอลดีแอลวัดโดยวิธีวัดตรงกับวัดโดยการคำนวณในกลุ่มการคัดกรองให้ผลลบ, ฮีโมโกลบินอีแฝง และโรคโลหิตจางธาลัสซีเมียชนิดอี คือ -8.640 ถึง 22.156 มิลลิกรัมต่อเดซิลิตร, -8.746 ถึง 23.446 มิลลิกรัมต่อเดซิลิตร และ -6.748 ถึง 21.197 มิลลิกรัมต่อเดซิลิตร ตามลำดับ พบจำนวนผู้มีค่าไขมันแอลดีแอลโดยวิธีคำนวณน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร มากกว่าจำนวนผู้มีค่าไขมันแอลดีแอลโดยวิธีวัดตรงน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร ในกลุ่มย่อยที่มีค่าไตรกรีเซอไรด์สูงตั้งแต่ 100 มิลลิกรัมต่อเดซิลิตร ขึ้นไปแทบทุกกลุ่มย่อยอย่างมีนัยสำคัญทางสถิติ ผู้ป่วยเบาหวานที่มีโรคโลหิตจางธาลัสซีเมียชนิดอีพบอัตราผู้มีค่าไขมันแอลดีแอลโดยวิธีวัดตรงน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร และอัตราผู้มีค่าไขมันแอลดีแอลโดยวิธีคำนวณน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร มากกว่ากลุ่มการคัดกรองให้ผลลบ โดยค่า adjusted odds ratio และค่าความเชื่อมั่นร้อยละ 95 เป็น 1.383 (1.022 ถึง 1.871) และ 1.838 (1.375 ถึง 2.456) โดยมีค่า $p = 0.036$ และ $p<0.001$ ตามลำดับ*

สรุป: *ค่าไขมันแอลดีแอลโดยวิธีวัดตรงสูงกว่าค่าไขมันแอลดีแอลโดยวิธีคำนวณตามสูตรของ Friedewald ในผู้ป่วยเบาหวานทั้งมีและไม่มีฮีโมโกลบินอี โดยเริ่มพบความแตกต่างอย่างมีนัยสำคัญทางสถิติที่ระดับไตรกรีเซอไรด์ตั้งแต่ 100 มิลลิกรัมต่อเดซิลิตร ขึ้นไป พบ systematic bias เมื่อเปรียบเทียบการวัดระหว่างสองวิธีในทุกกลุ่ม แต่ proportional difference พบเฉพาะในกลุ่มผู้ป่วยเบาหวานที่ไม่มีฮีโมโกลบินอี*