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RESEARCH ARTICLE

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Association between measured or calculated small dense low-density lipoprotein cholesterol and oxidized low-density lipoprotein in subjects with or without type 2 diabetes mellitus

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Abstract

Background: Small dense low-density lipoprotein (sdLDL) possesses atherogenic potential and is predicted to be susceptible to atherogenic modifications, which further increases its atherogenicity. However, studies on the association between measured or estimated sdLDL cholesterol (sdLDL-C) levels and atherogenic modification in diverse population groups are lacking.

Methods: Surplus serum samples were collected from male subjects with type 2 diabetes mellitus (DM) under treatment ($n = 300$) and without DM (non-DM; $n = 150$). sdLDL and oxidized LDL (oxLDL) levels were measured using the Lipoprint LDL subfractions kit (Quantimetrix Corporation) and the Mercodia oxidized LDL competitive enzyme-linked immunosorbent assay kit (Mercodia), respectively. The estimated sdLDL-Cs were calculated from two relevant equations. The effects of sdLDL-C on oxLDL were assessed using multiple linear regression (MLR) models.

Results: The mean (±SD) of measured sdLDL-C and oxLDL concentrations were 11.8 ± 10.0 mg/dl and 53.4 ± 14.2 U/L in the non-DM group and 0.20 ± 0.81 mg/dl and 46.0 ± 15.3 U/L in the DM group, respectively. The effects of measured sdLDL-Cs were significant ($p = 0.031$), whereas those of estimated sdLDL-Cs were not ($p = 0.060$, $p = 0.116$) in the non-DM group in the MLR models. The effects of sdLDL-Cs in the DM group were not significant.

Conclusion: In the general population, high level of sdLDL-C appeared to be associated with high level of oxLDL. The equation for estimating sdLDL-C developed from a general population should be applied with caution to a special population, such as patients with DM on treatment.

KEYWORDS
atherogenesis, diabetes mellitus, low-density lipoprotein, oxidized low-density lipoprotein, small dense low-density lipoprotein

1 | INTRODUCTION

Low-density lipoprotein (LDL) is broadly defined as a lipoprotein fraction with density ranging from 1.006 to 1.063 g/ml, and circulating LDL particles can be further classified based on their density or size.[1] Among LDL subfractions, small dense LDL (sdLDL) is strongly associated with the risk of developing atherosclerotic cardiovascular disease (ASCVD).[2–4] The association between ASCVD and sdLDL is presumably because sdLDL penetrates the vessel wall easily and tends to undergo atherogenic modifications, such as oxidation.[5] Oxidized LDL (oxLDL) has been demonstrated to be involved in foam cell formation and stimulation of immune responses, which explains its atherogenic property.[6] As sdLDL has lower affinity for LDL receptors and longer circulation time than large LDL particles,[7,8] it is susceptible to modifications, such as glycation and oxidation.[9,10] Moreover, glycated LDL is more susceptible to oxidation,[11] and differences in the lipid composition of sdLDL may also contribute to the increased susceptibility to oxidation.[12]

Patients with type 2 diabetes mellitus (T2DM) are at a 2- to 4-fold higher risk of developing coronary heart disease and stroke than healthy individuals.[13] Atherogenic dyslipidemia is one of the reasons underlying the increased risk of T2DM patients. Patients with T2DM have higher proportion of sdLDL than in normal subjects,[14] and increase in sdLDL level is associated with ASCVD risk in patients with T2DM or prediabetes even after adjusting for traditional risk factors.[15] Patients with T2DM also have high oxLDL levels, although their LDL cholesterol is maintained at a desirable level.[16] Statins are widely used to treat atherogenic dyslipidemia.[17] However, the specific effect of statins on lowering sdLDL or oxLDL levels is not completely understood.[1,18]

Various methods are used to measure sdLDL levels. Ultracentrifugation, nuclear magnetic resonance spectroscopy, and gradient-gel electrophoresis have been commonly used for research purposes; however, these methods are either too laborious or expensive for general clinical use.[19] A less complex LDL subfractionation procedure using polyacrylamide gel electrophoresis can be used to determine sdLDL content.[20] Recently, a homogeneous direct assay for sdLDL-cholesterol (sdLDL-C) that can be adapted for use with autoanalyzers has been introduced.[21,22] More recently, equations for sdLDL-C based on classic lipid panel measures have been suggested using a homogeneous direct assay as a reference (23,24) OxLDL content is usually measured using enzyme-linked immunosorbent assay (ELISA) with various monoclonal antibodies.[25,26]

Although the correlation between sdLDL and oxLDL is well known and possible mechanisms have been studied, it has not been well studied in patients with DM. Furthermore, data on sdLDL and oxLDL are not necessarily comparable across various methods, and their relationship in the general population should be confirmed using different combinations of assays. In addition, equations for sdLDL-C based on classic lipid panel measures have recently been introduced, and the characteristics of their calculated values should be assessed.

In this study, we attempted to determine (1) the distribution of measured or estimated sdLDL-C and oxLDL in subjects with T2DM and without DM, (2) the relationship between measured and estimated sdLDL-C levels in the two groups, and (3) the relationship between sdLDL-C and oxLDL in the two groups.

2 | MATERIALS AND METHODS

2.1 | Study subjects and samples

Surplus serum samples from male subjects undergoing complete blood count (CBC) with reticulocyte count, and assessment of glycated hemoglobin (HbA1c) level and lipid panel (total cholesterol [TC], high-density lipoprotein-cholesterol [HDL-C], directly measured low-density lipoprotein-cholesterol [dLDL-C], and triglyceride [TG]) were collected at the Asan Medical Center from April 2018 to October 2018. The samples were sequentially collected from the subjects meeting the criteria for two groups: one without diabetes mellitus (non-DM group), the criteria for which were as follows: (1) the HbA1c and lipid panel tests were requested from the health screening and promotion center, (2) HbA1c $<6.0\%$ (42 mmol/mol), and (3) the subject did not have any diagnosis or treatment history for DM, and dyslipidemia on the health checkup questionnaire; group with DM (DM group), the inclusion criteria for which were as follows: (1) the HbA1c and lipid panel tests were requested from the outpatient clinic of the Department of Endocrinology and Metabolism, (2) the subject had diagnosis codes of T2DM, and (3) the subject was treated for DM (not in the first visit). The samples were stored at −70°C before analysis of sdLDL, oxLDL, and glycated albumin levels and total antioxidant status (TAS). Clinical and laboratory data, including age, sex, height, weight, fasting glucose level, HbA1c level, CBC, reticulocyte count, and lipid panel test results, were collected from electronic medical records. The study protocol was approved by the Institutional Review Board of the Asan Medical Center (reference number: 2018-0123).

2.2 | sdLDL-C measurement and estimation

sdLDL was measured using a Lipoprint LDL subfractions kit (Quantimetrix Corporation; Redondo Beach, CA, USA) based on polyacrylamide gel electrophoresis (PGE). From the acquired lipoprotein profile, the concentration of measured sdLDL-C (M-sdLDL-C; mg/dl) was calculated by multiplying the relative area of small and dense subfractions (LDL-3 through LDL-7) by the TC concentration of the sample following the manufacturer's instructions. Additionally, sdLDL-C level was estimated from the lipid panel result using two equations based on the results obtained from the direct Denka sdLDL-C assay (Denak Seiken, Tokyo, Japan).[21,23,24] The

first equation used indirect LDL-C determined using the Sampson equation, suggested by Sampson et al[23]: E(MS)-sdLDL-C (mg/dl) = LDL-C−(1.43×LDL-C−(0.14×ln(TG)×LDL-C)−8.99). The other was an equation using both directly measured LDL-C (dLDL-C) and indirect LDL-C determined using the Friedewald equation (cLDL-C), suggested by Pornpen Srisawasdi et al[24]: E(PS)-sdLDL-C (mg/dl) = 0.580×non-HDL-C+ 0.407×dLDL-C−0.719×cLDL-C−12.05, where non-HDL-C was calculated by subtracting the HDL-C from the TC concentration.

2.3 | Other biochemical analyses

Oxidized LDL level was measured using the Mercodia oxidized LDL competitive ELISA kit (Mercodia, Uppsala, Sweden), which uses the 4E6 monoclonal antibody. TAS was measured using the Randox TAS assay (Randox Laboratories Ltd., Crumlin, UK) based on the generation of the radical cation from 2,2′-azino-di-(3-ethylbenz thiazoline sulfonate). Glycated albumin, glucose, and lipid panel tests were performed using an AU5800 clinical chemistry analyzer (Beckman Coulter, Brea, CA, USA). HbA1c levels were measured using a Tosoh HLC-723 G11 analyzer (Tosoh Corporation, Tokyo, Japan). CBC and reticulocyte counts were measured using a Sysmex XE-2100 automated hematology analyzer (Sysmex, Kobe, Japan).

2.4 | Statistical analysis

With the exception of TG, all other parameters were compared between the groups using *t*-tests. As TG was non-normally distributed, TG data were presented as median and interquartile range and compared using the Mann–Whitney U test between groups. Pearson's correlation, linear regression, and scatterplots were used to examine bivariate relationships between parameters. TG levels were log-transformed to obtain a linear relationship. A multiple linear regression (MLR) model was used to assess the association between sdLDL-C and oxLDL levels, adjusted by dLDL-C and other parameters related to LDL oxidation. The variable was selected mainly based on the Akaike information criterion value and adjusted R^2 among the candidate markers (age, body mass index (BMI), CBC parameters, reticulocyte (%), HbA1c, and TAS). Finally, MLR analysis was performed with oxLDL as the dependent variable, and with dLDL-C, age, and reticulocytes in the non-DM group, or with dLDL-C, age, hemoglobin, and HbA1c in the DM group as independent variables, along with sdLDL-Cs. A *t*-test on the regression coefficient was performed to determine whether the effect of sdLDL-C was significant. The partial F-test was used to assess the effect of the subject group (non-DM or DM), including the interactive term, on the relationship between E(MS)-sdLDL-C and E(PS)-sdLDL-C. Statistical significance was set at $p < 0.05$. Data integration, analysis, and visualization were performed using R version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

3.1 | Subject characteristics

Samples were collected from 150 subjects in the non-DM group and 300 subjects in the DM group. For four subjects in the DM group, oxLDL and TAS results could not be obtained because of insufficient sample volume for valid results. The clinical and laboratory test results are presented in Table 1. The DM and non-DM groups differed significantly in age, height, hematocrit, reticulocyte, and levels of glucose, HbA1c, glycated albumin, hemoglobin, TC, HDL-C, dLDL-C, non-HDL-C, TG, oxLDL, TAS, M-sdLDL-C, E(MS)-sdLDL-C, and E(PS)-sdLDL-C (except for weight and BMI). When the DM group was subdivided into two subgroups based on the HbA1c value of 6.5% (48 mmol/mol; the general target for DM management), glucose, glycated albumin, TG, and E(PS)-sdLDL-C levels were significantly higher in the group with HbA1c ≥ 6.5% than in the group with HbA1c < 6.5%.

3.2 | Distribution of measured sdLDL-C and relationship with other lipid measures

In the non-DM group, M-sdLDL-C was detected in 94.7% samples; however, in the DM group, it was detected in only 7.3% samples. The mean (±standard deviation: median [IQR]) of measured sdLDL-C concentration was 11.8 ± 10.0 (8.6 [12.8]) mg/dl in the non-DM group and 0.20 ± 0.81 (0.0 [0.0]) mg/dl in the DM group. The detailed distribution of M-sdLDL-C levels is shown in Table 2. M-sdLDL-C correlated positively with non-HDL-C, triglyceride (log-transformed), dLDL-C, total cholesterol, and oxLDL levels, with correlation coefficients of 0.634 ($p < 0.001$), 0.580 ($p < 0.001$), 0.466 ($p < 0.001$), 0.466 ($p < 0.001$), and 0.392 ($p < 0.001$), respectively. M-sdLDL-C showed negative correlation with HDL-C level, with a correlation coefficient of −0.352 ($p < 0.001$).

3.3 | Relationship between measured and estimated sdLDL-Cs

Figure 1 shows the linear associations between M-sdLDL-C, E(MS)-sdLDL-C, and E(PS)-sdLDL-C levels. The coefficient of determination from simple linear regression was higher between the estimated sdLDL-Cs ($R^2 = 0.726$) than between the measured and estimated sdLDL-Cs ($R^2 = 0.532$ and $R^2 = 0.518$). The effect of group (non-DM or DM) on the relationship between estimated sdLDL-Cs was not significant ($p = 0.066$) in the MLR model using the *F*-test. The mean (standard deviation) of the differences between estimates in the non-DM group were 19.03 (7.00) mg/dl between E(MS)-sdLDL-C and M-sdLDL-C, 25.48 (8.51) mg/dl between E(PS)-sdLDL-C and M-sdLDL-C, and 6.45 (5.46) mg/dl between E(PS)-sdLDL-C and E(MS)-sdLDL-C. Those in the DM group were 27.84 (8.27) mg/dl between E(MS)-sdLDL-C and M-sdLDL-C, 31.97 (13.52) mg/dl between

TABLE 1 Subject characteristics and differences in parameters among groups.

Characteristics	Non-DM ($n = 150$)	DM ($n = 300$)				
		Total	HbA1c <6.5% ($n = 110$)	HbA1c ≥6.5% ($n = 190$)	p value[a]	p value[b]
Age, years	57.6 ± 8.6	60.8 ± 9.6	60.5 ± 9.7	61.0 ± 9.6	0.644	<0.001
Height, cm	172.3 ± 5.7	168.8 ± 5.6	169.1 ± 5.9	168.6 ± 5.5	0.486	<0.001
Weight, kg	74.3 ± 8.7	72.5 ± 10.2	72.0 ± 10.7	72.8 ± 9.9	0.558	0.054
BMI, kg/m^2	25.0 ± 2.4	25.4 ± 3.2	25.2 ± 3.3	25.5 ± 3.1	0.317	0.143
Glucose, mg/dl	103.7 ± 10.9	138.6 ± 36.9	124.3 ± 20.3	146.9 ± 41.6	<0.001	<0.001
HbA1c, %	5.43 ± 0.27	6.96 ± 1.21	5.84 ± 0.46	7.60 ± 1.02	–	–
HbA1c, mmol/mol	35.8 ± 2.9	52.5 ± 13.2	40.4 ± 5.1	59.6 ± 11.2	–	–
Glycated albumin, %	12.5 ± 1.3	18.2 ± 5.2	15.0 ± 2.8	20.1 ± 5.3	<0.001	<0.001
Hemoglobin, g/dl	15.0 ± 1.1	14.0 ± 1.8	13.8 ± 1.71	14.2 ± 1.80	0.101	<0.001
Hematocrit, %	45.3 ± 3.1	42.6 ± 5.5	41.9 ± 4.8	42.9 ± 5.9	0.098	<0.001
Reticulocyte, %	1.71 ± 0.36	1.80 ± 0.52	1.77 ± 0.56	1.82 ± 0.50	0.460	0.027
TC, mg/dl	171.3 ± 29.8	142.8 ± 29.6	142.9 ± 27.7	142.8 ± 30.7	0.982	<0.001
HDL-C, mg/dl	56.3 ± 15.2	42.9 ± 10.9	43.2 ± 12.1	42.7 ± 10.2	0.702	<0.001
dLDL-C, mg/dl	114.4 ± 29.8	89.0 ± 23.6	89.6 ± 22.1	88.7 ± 24.6	0.737	<0.001
Non-HDL-C, mg/dl	115.0 ± 30.4	99.9 ± 28.0	99.7 ± 25.9	100.1 ± 29.2	0.891	<0.001
TG, mg/dl (median [IQR])	110.5 (65.5)	133.0 (84.3)	120.5 (74.8)	142.0 (92.8)	0.006	<0.001
oxLDL, U/L	53.4 ± 14.2	46.0 ± 15.3	43.7 ± 15.0	47.2 ± 15.4	0.054	<0.001
TAS, mmol/L	1.69 ± 0.13	1.61 ± 0.14	1.65 ± 0.12	1.59 ± 0.15	<0.001	<0.001
M-sdLDL-C, mg/dl	11.8 ± 10.0	0.20 ± 0.81	0.07 ± 0.39	0.28 ± 0.97	–	–
E(MS)-sdLDL-C, mg/dl	30.8 ± 8.9	28.0 ± 8.6	27.5 ± 8.0	28.3 ± 8.9	0.417	0.002
E(PS)-sdLDL-C, mg/dl	37.3 ± 12.1	32.2 ± 13.7	29.8 ± 11.0	33.5 ± 15.0	0.016	<0.001

Note: Data are shown as mean ± standard deviation, except for triglycerides (median [interquartile range]). p values were calculated using Student's t-test, except for triglycerides (Mann–Whitney U test). p values for HbA1c (grouping factor) and M-sdLDL-C (not detected in most subjects in the DM group) were not calculated.

[a]Statistical significance between HbA1c <6.5% and ≥6.5% groups.

[b]Statistical significance between the non-DM and DM groups.

TABLE 2 Distribution of M-sdLDL-C according to groups.

Group	Detection rate (%)	Percentiles (mg/dl)							
		Min	10th	25th	50th	75th	90th	95th	Max
Non-DM	142/150 (94.7)	0.0	1.9	3.9	8.6	16.7	27.1	30.6	46.0
DM	22/300 (7.3)	0.0	0.0	0.0	0.0	0.0	0.0	2.0	6.4

E(PS)-sdLDL-C and M-sdLDL-C, and 4.13 (8.31) mg/dl between E(PS)-sdLDL-C and E(MS)-sdLDL-C.

3.4 | Relationship between sdLDL-C and oxLDL levels

dLDL-C and oxLDL levels correlated moderately ($R = 0.534$, $p < 0.001$ in the non-DM group; $R = 0.603$, $p < 0.001$ in the DM group). The relationship between dLDL-C and oxLDL in the non-DM group was affected by sdLDL-Cs, as shown in Figure 2A. However, the effect of sdLDL-Cs was not observed in the DM group (Figure 2B). For the MLR model of the DM group, M-sdLDL-C level was converted into a categorical variable (detected or not) because of the low detection rate. TAS did not improve the quality of the models and was not selected as a variable. Consistent with the observations in the scatter plots, M-sdLDL-C level was significant in the model of the non-DM group (Table 3), whereas sdLDL-C levels were not significant in the models of the DM group (Table 4).

4 | DISCUSSION

In this study, the DM group showed lower TC, HDL-C, dLDL-C, oxLDL, and M-sdLDL-C levels, but higher TG levels than in the non-DM group. Linear associations were observed between M-sdLDL-C,

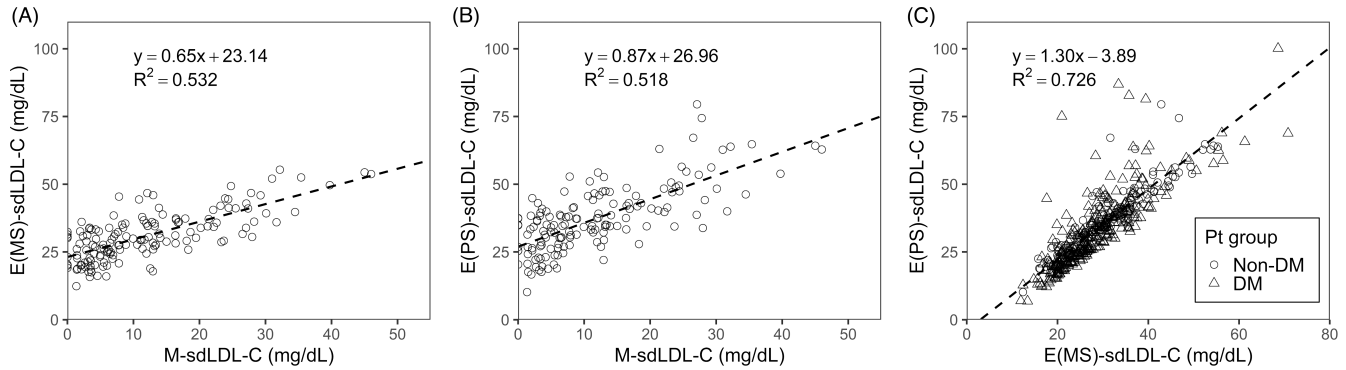

FIGURE 1 Relationship between sdLDL-Cs shown as scatter plots. (A) M-sdLDL-C vs. E(MS)-sdLDL-C in the non-DM group. (B) M-sdLDL-C vs. E(PS)-sdLDL-C in the non-DM group. (C) E(MS)-sdLDL-C vs. E(PS)-sdLDL-C in total number of patients (circle for non-DM subject and triangle for patient with DM). Regression lines are shown as dashed lines.

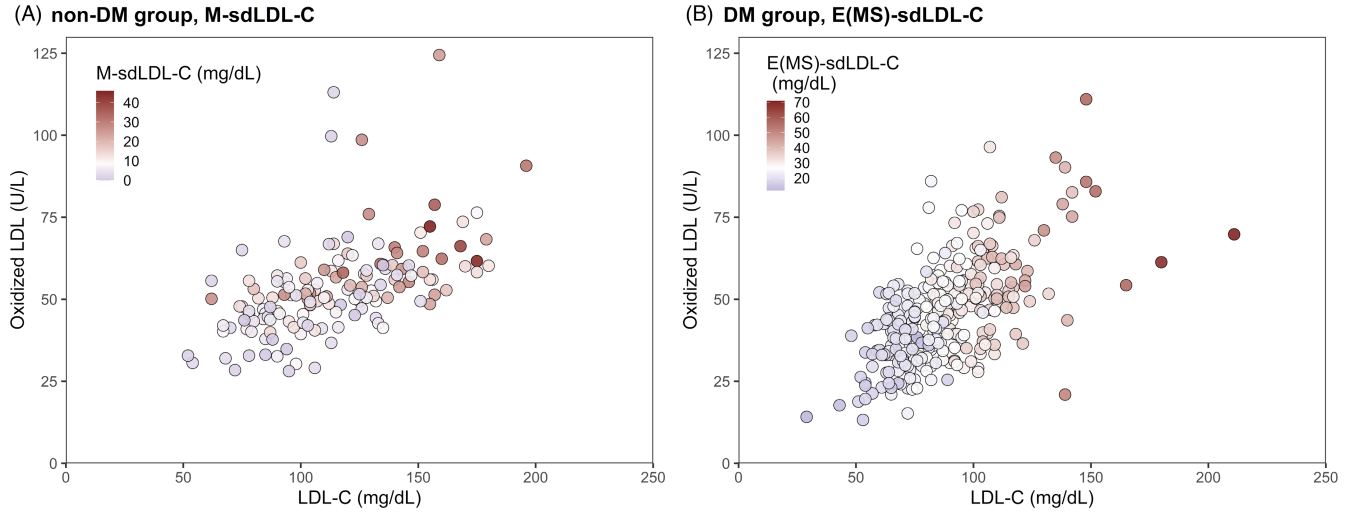

FIGURE 2 Distribution of sdLDL-Cs in the relationship between dLDL-C and oxLDL. (A) dLDL-C and oxLDL of subjects in the non-DM group were plotted, and the points were colored according to M-sdLDL-C concentration. (B) dLDL-C and oxLDL of subjects in the DM group were plotted, and the points were colored according to E(MS)-sdLDL-C concentration.

E(MS)-sdLDL-C, and E(PS)-sdLDL-C levels. The effects of sdLDL-Cs on oxLDL were less significant in the DM group, although the number of subjects was twice of that in the non-DM group. These results suggested that the relationships among measured and estimated sdLDL-Cs and other proatherogenic modifications, such as oxidation, might differ according to population groups with different lipid profiles. Therefore, extrapolation of the estimated sdLDL-C equation developed from the results of the general population should be used with caution.

The M-sdLDL-C concentrations were lower than the estimated sdLDL-C concentrations. A previous report comparing the Lipoprint PGE method and the direct Denka method showed the mean difference between the methods to be 0.62 mmol/L, which is 23.98 mg/dl when multiplied by 38.67.[27] The direct Denka method is speculated to recognize a wider range of atherogenic lipoprotein particles as sdLDL.[27,28] As the formulas for calculating E(MS)-sdLDL-C and E(PS)-sdLDL-C were derived from the results of the direct Denka method, the differences (19.03 and 25.48 mg/dl) with the M-sdLDL-C levels obtained using the Lipoprint PGE method in this study are not unusual. Despite this bias, the two estimated sdLDL-Cs showed good correlation and a linear relationship with M-sdLDL-C in the non-DM group.

M-sdLDL-C levels were not detectable in most patients of the DM group. The patient's disease status and medications might have affected the results. However, T2DM itself is believed to be associated with increased levels of sdLDL.[29] A study reported that the sdLDL-C levels of patients with T2DM were higher than those of non-DM subjects (9.10 mg/dl vs. 6.97 mg/dl) measured using the Lipoprint PGE method.[30] Although the effect of lipid-lowering treatment on the specific lowering of sdLDL level is not clear,[1] the absolute decrease in sdLDL-C concentration appears to have been acquired by lipid-lowering medications, such as statins, ezetimibe, and fibrates.[31,32] Considering that a high proportion of DM patients visiting tertiary hospitals in Korea are on lipid-lowering medications,[33] it is possible that the DM patients in our study had low sdLDL-C levels because of the effect of lipid-lowering treatment. The estimated sdLDL-C levels were also lower in the DM group, although the differences were considerably lower than

TABLE 3 Multiple linear regression statistics in the models for interpreting the relationships between sdLDL-C and oxLDL in the non-DM group ($n = 150$).

Variables	β[a] (SE)	p value
MLR model without sdLDL-C variables (adjusted $R^2 = 0.327$)		
dLDL-C (mg/dl)	0.244 (0.032)	<0.001
Age (years)	−0.302 (0.113)	0.008
Reticulocyte (%)	5.904 (2.654)	0.028
MLR model with M-sdLDL-C (adjusted $R^2 = 0.348$)		
M-sdLDL-C (mg/dl)	0.235 (0.108)	0.031
dLDL-C (mg/dl)	0.208 (0.036)	<0.001
Age (years)	−0.296 (0.111)	0.009
Reticulocyte (%)	5.424 (2.629)	0.041
MLR model with E(MS)-sdLDL-C (adjusted $R^2 = 0.339$)		
E(MS)-sdLDL-C (mg/dl)	0.336 (0.177)	0.060
dLDL-C (mg/dl)	0.169 (0.051)	0.001
Age (years)	−0.245 (0.115)	0.035
Reticulocyte (%)	4.757 (2.699)	0.080
MLR with E(PS)-sdLDL-C (adjusted $R^2 = 0.334$)		
E(PS)-sdLDL-C (mg/dl)	0.175 (0.111)	0.116
dLDL-C (mg/dl)	0.198 (0.044)	<0.001
Age (years)	−0.265 (0.114)	0.022
Reticulocyte (%)	4.847 (2.723)	0.077

[a]Estimated coefficient (standard error, SE).

TABLE 4 Multiple linear regression statistics in the models for interpreting the relationships between sdLDL-C and oxLDL in the DM group ($n = 296$).

Variables	β[a] (SE)	p value
MLR model without sdLDL-C variables (adjusted $R^2 = 0.391$)		
dLDL-C (mg/dl)	0.416 (0.031)	<0.001
Age (years)	0.171 (0.076)	0.025
Hemoglobin (g/dl)	1.089 (0.411)	0.008
HbA1c ≥6.5%	3.658 (1.450)	0.012
MLR model with M-sdLDL-C (adjusted $R^2 = 0.389$)		
M-sdLDL-C (Detected)	−0.262 (2.800)	0.926
dLDL-C (mg/dl)	0.416 (0.032)	<0.001
Age (years)	0.170 (0.076)	0.027
Hemoglobin (g/dl)	1.090 (0.412)	0.009
HbA1c ≥6.5%	3.675 (1.463)	0.013
MLR model with E(MS)-sdLDL-C (adjusted $R^2 = 0.393$)		
E(MS)-sdLDL-C (mg/dl)	0.293 (0.222)	0.188
dLDL-C (mg/dl)	0.316 (0.082)	<0.001
Age (years)	0.170 (0.076)	0.026
Hemoglobin (g/dl)	1.132 (0.412)	0.006
HbA1c ≥6.5%	3.313 (1.471)	0.025
MLR with E(PS)-sdLDL-C (adjusted $R^2 = 0.390$)		
E(PS)-sdLDL-C (mg/dl)	0.053 (0.071)	0.457
dLDL-C (mg/dl)	0.394 (0.042)	<0.001
Age (years)	0.177 (0.077)	0.021
Hemoglobin (g/dl)	1.119 (0.413)	0.007
HbA1c ≥6.5%	3.432 (1.482)	0.021

[a]Estimated coefficient (standard error, SE).

those of M-sdLDL-C. As the studies that developed the equations for sdLDL-C estimation did not include DM patients with lipid-lowering treatment,[23,24] further research is required to determine whether the estimation is relevant in these patients with different lipid profiles.

In the MLR models explaining oxLDL levels, the M-sdLDL-C level was a significant variable in the non-DM group. Studies have suggested that sdLDL is highly susceptible to oxidation,[1,12] and our results are consistent with this idea. Although we could not show the superiority of M-sdLDL-C in predicting oxLDL by direct comparison, the estimated sdLDL-Cs were not significant in the MLR models. Further studies are required to determine whether estimated sdLDL-C can replace measured sdLDL-C or whether measured sdLDL-C has an advantage over estimated sdLDL-C. In the MLR models for the DM group, M-sdLDL-C and estimated sdLDL-Cs were not significant. This may be due to measurement issues or differences in the mechanism underlying oxLDL production. As mentioned above, M-sdLDL-C was not detectable, making quantitative analysis difficult, and the estimated sdLDL-C levels might differ from the actual direct Denka assay value. As glycation also renders LDL susceptible to oxidation,[11] the influence of sdLDL may be relatively small in the DM population, especially when the sdLDL level is lowered with lipid-lowering medications. Although the applicability of estimated sdLDL-C has been suggested to predict residual risk after statin treatment,[23] further studies are required to demonstrate the effectiveness and feasibility of such monitoring.

In the MLR models, some variables other than dLDL-C and age were selected: reticulocytes in the models for the non-DM group and hemoglobin and HbA1c in the models for the DM group. Although both reticulocytes and erythrocytes possess antioxidant activities, reticulocytes are more active than mature erythrocytes.[34] The lifespan of erythrocytes is also associated with oxidative stress and antioxidant responses.[35] However, in our models, higher hemoglobin concentrations were associated with higher oxLDL levels, which was not explained by such mechanisms. However, the association between higher hemoglobin concentration and unfavorable lipoprotein particle profile has also been reported in a previous study,[36] suggesting that our findings are not unusual. In addition, as the results regarding the significance of the sdLDLs did not differ between models with and without them, we included reticulocytes or hemoglobin in the final models for obtaining higher coefficient of determination. The association between HbA1c and oxLDL has been reported previously.[37,38] This association could be interpreted as a result of the increased susceptibility of LDL to oxidation in DM patients with high HbA1c levels[39] and/or increased oxidative stress, which may contribute to both increase in oxLDL and HbA1c levels.[40,41] The association

between HbA1c and oxLDL levels observed in this study is consistent with these mechanisms. TAS was expected to be associated with oxLDL,[42,43] but was not a significant variable in our study. This can be because of the difficulty in measuring TAS using a single test,[44] which may not completely reflect the actual in vivo antioxidant status.

Our study has several limitations. First, as a cross-sectional study utilizing residual samples, clinical information, such as factors related to environment, dietary or behavioral characteristics, and detailed disease and medication history, was limited. Second, some measurements, such as assessment of enzymatic antioxidants, direct Denka sdLDL-C assay (the reference for estimated sdLDL-C levels), and determination of sdLDL-C levels using ultracentrifugation (the gold standard), which could be beneficial for the analysis, were not performed. Our results may have been biased by the unmeasured confounding factors. Finally, the cross-sectional nature of this study does not fundamentally allow for the determination of causality, and the presence of group heterogeneity (for example, DM patients with a wide spectrum of disease severity and medication) may render its estimation difficult. However, our study clearly shows that the distribution of sdLDL-Cs and the relationships among M-sdLDL-C, estimated sdLDL-Cs, and oxLDL in DM outpatients visiting tertiary hospitals differ from those in the general non-DM population.

In conclusion, we showed that high sdLDL-C level was associated with increase in oxLDL level in the general population. Although the estimated sdLDL-Cs showed good correlations with M-sdLDL-C in the non-DM group, their relationships with M-sdLDL-C and oxLDL differed in the DM group. Therefore, the sdLDL-C estimating equation developed from a general population should be applied with caution to a special population, such as patients with DM on treatment.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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