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Abnormally low LDL-Cholesterol Level in Cholestasis or Hypertriglyceridemia

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= 국문초록 =

담즙울체나 고지혈증에서 동반되는 비정상적으로 낮은 혈중 LDL-Cholesterol 농도

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조성은 · 홍기숙 · 정화순

목 적 : 최근에 LDL-C를 직접 측정하는 방법들이 개발되었다. 이 방법들은 정확하고 정밀하게 LDL-C를 측정할 수 있으며, 기존 방법들에 비해 다양한 간섭물질에 의한 영향을 덜 받는 것으로 알려졌다. 그러나, 저자들은 이대목동병원 입원 환자 중에서 Kyowa reagents(Kyowa Medex Co., Tokyo, Japan)에 의해 polyethylene/cyclodextrin(PC) 방법으로 LDL-C를 측정했을 때 비정상적으로 LDL-C 수치가 낮거나 측정되지 않는 11명의 환자를 발견하고, 그 원인을 분석하였다.

방 법 : 2002년 4월부터 12월까지 이대목동병원에 내원하여 PC 방법으로 LDL-C를 측정했던 7872명의 환자들 중 비정상적으로 낮거나 측정되지 않는 LDL-C 결과를 보인 11명의 환자를 대상으로 하였다. 11명 환자의 혈청을 같은 방법으로 재검하였고, 이 중 8명의 LDL-C는 Wako reagents(Wako Pure Chemical Industries, Osaka, Japan)을 사용하여 detergent(D) 방법으로 재측정하였다. 측정된 LDL-C 결과는 Friedewald equation 계산치와 비교하였고, 5명의 환자에서는 지단백 전기영동을 실시하였다.

결 과 : Cholestasis를 동반한 8명의 환자에서는 PC 방법으로는 LDL-C가 비정상적으로 낮거나 측정되지 않았다. 하지만 D 방법으로 측정한 이들의 LDL-C 결과는 모두 30mg/dL 보다 높은 값을 보였고, Friedewald 공식으로 계산한 LDL-C도 30mg/dL보다 높았다. 지단백 전기영동을 실시한 4명에서 LDL-C band가 존재하거나(2명), LpX가 관찰되었다(2명). Hypertriglyceridemia를 동반한 4명의 환자에서 (1명은 cholestasis와 hypertriglyceridemia를 동시에 동반한 경우로서 양쪽 group에 중복 포함되었음) PC 방법으로는 LDL-C가 비정상적으로 낮거나 측정되지 않았으나 D 방법으로는 40mg/dL보다 높은 결과를 보였다. 지단백 전기영동을 실시한 2명에서 모두 LDL-C이 존재하였다.

결 론 : PC 방법으로 LDL-C 측정시 LDL-C가 비정상적으로 낮거나 측정되지 않을 경우에는 cholestatic sera에 존재하는 비정상적인 LDL-C나 Lpx 또는 hypertriglyceridemia에 의한 간섭을 고려하여야 하며 그 외의 가능한 간섭 물질들을 고려해야 한다. 이러한 경우, D 방법이나 계산법, 전기영동법과 같은 다른 측정법에 의한 재검이 필요하다. 또한 측정법 차이에 따라 정확하게 정상 LDL-C만 LDL-C로 측정하는 방법과 모든 비정상적인 LDL-C까지도 LDL-C에 포함시켜 측정하는 방법 중에서 어느 방법이 우월한지는 임상 경과와 연관시켜 고려해야 할 것이다.

중심 단어 : LDL-C · 담즙울체 · 고지혈증 · PC assay · D assay.

Introduction

A wide variety of methods has been developed to determine low density lipoprotein-cholestserol (LDL-C) in serum, including a precipitation-based method[1], electrophoresis[2], high-performance liquid chromatography[3], sequential and density-gradient ultracentrifugation[4], the Friedewald formula[5], and immunoseparation[6][7]. Beta-quantification by ultracentrifugation is the currently accepted reference method for measuring LDL-C in serum[8].

Direct LDL-C assays are available in clinical laboratories, and they are less expensive than ultracentrifugation methods, and provide greatly improved turn-around times[9-11].

Several reagents are commonly used in these direct LDL-C assays. Kyowa reagents (Kyowa medex Co., Tokyo, Japan) designated as a polyethylene/cyclodextrin (PC) assay utilizes surfactant and quencher. Because the combination of polyoxyethylene-polyoxypropylene block polyether and α-cyclodextrin sulfate quenches the reactivity of high density lipoprotein-cholesterol (HDL-C), chylomicron-cholesterol, and very low density lipoprotein-cholesterol (VLDL-C), the enzymatic reaction for cholesterol occurs only with the LDL-C[9].

Using Wako reagents (Wako Pure Chemical Industries, Osaka, Japan) designated as a detergent (D) assay, specific detergent separates LDL particles from other lipoproteins, then these LDL particles are measured by conventional enzymatic reactions[10].

It has been reported that these assays are reliable and suitable even for sera with various interferences. No adverse effect was observed using the PC assay in the presence of TG (up to 2000mg/dL), free bilirubin (up to 60mg/dL), ascorbic acid (up to 250mg/dL), Hb (up to 0.5g/dL), Intralipos (up to 1g/dL), EDTA-2Na (up to 24 mg/dL), citrate (up to 30mg/dL). Presence of conjugated bilirubin up to 60mg/dL, a slightly negative error of up to 10% can influence on the assay result using the PC assay[9].

It has also been reported that EDTA-treated plasma was 3% lower than that of serum or heparinized samples in the D assay. But, this negative trend was not statistically significant. No adverse effect was observed in the presence of TG (up to 1132mg/L), bilirubin (up to 25.8 mg/dL), ascorbic acid (up to 15mg/dL), hemoglobin using the D assay[10].

However, we detected 11 patients whose LDL-C levels were abnormally low or absent at all measured by the PC assay. So, we evaluated possible causes of these abnormal results. Possible interferences were considered. We reanalyzed these 11 sera with the D assay. LDL-C values from the PC assay and from the D assay were compared.

Materials and Methods

LDL-C were measured in 7872 serum samples at Mokdong Hospital, Ewha Womans University from April to December 2002 with Hitachi 747 automated analyzer (Hitachi, Tokyo, Japan) using Kyowa reagents (Kyowa Medex Co.). From this group, we selected 11 specimens which showed abnormally low or absent LDL-C concentrations.

The definition of 'abnormally low LDL-C' is 'less than 10 % of the lower limit of reference range of LDL-C in Mokdong Hospital'. The reference range of LDL-C is less than 120 mg/dL. 'Absent LDL-C' meant 0mg/dL of LDL-C.

We reviewed hospital records of these patients to know clinical diagnoses and drug histories which could influence on concentrations of the LDL-C.

Levels of total cholesterol, high density lipoprotein-cholesterol (HDL-C), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and direct bilirubin were measured by Hitachi 747 automated analyzer (Hitachi).

LDL-C levels in these 11 samples were reanalyzed on Hitachi 747 (Hitahi) using the Kyowa reagents (Kyowa Medex Co.).

Eight of these 11 samples were also analyzed by the D assay on Hitachi 7600-110 automated analyzer (Hitachi, Tokyo, Japan) using the Wako reagents (Wako Pure Chemical Industries) at Dongdaemun hospital. In this assay, non-LDL lipoproteins are disrupted by a detergent, the released cholesterol is hydrolyzed by cholesterol esterase, and the resulting free cholesterol reacts with cholesterol oxidase to generate hydrogen peroxide. After

hydrogen peroxide is consumed by a peroxidase in the presence of 4-aminoantipyrine to generate a colorless product, a second detergent releases the cholesterol from LDL particles. In an enzymatic reaction series similar to that described above for non-LDL lipoproteins, except that hydrogen peroxide reacts with N,N'-bis-(4-sulfobutyl)-m-toluidine disodium salt, a colored product is generated from the LDL-C[10].

LDL-C concentrations were compared with those estimated by the Friedewald equation[8]. Lipoprotein electrophoresis was performed to confirm the existence of LDL-C in sera from five of the patients by Ewon Reference Laboratory with reagents of Helena (Helena laboratories, Texas, USA).

Results

Among 11 patients with abnormally low or absent LDL-C by the PC assay, 10 patients were male and one patient was female. Mean age was 50 years old (36-74 years old). No one has taken specific medication which can affect the level of the LDL-C.

Of these 11 patients, 8 patients showed elevated direct bilirubin levels. LDL-C levels of 6 patients among these 8 patients were 30-50mg/dL by the D assay. LDL-C levels of these 8 sera calculated by the Friedewald equation were 39.2-54.6mg/dL. Lipoprotein EP of 4 samples among these 8 patients showed presence of LDL-C fraction in two cases and LpX fraction in the other two cases (Table 1).

Four samples with abnormally low or absent LDL-C were hypertriglyceridemic (TG concentrations >400 mg/dL). Three sera among these four samples showed LDL-C concentrations higher than 40mg/dL by the D assay. Lipoprotein EP of two sera among these four samples showed presence of LDL-C fraction (Table 2).

Discussion

There are several reports claiming the merits of direct LDL-C assays. They say that direct LDL-C assays overcome the TG and fasting limitations of cacluation me-

Table 1. LDL-C concentrations in patients with cholestatic hepatobiliary dysfunction

Patient No.	Sex/ Age	LDL-C (mg/dL) PC	D	Friedewald	Lipoprotein EP	Total cholesterol (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	DB (mg/dL)	Diagnosis
1	F/62	1	43	50	NT*	93	6	185	2.6	Chronic liver disease
2	M/74	11	33	50.4	NT	75	9	78	21.3	Hepatocellular carcinoma
3	M/45	8	50	NT	LpX band	130	NT	226	14.4	Gallstone colic
4	M/43	0	36	18.8	NT	40	3	91	16.3	Alcoholic liver cirrhosis
5	M/43	3	30	51.2	LpX band	91	7	164	25.4	Alcoholic liver cirrhosis
6	M/40	7	44	39.2	LDL-C band	139	9	454	2.6	Chronic liver disease
7	M/36	3	NT	54.6	NT	103	14	172	15.3	Chronic liver disease
8	M/47	3	NT	45.4	LDL-C band	88	10	163	22.7	Chronic liver disease

* : not tested
PC : polyethylene/cyclodextrin assay, D : detergent assay, EP : electrophoresis, LDL-C : low-density lipoprotein-cholesterol, HDL-C : high-density lipoprotein-cholesterol, TG : triglyceride, DB : direct bilirubin, Lpx : Lipoprotein X

Table 2. LDL-C concentrations in the patients with hypertriglyceridemia

Patient No.	Sex/ Age	LDL-C (mg/dL) PC	D	Friedewald	LDL-C EP	Total cholesterol (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	DB (mg/dL)	Diagnosis
6	M/40	7	44	39.2	LDL (+)	139	9	454	2.6	Alcoholic liver cirrhosis
9	M/46	1	55	NI*	NT†	279	23	1634	1.1	Pancreatitis
10	M/72	3	51	NI	NT	122	22	595	1.0	Fatty liver
11	M/54	0	NT	NI	LDL (+)	89	13	574	0.8	Nonsymptomatic gallstone

* : not indicated, † : not tested

thods, are readily adapted to clinical laboratories, are less expensive than ultracentrifugation methods, and provide greatly improved turnaround time. It has also been reported that these assays are reliable and suitable even for sera with various interferences such as TG, bilirubin, ascorbic acid, hemoglobin[9-11].

But, in real patients' sera, we found abnormally low or absent LDL-C concentrations by the PC assay. On the other hand, LDL-C concentrations of these sera by the D assay were much higher. Results of the D assay were agreed well on the Friedewald calculation data. LDL-C was also revealed in some of these samples upon lipoprotein EP.

We divided these samples into two groups according to their lipoprotein profiles.

One group of samples came from patients with cholestatic hepatobiliary dysfunction. In these samples, LpX, a clinical marker for cholestasis, was observed upon lipoprotein EP. We hypothesized that the LpX present in the cholestatic sera[12] would be mistakenly measured as LDL-C by the D assay and would not be measured as LDL-C by the PC assay. This supposition was based on a previous report in which the D assay mistakenly measured 51% of the LpX cholesterol as LDL-C, whereas the PC assay measured none of the LpX cholesterol as LDL-C[13]. But, this hypothesis is not sufficient to explain the abnormally low or absent LDL-C itself, setting aside LpX.

We also hypothesized that measurement of LDL-C by either assay might be hindered by LDL-C molecules altered by oxidation, glycation, or some other modification. One such modified LDL-C is small, dense LDL (SD-LDL), which is emerging as an important risk factor for coronary artery disease. In a study by Miller, the presence of abnormal lipoproteins such as SD-LDL caused the D assay to overestimate and the PC assay underestimate LDL-C, as compared with the beta quantification reference method[14]. But, this hypothesis is not sufficient to explain the abnormally low or absent LDL-C, either. Because, concentrations of modified LDL-C such as SD-LDL would not be so high as to influence on measuring LDL-C concentrations.

Second group of samples exhibited high TG concentrations. We hypothesized that high TG concentrations would interfere the measurement of LDL-C by the PC assay rather than the D assay. This hypothesis is partially consistent with previous reports evaluating the PC and the D assays. Several studies have shown that high TG(700-1000mg/dL) causes negative interference in the PC assay and positive interference in the D assay. But, no interference was observed either in the PC or in the D assay at TG concentrations less than 700mg/dL[15]. In another study, a negative bias of 10% was observed for the PC assay at TG concentrations exceeding 1000 mg/dL[16]. Though, the PC assay is more prone to be influenced by high TG concentrations than is the D assay, this hypothesis is not sufficient. Because TG concentrations were less than 700mg/dL except in one patient in our case.

The difference in methods between the PC and the D assay would influence on measuring LDL-C. While surfactant and quencher are used in the PC assay, detergent is used in the D assay. But, this difference could not completely explain the abnormally low or absent LDL-C by the PC assay.

Though we could exclude the effects of ascorbic acid, drug interference or hemolysis in these 11 cases, there remain a lot of possible interferences which are not revealed yet. There could be matrix effect, for example. In fact, previous reports about interferences of measuring the LDL-C were performed using pooled sera or control materials in artificial conditions. Therefore, another factors could influence on measuring the LDL-C in real patients' sera.

There is similar report by Lim. According to this report, direct LDL-C measurement by immunoseparation method known not to be influenced by food, is revealed to be actuallly influenced by food ingestion in real sera[17].

Consequently, upper limits of concentrations of interferences should be newly evaluated in real patients' sera. Another intereference which is not yet known should be revealed in real patients' sera, too. LDL-C subfraction or the component of abnormal LDL-C in pathologic conditions should also be evaluated.

We signify that our data is the first case report in real patients about discrepancy of the LDL-C concentrations between the PC and the D assay in Korea.

When the LDL-C concentration is abnormally low or absent by the PC assay, the presence of abnormal LDL-C or LpX in cholestatic sera or the interference by high TG concentration should be considered. In this case, the LDL-C should be reanalyzed by another method such as the D assay, the calculation method or EP. We cannot conclude the superiority of one assay which measures only the normal portion of the LDL-C as the LDL-C to the other which measures the LDL-C portion more broadly.

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