High-Density Lipoprotein Subfractions and Their Oxidized Subfraction Particles in Patients with Chronic Kidney Disease

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Aim: Chronic kidney disease (CKD) may lead to reduced concentrations of high-density lipoprotein (HDL) and its subfractions (HDL2 and HDL3), and damage them via inflammation and oxidative stress. The present study aimed to determine the contribution of such changes to cardiovascular disease (CVD) in patients with CKD.

Methods: The levels of total cholesterol, low-density lipoprotein cholesterol, HDL-C, HDL2, HDL3, apolipoproteins, malondialdehyde-modified LDL (MDA-LDL), oxidized (ox) HDL, oxHDL2, and oxHDL3 were measured in blood samples from patients with CKD (stages 2–5, n = 86) who were not on dialysis and from patients undergoing hemodialysis (CKD stage 5D, n = 25). The patients were followed up for 28 ± 9 months after baseline examinations and CVD events were recorded.

Result: The levels of HDL3 and ApoA1 in HDL3 fraction decreased according to CKD severity, whereas those of HDL2 and ApoA1 in HDL2 fraction did not differ. The levels of oxHDL were similar across CKD stages. The levels of oxHDL2 were increased according to CKD severity. Multivariate analyses using the Cox proportional hazards model selected high levels of oxHDL and its subfractions, and those adjusted with HDL-C and HDL subfractions or ApoA1 in HDL fractions respectively, compared with HDL-C and HDL subfractions or ApoA1 in HDL fractions alone as independent risk factors for CVD events.

Conclusion: The levels of HDL subfractions and their oxidized subfraction particles differed among patients with CKD. The increasing levels of oxHDL subfractions might cause a high frequency of CVD events in such patients.

J Atheroscler Thromb, 2016; 23: 81-94.

Key words: Oxidized HDL, HDL subfraction, CVD events

Introduction

The progression of atherosclerosis and cardiovascular disease (CVD) is usually closely associated with lipid abnormalities such as increased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C). However, these associations differ in patients with chronic kidney disease (CKD) compared with the general population. Relationships between TC or LDL-C levels and CVD events are reciprocal and J-shaped in patients with CKD, and high HDL-C levels are not always associated with the suppressed progression of atherosclerotic lesions and lower cardiovascular mortality in such patients. These differences are closely associated with lipid abnormalities in CKD that are characterized by more qualitative
than quantitative abnormalities. The metabolism of LDL is altered and triglyceride-rich lipoproteins accumulate with a predominant small, dense LDL phenotype in patients with CKD7, 8. Increased fractional catabolism decreases the serum levels of HDL-C, which does not mature normally and becomes cholesterol ester-poor due to decreased levels of lecithin cholesterol acyltransferase (LCAT) in patients with CKD7, 8).

Inflammation and oxidative stress are consistent features of CKD and important causes of reduced HDL as well as impaired and denatured HDL particles that become oxidized (oxHDL)8. Oxidized HDL behaves in the bloodstream as dysfunctional HDL8,11 and consequently accelerates atherosclerosis11, 12. Several studies have investigated dysfunctional HDL in patients with CKD who are under dialysis. Yamamoto et al. showed that the capacity of HDL for accepting cholesterol from macrophages is less effective in patients on hemodialysis (HD) than in individuals with normal HDL13. Kalantar-Zadeh reported that dysfunctional HDL that has lost its anti-oxidant and anti-inflammatory abilities is associated with a poor outcome in patients on HD14. We also showed that oxHDL is closely associated with CVD outcomes and nutritional status in patients with CKD, particularly those with inflammation15, 16. These findings suggest that the composition of denatured HDL contributes to an increased risk of CVD in patients on HD.

HDL can be separated into HDL2 and HDL3 subfractions, which have anti-atherogenic and anti-inflammatory effects, respectively, that protect against CVD. Thus, measuring and estimating changes in HDL subfractions and denatured particles of HDL subfractions should be important for predicting CVD in populations with CKD. Although several studies have analyzed HDL2 and HDL3 profiles in patients with CKD17-20, associations between oxHDL subfractions and kidney function, and the prediction of CVD events in patients with CKD have not been fully evaluated.

Table 1. Patients’ characteristics

|                      | Total (n = 111) | CKD stage 2-3 (n = 35) | CKD stage 4 (n = 26) | CKD stage 5 (n = 25) | CKD stage 5D (n = 25) | p \(^2\) |
|----------------------|----------------|------------------------|----------------------|----------------------|----------------------|---------|
| Age (y)              | 64 ± 14\(^3\)  | 59 ± 16                | 66 ± 13              | 66 ± 14              | 68 ± 12              | 0.09    |
| Sex (male, %)        | 71             | 77                     | 76                   | 66                   | 64                   | 0.62    |
| Diabetes mellitus (%)| 38             | 27                     | 28                   | 54                   | 48                   | 0.09    |
| Cause of CKD (%)     |                |                        |                      |                      |                      | 0.09    |
| Chronic glomerulonephritis | 19           | 24                     | 20                   | 19                   | 16                   |         |
| Diabetic nephropathy | 32             | 17                     | 20                   | 42                   | 48                   |         |
| Nephrosclerosis       | 34             | 41                     | 40                   | 23                   | 22                   |         |
| Other or Unknown      | 15             | 18                     | 20                   | 16                   | 14                   |         |
| Cardiovascular disease (%) | 24          | 12                     | 12                   | 31                   | 52                   | 0.002   |
| Body mass index (kg/m\(^2\)) | 24.6 ± 4.1 | 25.7 ± 4.4             | 23.6 ± 3.3           | 23.8 ± 3.8           | 21.4 ± 4.1           | 0.002   |
| ARB/ACE-I\(^4\) (%)  | 100            | 100                    | 100                  | 100                  | 100                  |         |
| Lipid lowering drugs (%) |            |                        |                      |                      |                      |         |
| Statins              | 41             | 32                     | 28                   | 54                   | 56                   | 0.08    |
| Other                | 15             | 13                     | 21                   | 20                   | 8                    | 0.52    |
| Urine albumin (mg/g Cr) | 737 (20, 6109)\(^5\) | 123 (20 –3690) | 320 (23–4550) | 1606 (25–6109) | –                     | <0.0001 |
| Serum creatinine (mg/dL) | 2.1 ± 1.2 | 1.0 ± 0.3               | 2.2 ± 0.6            | 4.6 ± 1.7            | 11.2 ± 2.9           | <0.0001 |
| eGFR\(^6\) (mL/min./1.73 m\(^2\)) | 34.3 ± 23.8 | 59.0 ± 18.2            | 22.5 ± 5.5           | 10.6 ± 3.1           | –                    | –       |
| Serum albumin (g/dL)  | 3.9 ± 0.5      | 4.2 ± 0.4              | 3.8 ± 0.5            | 3.8 ± 0.5            | 3.7 ± 0.3            | <0.0001 |
| High sensitive-CRP (mg/dL) | 0.06 (0.007–2.2) | 0.05 (0.01–0.54) | 0.06 (0.017–0.5) | 0.06 (0.016–0.75) | 0.11 (0.007–2.2) | 0.11    |

1: chronic kidney disease; 2: P values for differences of the variables among CKD stages; 3 mean ± standard deviation; 4: angiotensin receptor blocker/angiotensin converting enzyme inhibitor; 5: median (range); 6: estimated glomerular filtration rate.
among HDL-C and HDL subfractions, oxHDL and oxHDL subfractions, other lipid parameters, and CKD stages, and these factors were compared with CVD or diabetes mellitus (DM) status. Clinical factors consisting of the cause of CKD, the presence of DM, and a history of CVD were recorded. A history of CVD was determined from medical records, clinical symptoms, or findings indicating cerebrovascular (stroke) and/or peripheral vascular disease. Medical prescriptions for antihypertensive and lipid-lowering drugs were recorded.

The patients were followed up for 36 months to estimate composite CVD events. Major CVD events were defined as non-fatal myocardial infarction (MI), non-fatal stroke, or death from CVD events. The time to each event was determined by analyzing composite CVD events consisting of non-fatal CVD events, fatal MI and unstable angina pectoris (UAP), fatal cerebral infarction, or peripheral artery disease (PAD).

### Measured Factors

The baseline levels of albumin, creatinine, TC, HDL-C, LDL-C, apolipoproteins (ApoA1, ApoA2, ApoB, ApoC2, ApoC3, ApoE), HDL subfractions (HDL2 and HDL3), malondialdehyde-modified LDL (MDA-LDL), oxHDL and the oxHDL subfractions,

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**Table 2. Factors associated with CVD status**

|                           | Model 1 (r²=0.35) | Model 2 (r²=0.42) | Model 3 (r²=0.42) |
|---------------------------|-------------------|--------------------|--------------------|
| Intercept                 | -11.44, 3.07, 0.0002 | -7.23, 3.07, 0.02 | -8.20, 3.19, 0.01 |
| Age                       | 0.11, 0.03, 0.003  | 0.11, 0.04, 0.004 | 0.12, 0.04, 0.002 |
| Hemodialysis therapy      | 0.90, 0.32, 0.004  | 0.76, 0.38, 0.04  | 0.95, 0.37, 0.01  |
| Log hs-CRP                | 0.56, 0.24, 0.02  | 0.48, 0.25, 0.05  | 0.44, 0.04, 0.07  |
| Oxidized HDL              | 0.02, 0.006, 0.0009 | –                  | –                  |
| Oxidized HDL2             | –                 | Not selected       | Not selected       |
| Oxidized HDL3             | –                 | 0.03, 0.009, 0.002 | 0.03, 0.009, 0.001 |
| HDL-C                     | Not selected      | –                  | –                  |
| HDL2                      | –                 | Not selected       | –                  |
| HDL3                      | –                 | -0.26, 0.09, 0.003 | –                  |
| Total ApoA1               | Not selected      | –                  | –                  |
| ApoA1-HDL2f               | –                 | –                  | Not selected       |
| ApoA1-HDL3f               | –                 | –                  | -0.08, 0.03, 0.006 |

Model 1 is including history of cardiovascular disease (CVD) as a dependent factor, and age, sex, body mass index, diabetes status, hemodialysis therapy, statin treatment, log high-sensitive (hs) CRP, HDL-C (or ApoA1) and oxidized HDL as independent factors. Factors were selected by forward stepwise analysis.

Model 2 and 3 is including history of CVD as a dependent factor, and factors adjusted in Model 1 and HDL2 and HDL3 instead of HDL-C (ApoA1-HDL2f and ApoA1-HDL3f instead of ApoA1) and oxidized HDL2 and oxidized HDL3 instead of oxidized HDL as independent factors. Factors were selected by forward stepwise analysis.

Total ApoA1, ApoA1 in sera before separation of HDL3 fraction; ApoA1-HDL3f, ApoA1 in sera from HDL3 fraction; ApoA1-HDL2f, total ApoA1 - ApoA1-HDL3f.

Thus, the present study compared the levels of HDL and oxHDL subfractions according to CKD severity to define the impact of oxHDL subfractions as well as HDL subfractions on new CVD events in patients with CKD.

### Methods

#### Patients

This study included 111 patients with CKD i.e., 86 patients with CKD stages 2–3 who were not on dialysis (n=35), 4 (n=26), and 5 (n=25) who were managed at the Showa University Hospital and 25 patients with CKD stage 5D who were undergoing HD at a clinic. The patients who did not provide blood samples, had an anticipated life expectancy of <6 months, or who presented with clinical signs of overt infection, acute vasculitis, or liver disease at the time of recruitment were excluded from the study. All patients provided written informed consent to participate in this study, which was approved by the Ethics Committee at Showa University School of Medicine.

#### Study Design

This prospective cohort study was designed after a baseline cross-sectional assessment of associations among HDL-C and HDL subfractions, oxHDL and oxHDL subfractions, other lipid parameters, and CKD stages, and these factors were compared with CVD or diabetes mellitus (DM) status. Clinical factors consisting of the cause of CKD, the presence of DM, and a history of CVD were recorded. A history of CVD was determined from medical records, clinical symptoms, or findings indicating cerebrovascular (stroke) and/or peripheral vascular disease. Medical prescriptions for antihypertensive and lipid-lowering drugs were recorded.

The patients were followed up for 36 months to estimate composite CVD events. Major CVD events were defined as non-fatal myocardial infarction (MI), non-fatal stroke, or death from CVD events. The time to each event was determined by analyzing composite CVD events consisting of non-fatal CVD events, fatal MI and unstable angina pectoris (UAP), fatal cerebral infarction, or peripheral artery disease (PAD).

### Measured Factors

The baseline levels of albumin, creatinine, TC, HDL-C, LDL-C, apolipoproteins (ApoA1, ApoA2, ApoB, ApoC2, ApoC3, ApoE), HDL subfractions (HDL2 and HDL3), malondialdehyde-modified LDL (MDA-LDL), oxHDL and the oxHDL subfractions,
oxHDL2 and oxHDL3, and high-sensitivity C-reactive protein were measured in venous blood samples from non-fasting patients. Urine albumin-to-creatinine ratios (UACR) were measured in the non-dialysis patients.

**HDL Subfractions**

We measured HDL subfractions in frozen sera as follows. Firstly, HDL-C or ApoA1 levels were measured in sera before separation. The serum samples (300 μL) were precipitated with heparin containing MnCl₂ and dextran sulfate and separated by centrifugation at 10,000 rpm for 10 min. The amounts of HDL3 in the supernatant were measured using homogeneous HDL-EX HDL-C assays (Denka Seiken, Tokyo, Japan). The amount of ApoA1 in HDL3 fractions was measured in sera.

Levels of HDL2 were derived from the formula:

\[
\text{HDL2} = \text{HDL-C} - \text{HDL3}. 
\]

The serum levels of ApoA1 and ApoA2 derived from HDL2 fractions (ApoA1-HDL2f and ApoA2-HDL2f, respectively) were estimated using the formula: ApoA1 or ApoA2 in sera before separation of HDL3 fraction (total ApoA1 or total ApoA2) - ApoA1 or ApoA2 in the sera from HDL3 fraction (ApoA1-HDL3f and ApoA2-HDL3f, respectively).

**Oxidized HDL and oxHDL Subfractions**

We analyzed oxHDL using an ELISA with anti-oxidized ApoA1 antibody as described previously. We measured oxHDL in whole blood and oxHDL3 in separated serum. Oxidized HDL2 was derived from the formula: oxHDL2 = oxHDL - oxHDL3.

**Statistical Analyses**

Data are expressed as mean ± standard deviation or as medians (range) unless otherwise noted, and values with \( p < 0.05 \) were considered statistically significant. Normally distributed variables between two groups were compared using the Student's \( t \)-test, and non-normally distributed variables were assessed using the Wilcoxon rank-sum test. Nominal variables were compared between two groups using Fisher's exact test, and among more than two groups using the \( \chi^2 \) test. Correlations were calculated using the Spearman rank test (Rho, \( \rho \)) for non-parametric data. Paired samples were compared using the Wilcoxon signed-rank test. Independent associations between one dependent variable and more than two independent variables were assessed using forward stepwise multivariate regression analysis. The maximum \( P \) value required for an effect to be entered into the model.

**Fig. 1.** The levels of total cholesterol (TC), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and non-HDL-C according to the stages of chronic kidney disease.
and their respective subfractions according to DM and CVD status in all patients.

The levels of HDL3 correlated with oxHDL3 ($\rho = 0.27$, $p = 0.003$), whereas HDL-C and HDL2 did not correlate with either oxHDL ($\rho = 0.08$, $p = 0.41$) or oxHDL2 ($\rho = 0.07$, $p = 0.44$).

The levels of HDL-C, HDL2, and HDL3 were lower in patients with than without DM (Supplementary Fig. 1). Oxidized HDL levels tended to decrease in patients with DM, whereas those of oxHDL2 and oxHDL3 did not differ between patients with and without DM (Supplementary Fig. 1). The levels of HDL3 were lower, and those of oxHDL and oxHDL2 were higher in patients with than in those without a

during a forward step was 0.1. Independent predictors of composite CVD events and the amount of time to the first event when patients had more than one CVD event during the observation period were determined using the Cox proportional hazards model. The data were analyzed using JMP Pro version 11.0.0 (SAS Institute, Cary, NC, USA).

Results

Table 1 shows the characteristics of all the included patients. We initially assessed the associations between HDL-C and HDL subfractions and oxidized properties, and between the levels of HDL-C, oxHDL,
lowering drugs than patients with CKD stage 2–3 and 4.

Levels of Cholesterol and Apolipoproteins According to CKD Stage

The levels of TC, LDL-C, and non-HDL cholesterol were significantly decreased in patients with CKD stage 5 and 5D compared with those who had CKD stage 2–3 (Fig. 1). The levels of HDL-C were significantly lower in patients with CKD stage 5D compared with those who had CKD stage 2–3 (Fig. 2). ApoE levels were the lowest in stage 5 among
the CKD stages (Fig. 2).

The levels of HDL2 did not differ among CKD stages (Fig. 3A), whereas those of HDL3 decreased according to the severity of CKD (Fig. 3D). The changes in ApoA1 and A2 in HDL2 or HDL3 fraction were similar to those in HDL2 or HDL3 (Fig. 3).

**Levels of oxHDL, oxHDL Subfractions, and MDA-LDL According to CKD Stage**

The levels of oxHDL did not differ among CKD stages (Fig. 4A) but those of oxHDL3 decreased according to the severity of CKD and those of oxHDL2 were significantly increased in patients on HD (Fig. 4B, C). The levels of MDA-LDL were lower in patients with CKD stages 5 and 5D (Fig. 4D).

**Associations between New CVD Events and HDL, oxHDL, Their Respective Subfractions and ApoA1 in HDL and Respective HDL Fractions**

At least one new CVD event developed in 21 patients during a mean follow-up period of 28 ± 9 months. The events consisted of non-fatal or fatal MI (n=7) and UAP (n=5) and non-fatal or fatal cerebral infarction (n=8) and PAD (n=4).

Table 3 and Supplementary Table 1 show associations between composite CVD events and HDL-C, ApoA1, oxHDL, their respective subfractions, and ApoA1-HDL2f and ApoA1-HDL3f. HDL-C, HDL subfractions, ApoA1, ApoA1-HDL2f, and ApoA1-HDL3f did not predict CVD events (Supplementary Table 1). However, the hazards ratios (HR) for oxHDL and their respective subfractions indicated that the oxidized particles were independent predictors of CVD events. The HRs for oxHDL or oxHDL subfractions were significantly increased in multivariate models 1–4 (Table 3). Neither LDL-C nor MDA-LDL predicted CVD events in the present study.

Table 4 shows associations between CVD events and the ratios of oxHDL to HDL-C and ApoA1, and of oxHDL subfractions to their respective HDL subfractions and to ApoA1 in the respective HDL2 and 3 fractions. The HR for these ratios were significantly increased in multivariate models 1, 2, 4, and 5 (Table 4). The HR for the ratio of oxHDL2 to HDL2 adjusted with the ratio of oxHDL3 to HDL3 and confounders did not reach statistical significance (Table 4, model 3), although the ratios of oxHDL2 to HDL2 could predict CVD events in that model (Table 4, model 1, 2). However, the HR for the ratio of oxHDL2 to ApoA1-HDL2f adjusted with the ratios of oxHDL3 to ApoA1-HDL3f and confounders was significantly increased (Table 4, model 6).

![Fig. 4](image.png) The levels of oxidized high-density lipoprotein (oxHDL), oxidized HDL subfractions (oxHDL2 and oxHDL3), and malondialdehyde-modified LDL (MDA-LDL) according to the stages of chronic kidney disease.
lipase and LCAT activities are significantly reduced in patients with CKD, particularly when they are on HD. Therefore, such decreases might influence the metabolism of HDL2 and HDL3, the similar serum HDL2 levels among CKD stages, the significantly decreased serum HDL3 levels in severe CKD stages, discrepant serum levels between oxidized HDL subfractions, and increased oxHDL2 and decreased oxHDL3 levels in patients with CKD stages 5 and 5D.

Paraoxonase 1 (PON1) is transported via HDL binding to ApoA1 as an athero-protective protein with anti-oxidative properties. Low PON1 activity is associated with an increased risk of CVD. Recent studies have shown that oxidative stress alters PON1 as well as ApoA1 in HDL that becomes dysfunctional and PON1 activity is decreased in patients with CKD. Thus, altered PON1 levels may be involved in impaired and denatured HDL particles via oxidation in patients with CKD.

Evidence indicates that low HDL-C levels impose a risk for CVD events in general populations. The function of HDL is important in lowering the incidence of CVD events and the decreasing levels of HDL2 and HDL3 that result in diminished specific functions could be associated with a greater likelihood of developing CVD events. Thus, the low levels of HDL2 and HDL3 may be associated with CVD events in patients with CKD. Several studies have measured the levels of HDL subfractions in patients with CKD under HD and found that the levels of HDL3 are decreased compared with those of healthy controls, whereas those of HDL2 are controversial 18, 20. Although the methods used to measure HDL subfractions might have influenced HDL2 and HDL3 values in the present study, we nevertheless found similar low HDL3 levels in patients with CKD under HD. While the HDL-C levels were similar among these stages, the levels of HDL3 gradually decreased in patients with CKD, particularly when they are on HD.

**Discussion**

High levels of oxHDL and their oxidized subfractions were associated with an increased risk for CVD events in patients with CKD. Uremia including inflammation and oxidative stress alters HDL concentrations and causes dysfunctional HDL in patients with CKD. Thus, uremia with oxidation may alter the anti-atherogenic and anti-inflammatory properties of HDL2 and HDL3, thus contributing to atherosclerotic progression and an increased incidence of CVD events arising in patients with CKD, particularly those on dialysis.

On the other hand, the ability of oxHDL or its subfractions alone to predict CVD events did not seem to be any better than the ratios of oxHDL to HDL-C or ApoA1, or of oxHDL subfractions to their respective HDL subfractions, or of ApoA1 to their respective HDL fractions. The total oxHDL levels did not change with CKD stage, whereas the levels of oxHDL2 and oxHDL3 were increased and decreased, respectively, in patients with CKD stages 5 and 5D. The association between HDL2 or ApoA1-HDL2f and oxHDL2 differed from that between HDL3 or ApoA1-HDL3f and oxHDL3. Thus, the predictive ability of oxHDL and their subfractions to CVD events might be influenced by the amount of HDL-C and ApoA1 or of HDL subfractions and ApoA1 in each respective HDL fraction.

Hepatic lipase and LCAT might influence the metabolism of HDL subfractions and levels of their oxidized particles in this setting. LCAT is essential for HDL maturation of lipid-poor HDL to lipid-rich spherical HDL that becomes HDL3 and then HDL2. Hepatic lipase is inversely associated with the buoyancy and size of HDL-C and it plays an important role in remodeling HDL particles in a process that involves the catabolism of HDL2 particles. Hepatic lipase and LCAT activities are significantly reduced in patients with CKD, particularly when they are on HD.

**Table 3.** Cox proportional hazards models for composite CVD events

| Model | Oxidized HDL | Oxidized HDL2 | Oxidized HDL3 |
|-------|--------------|---------------|---------------|
| 1     | 1.01 (1.01, 1.02), <0.0001 | 1.02 (1.00, 1.03), 0.001 | 1.01 (1.00, 1.02), 0.0006 |
| 2     | 1.01 (1.00, 1.02), 0.0004 | 1.01 (1.00, 1.02), 0.02 | 1.01 (1.00, 1.02), 0.002 |
| 3     | 1.01 (1.00, 1.02), 0.0004 | 1.01 (1.00, 1.02), 0.02 | 1.01 (1.00, 1.01), 0.003 |
| 4     | 1.01 (1.00, 1.01), 0.0003 | 1.01 (1.00, 1.02), 0.02 | 1.01 (1.00, 1.01), 0.003 |

Model 1: Age, sex, diabetes mellitus (DM) status, hemodialysis therapy are independent factors.
Model 2: Age, DM status, history of cardiovascular disease (CVD) and hemodialysis therapy are independent factors.
Model 3: Age, history of CVD, hemodialysis therapy, log hs-CRP are independent factors.
Model 4: Age, DM status, history of CVD, hemodialysis therapy and log hs-CRP are independent factors.

CI, confidence interval; HR, hazards ratio.
parallel with a decrease in eGFR but those of HDL2 did not. HDL3 correlated with a history of CVD, but low levels of HDL subfractions and of HDL-C did not predict CVD events in the present study. Thus, the controversy over the ability of HDL-C and HDL subfractions to predict CVD between patients with and without CKD might be associated with altered HDL metabolism in CKD, particularly at the advanced stages.

We measured the serum levels of LDL-C and MDA-LDL as one type of oxidized LDL (oxLDL) and estimated their ability to predict CVD events. Values for LDL-C decreased in accordance with CKD severity and were similar to those of MDA-LDL. Neither LDL-C nor MDA-LDL predicted CVD events. The atherogenicity of LDL is increased in patients with advanced CKD, but when LDL metabolism changes, the amount of small dense LDL particles increases and the amount of total LDL-C relatively decreases in patients with advanced CKD8). Noori et al. assessed the ability of LDL to predict mortality in a cohort of patients on HD and found that an increase in the amount of very small LDL particles increases the risk for mortality whereas large LDL molecules are associated with better survival for these patients38). Moreover, a low oxLDL concentration is a risk factor for CVD events in such populations, whereas a high concentration is not39). Therefore, LDL-C and oxLDL may not be as reliable as oxHDL and its subfractions in terms of serving as biomarkers for CVD events in patients with advanced CKD, despite having atherogenic properties.

The present results must be considered with the following caveats. The number of patients was relatively small and thus our findings might have been influenced by low statistical power. The HDL-C and HDL subfractions as well as their oxidized properties were measured only at baseline. Our patients with CKD were heterogeneous in that they were not all under dialysis. We did not estimate different sizes or features of LDL or oxLDL. Therefore, a prospective large cohort study is required to reveal the associations between these molecules and CVD events in patients with CKD who are being treated with and without dialysis.

In conclusion, the changes in the HDL subfractions and oxidized HDL particles differ depending on the severity of CKD, and increased amounts of oxidized subfractions of HDL may cause a high frequency of CVD events in patients with advanced CKD.

**Conflicts of Interest**

None.
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Supplementary Fig. 1.
The levels of high-density lipoprotein cholesterol (HDL-C) and HDL subfractions (HDL2 and HDL3) and their respective oxidized states according to the diabetes status.
Supplementary Fig. 2.
The levels of high-density lipoprotein cholesterol (HDL-C) and HDL subfractions (HDL2 and HDL3) and their respective oxidized states according to the history of cardiovascular disease.
## Supplementary Table 1.
Cox proportional hazards models of HDL-C, HDL subfractions, ApoA1 and ApoA1 in HDL subfractions for composite CVD events

| Model | Cox multivariate models (OR; 95% CI, p value) |
|-------|---------------------------------------------|
|       | HDL-C  | HDL2  | HDL3  | Total ApoA1 | ApoA1-HDL2f | ApoA1-HDL3f  |
| 1     | 1.02 (0.99, 1.04), 0.43 | 1.01 (0.97, 1.04), 0.98 | 0.90 (0.78, 1.02), 0.10 | 1.00 (0.98, 1.02), 0.97 | 1.00 (0.99, 1.02), 0.62 | 0.98 (0.95, 1.02), 0.38 |
| 2     | 1.01 (0.98, 1.03), 0.44 | 0.99 (0.96, 1.03), 0.91 | 0.94 (0.81, 1.08), 0.39 | 1.00 (0.98, 1.02), 0.86 | 1.00 (0.98, 1.02), 0.82 | 1.00 (0.96, 1.04), 0.89 |
| 3     | 1.01 (0.98, 1.03), 0.24 | 0.99 (0.96, 1.03), 0.59 | 0.90 (0.77, 1.03), 0.15 | 1.00 (0.97, 1.01), 0.59 | 1.00 (0.98, 1.01), 0.83 | 0.98 (0.94, 1.03), 0.42 |
| 4     | 0.99 (0.95, 1.02), 0.42 | 0.99 (0.95, 1.02), 0.66 | 0.90 (0.77, 1.05), 0.17 | 1.00 (0.98, 1.02), 0.65 | 1.00 (0.98, 1.01), 0.88 | 0.98 (0.94, 1.03), 0.45 |

Model 1: Age, sex, diabetes mellitus (DM) status, hemodialysis therapy are independent factors.
Model 2: Age, DM status, history of cardiovascular disease (CVD) and hemodialysis therapy are independent factors.
Model 3: Age, history of CVD, hemodialysis therapy, log hs-CRP are independent factors.
Model 4: Age, DM status, history of CVD, hemodialysis therapy and log hs-CRP are independent factors.
CI, confidence interval; OR, odds ratio. Total ApoA1, ApoA1 in sera before separation of HDL3 fraction; ApoA1-HDL3f, ApoA1 in sera from HDL3 fraction; ApoA1-HDL2f, total ApoA1 - ApoA1-HDL3f.