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Evaluation of an HPLC method for LDL-cholesterol determination in patients with various lipoprotein abnormalities in comparison with beta-quantification

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ABSTRACT

Background: The main purpose of this study was to evaluate an HPLC method for LDL-cholesterol determination in the presence of abnormal lipoproteins.
Methods: We compared LDL-cholesterol levels obtained by HPLC (HPLC-LDL), Friedewald (F-LDL), and β-quantification (BQ-LDL) methods on 47 healthy volunteers and 50 outpatients with lipid disorders, including apolipoprotein E2/2 phenotype, cholesteryl ester transfer protein deficiency and lipoprotein lipase deficiency.
Results: For the control group ($n=50$), the HPLC-LDL and the F-LDL correlated highly with the BQ-LDL ($r=0.984$ and 0.969, respectively), but the HPLC-LDL was lower than the BQ-LDL (mean bias: −4.0%, $P<0.001$). For the subjects with lipoprotein abnormalities, significant biases were found in HPLC-LDL for the hypertriglyceridemia (+25%, $n=17$, $P<0.01$), the hyper HDL (−15.2%, $n=10$, $P<0.01$) and the hyper lipoprotein(a) groups (−13.4%, $n=12$, $P<0.001$). The F-LDL was significantly higher than the BQ-LDL in the apolipoprotein E2/2 subjects (+92%, $n=8$, $P<0.001$), but not significantly different in other subjects with triglycerides <4000 mg/l.
Conclusions: There were several discrepancies in LDL-cholesterol levels determined by the HPLC and the BQ methods in samples with lipoprotein abnormalities. However, the HPLC method can be useful and informative for analysis of abnormal lipoproteins.

1. Introduction

The association between total cholesterol (TC) and risk of developing coronary heart disease (CHD) has been well established. Most of the cholesterol in circulation is carried by low-density lipoproteins (LDL), which has been conclusively shown by many prospective studies and randomized clinical trials to be primarily responsible for the association with CHD risk [1]. The Adult Treatment Panel (ATP) guidelines of the National Cholesterol Education Program consider LDL-cholesterol (LDL-C) the major indicator for initial classification of CHD risk status and identify lowering of LDL-C as the primary goal of therapy [2].

The most widely accepted reference method for LDL-C is a version of β-quantification (BQ) combining ultracentrifugation and heparin/$MnCl_2$ precipitation, as performed at the CDC [3]. The LDL-C values determined by the BQ method, which are calculated by subtracting HDL-cholesterol (HDL-C) from cholesterol concentration measured in the 1.006 kg/l bottom fraction obtained by ultracentrifugation, include intermediate-density lipoproteins (IDL; density, 1.006–1.019 kg/l) and lipoprotein(a) [Lp(a)] as well as LDL particles with a density of 1.019–1.063 kg/l. IDL and Lp(a) are also generally considered to be atherogenic. Therefore, LDL-C values by the BQ method can be considered to represent the cholesterol contained in several potentially atherogenic lipoproteins [3].

The most common approach to determining LDL-C in the clinical laboratory is the Friedewald calculation [4], but the calculated LDL-C can be inaccurate for serum triglyceride (TG) concentrations >4000 mg/l or in the presence of chylomicrons (CM) or type III hyperlipoproteinemia. Recently, a new generation of homogeneous methods capable of full automation has been introduced that uses various types of specific reagents to selectively expose and directly measure the cholesterol associated with LDL [5,6]. Such methods will

Abbreviations: TC, total cholesterol; CHD, coronary heart disease; LDL, low-density lipoprotein; ATP, Adult Treatment Panel; LDL-C, LDL-cholesterol; BQ, β-quantification; HDL-C, HDL-cholesterol; IDL, intermediate-density lipoprotein; Lp(a); lipoprotein(a); TG, triglyceride; CM, chylomicron; HPLC, high-performance liquid chromatography; apo, apolipoprotein; LPL, lipoprotein lipase; CETP, cholesteryl ester transfer protein; (CM+VLDL)-C, (CM+VLDL)-cholesterol; HPLC-LDL, LDL-cholesterol determined by the HPLC method; BQ-LDL, LDL-cholesterol determined by the beta-quantification or modified beta-quantification methods; F-LDL, LDL-cholesterol calculated by the Friedewald equation.

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Table 1
Lipoprotein characteristics of studied subjects

	Control	Hyper TG	Apo E2/2	Hyper HDL	Hyper Lp(a)
	(*n*=50)	(*n*=17)	(*n*=8)	(*n*=10)	(*n*=12)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
	Min/max	Min/max	Min/max	Min/max	Min/max
TG[a,b]	1224±752	9799±5184†	2334±655†	660±235†	1093±329
	410/3320	4880/23,050	1448/2969	381/1202	410/1554
TC[b,c]	1917±343	2953±1028†	2105±419	2663±348†	2048±610
	1220/2720	2047/6518	1480/2798	2040/3182	1350/3332
(CM+VLDL)-C[b,c]	248±188	1766±972†	1000±397†	107±81†	191±41
	40/950	804/5003	484/1582	18/279	126/246
LDL-C[b,c]	1151±319	870±503§	581±174†	1263±387	1372±536
	573/1815	210/1724	359/790	633/2025	842/2635
HDL-C[b,c]	519±105	316±121†	525±121	1292±242†	485±222
	265/737	113/600	386/693	1003/1700	217/976
Lp(a)[b,d]	109±89	73±86§	108±90	137±55	850±402†
	10/390	16/210	20/290	50/220	436/1632
Apo A-I[b,d]	1460±229	1176±287†	1436±342	2144±223†	1323±439
	900/1900	290/1550	1100/1920	1712/2480	719/2030
Apo B[b,d]	910±246	1606±600†	654±183‡	862±148	1010±316
	420/1510	710/2620	430/950	660/1070	610/1564
Apo E[b,d]	42±18	141±39†	149±67‡	100±39‡	48±26
	17/101	92/248	64/276	55/190	26/118
VLDL size[e]	Not observed	42.0±2.1[f]	31.3±1.3	Not observed	Not observed
		38.9/45.2	30.1/33.8		
LDL size[e]	25.6±0.5	23.9±0.6†[g]	Not observed	25.4±0.6	25.8 0.4
	24.5/26.5	22.6/24.8		24.7/26.9	25.1 26.4
HDL size[e]	10.7±0.3	10.1±0.3†	10.3±0.2†	12.5±0.6†	10.8±0.3
	10.1/11.2	9.4/10.7	10.0/10.6	11.8/13.6	10.4/11.6

†$P<0.001$, ‡$P<0.01$, §$P<0.05$ (compared to the control group).
[a] Automated enzymatic method.
[b] In mg/l.
[c] Reference method.
[d] Immunoturbidimetric method.
[e] Diameter in nm.
[f] $n=15$.
[g] $n=14$.

grow in use but still need careful examination on their reactivity to lipoprotein particles other than LDL [7,8]. We previously reported partial reactivity of 2 homogeneous LDL-C assay kits on small dense LDL and their nonspecific reactions to very-low density lipoproteins (VLDL) particles [9].

High-performance liquid chromatography (HPLC) with gel permeation columns is an alternative method for classifying lipoproteins on the basis on particles sizes [10,11]. We developed a new analytical tool of cholesterol levels in major and subclasses of lipoproteins from a small amount of serum or plasma within 16 min by gel permeation HPLC [12–14]. We defined 3 VLDL subclasses, 4 LDL subclasses, and 5 HDL subclasses by using Gaussian curve fitting technique [11,15,16]. Moreover, qualitative information about the particle sizes obtained from the observed peak detection time on HPLC patterns can be used for characterization of lipoproteins and a better understanding of lipoprotein metabolism [14]. In this study, LDL-C levels obtained by the HPLC, which were calculated from component peak area corresponding to particle size from 16 to 30 nm, were compared with those by the BQ method on samples from the subjects with lipid disorders.

2. Materials and methods

2.1. Serum samples

Blood samples were collected into glass tubes without anticoagulant after a fasting state of at least 8 h from 47 healthy male volunteers and 50 outpatients in Osaka University hospital including 9 patients with type V hyperlipidemia, 9 patients with hypertriglyceridemia, 8 patients with apolipoprotein (apo) E2/2 phenotype, 4 patients with low hepatic lipase activity, 2 patients with lipoprotein lipase (LPL) deficiency, 3 patients with cholesteryl ester transfer protein (CETP) deficiency, 2 patients with low CETP activity, 2 patients with apo E7/3 phenotype, 4 patients with familial hyperlipidemia, 1 patient with mild primary biliary cirrhosis, 1 patient with hypergammaglobulinemia, 2 patients with sleep apnea syndrome, 1 patient with hypertension, 1 patient with diabetes mellitus and 1 patient with gout. Among 8 patients with apo E2/2 phenotype, 4 patients represent type III hyperlipoproteinemia and 5 patients are under medication by lipid lowering drugs; 2 of them by bezafibrate and 3 of them by fenofibrate. The blood samples were allowed to clot at room temperature and were centrifuged at 2000 ×g for 15 min to obtain serum samples. All serum samples were stored at 4 °C and analyzed within 5 days after blood collection. All volunteers and outpatients gave informed consent to participate in this study, and this study was conducted according to the Osaka University Hospital ethics committees.

2.2. HPLC method

Serum lipoproteins were analyzed by HPLC, as previously described [11,14–16]. In brief, 5 μl of whole serum sample was injected into 2 connected columns (300×7.8 mm) of TSKgel LipopropakXL (Tosoh, Tokyo, Japan) and eluted by TSKeluent Lp-1 (Tosoh) on HPLC system consisting of an AS-8020 auto-injector, CCPS and CCPM-II pumps and a UV-8020 detector (Tosoh). The effluent from the columns was continuously monitored at 550 nm after an on-line enzymatic reaction with a commercial kit, Determiner L TC (Kyowa Medex, Tokyo, Japan).

We defined 3 VLDL subclasses, 4 LDL subclasses, and 5 HDL subclasses using 20 component peaks on the basis of lipoprotein particle size (diameter) [15]. Cholesterol levels in major classes were obtained by grouping of these subclasses as follows: CM (>80 nm), VLDL (30–80 nm), LDL (16–30 nm) and HDL (8–16 nm). The within-run precision of cholesterol measurement for major lipoprotein classes was very high and coefficient of variation (CV) values ($n=5$, normolipidemic and hyperlipidemic pooled sera) were 2.51% (208 mg/l on average) and 1.29% (400 mg/l) for VLDL-C, 0.60% (926 mg/l) and 0.81% (750 mg/l) for LDL-C and 0.20% (636 mg/l) and 0.78% (422 mg/l) for HDL-C, respectively [15].

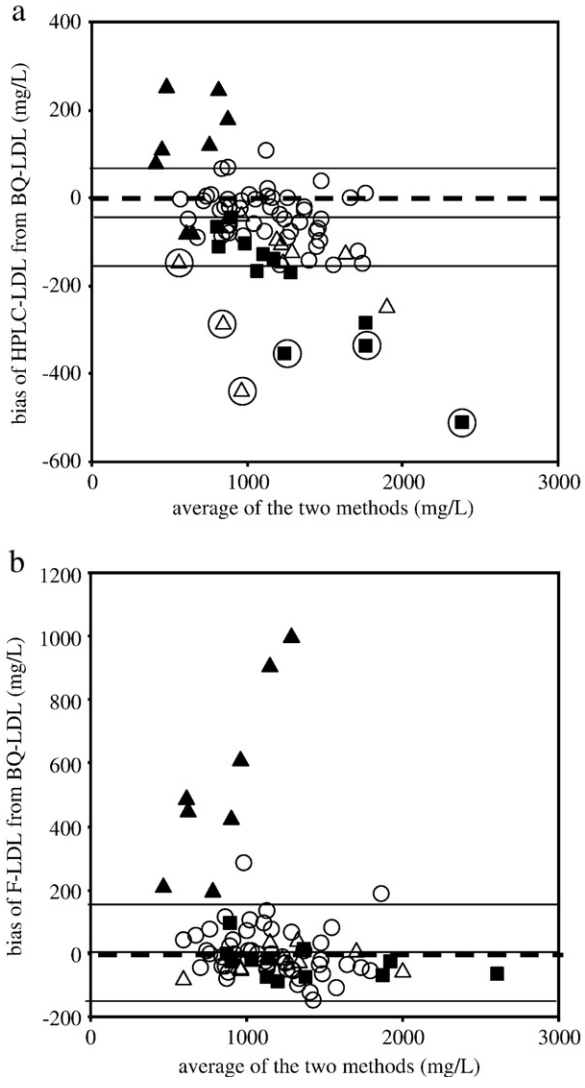

Fig. 1. Bland–Altman plots of biases of LDL-C levels by HPLC (HPLC-LDL) a) and Friedewald calculation (F-LDL) b) from those by BQ or modified BQ methods (BQ-LDL or mBQ-LDL) against average LDL-C levels by the two methods for controls (○, $n=50$) and subjects with apo E2/2 (▲, $n=8$), hyper HDL (△, $n=10$), and hyper Lp(a) (■, $n=12$). Dotted lines represent zero bias. Solid lines represent means of biases, means+2SD and means−2SD for the control subjects ($n=50$). Circled △, 3 subjects with CETP deficiency; Circled ■, 3 subjects with Lp(a) >1000 mg/l.