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# Association of cholesterol efflux capacity with plasmalogen levels of high-density lipoprotein: A cross-sectional study in chronic kidney disease patients

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ABSTRACT

*Background and aim:* Current research suggests that dysfunctional high-density lipoprotein (HDL) with low cholesterol efflux capacity may accelerate atherosclerosis, particularly in chronic kidney disease (CKD). We previously reported that serum levels of plasmalogens closely correlated with HDL concentration, and could serve as a novel biomarker for atherosclerosis. In the present study, we analyzed the association of cholesterol efflux capacity of HDL with clinical and biochemical parameters, including plasmalogens, in CKD patients.

*Methods:* We enrolled 24 mild-to-moderate CKD patients (CKD-3-4) and 33 end-stage renal disease (ESRD) patients nearing hemodialysis (CKD-5), and assessed physiological atherosclerotic scores, cholesterol efflux capacity, and plasmalogens levels in HDL. Furthermore, the effect of plasmalogen on cholesterol efflux capacity of HDL was examined by *in vitro* studies with re-constituted HDL (rHDL) and HDL prepared from CKD-5 patient (ESRD-HDL) with additional phospholipids.

*Results:* There were significant differences in many parameters between the two groups. In particular, plasmalogens levels and cholesterol efflux capacity of HDL were significantly reduced in the CKD-5 group compared to those in the CKD-3-4 group (-35.1%, $p < 0.001$, -36.8%, $p < 0.001$, respectively). Multivariate linear regression analyses revealed that ethanolamine plasmalogen levels of HDL were independently associated with cholesterol efflux capacity ($p = 0.045$) and plaque scores ($p = 0.035$). *In vitro* studies also indicated that additional plasmalogens augmented cholesterol efflux ability of HDL.

*Conclusions:* High plasmalogens concentrations in HDL may correlate with acceleration of cholesterol efflux and their decreased levels may promote atherosclerosis in advanced CKD patients.



## 1. Introduction

Major risk factors for atherosclerotic diseases include hypertension, dyslipidemia, obesity, diabetes mellitus, and cigarette smoking. The causality of these 'classic' risk factors is well established and they are commonly used to assess cardiovascular risk in the general population. Chronic kidney disease (CKD) also accelerates the development of atherosclerosis and increases the risk of cardiovascular disease (CVD) [1]. Noteworthy, the lipid profile induced by CKD has specific features distinct from the general population. Besides quantitative alterations, renal patients have qualitative lipid alterations that are responsible for the increase in cardiovascular risk [2].

*Abbreviations:* ABI, ankle-brachial index; ADMA, asymmetric dimethylarginine; Alb, albumin; apo A-I, apolipoprotein A-I; baPWV, brachial-ankle pulse wave velocity; %CH efflux, cholesterol efflux capacity; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; IL-6, Interleukin 6; IMT, intima media thickness; Ox-LDL, oxidized low-density lipoprotein cholesterol; PL, total phospholipids; Pls, plasmalogens; PlsCho, choline plasmalogen; PlsEtn, ethanolamine plasmalogen; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PS, plaque score; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; rHDL, re-constituted HDL; TC, total cholesterol; TG, triglyceride.

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Recent studies have suggested that dysfunctional high-density lipoprotein (HDL) particles and decreased plasma levels of HDL cholesterol (HDL-C) may contribute to accelerated atherosclerosis in CKD patients [3,4]. In particular, low cholesterol efflux capacity of HDL, which is the first step in reverse cholesterol transport and a key mechanism of the atheroprotective effect of HDL [5], has been characterized in advanced CKD [6,7].

Chronic and sterile inflammatory processes are known to be primarily involved in the modulation of the composition and function of HDL [8], as well as the reverse cholesterol transport system [9]. Proteomic and lipidomic analyses of HDL from uremic patients have revealed a significant increase in the amount of acute phase protein serum amyloid A [10,11], and a remarkable reduction in the contents of phospholipids, possibly due to the induction of secretory phospholipase $A_2$ [8]. Such altered lipid composition may well lead to HDL dysfunction, including reduced cholesterol efflux ability [12,13].

Several experimental studies have reported that phospholipid levels of HDL (HDL-PL) affect HDL cholesterol efflux capacity [14–17]. Furthermore, some clinical evidence supports the relevance of HDL-PL in the development of CVD [18], probably through their impact on cholesterol efflux capacity [19]. However, clinical characterization of the influence of the particular species of PL in HDL particles on cholesterol efflux capacity is still limited [20].

We demonstrated that serum plasmalogens (Pls), a subclass of glycerophospholipids with a vinyl-ether bond at the *sn*-1 position, were associated with diverse risk factors for metabolic syndrome and/or atherosclerosis [21] and CVD [22,23]. Of interest, serum levels of plasmalogen correlated positively and strongly with HDL-C concentration [21–23], despite the fact that plasmalogens exist almost ubiquitously in all lipoprotein fractions [24], suggesting that plasmalogen may be implicated in metabolism and/or function of HDL. A recent lipidomic analysis revealed that individuals with low HDL-C displayed changes in the quality of their HDL particles, along with decreased plasmalogen content [25]. The plasmalogen levels of HDL were inversely associated with both stable and acute coronary artery disease, and the association may reflect the anti-apoptotic effects of plasmalogen on endothelial cells [26]. In addition, statin treatment increased the content of polyunsaturated phospholipids and plasmalogens and antioxidative function of HDL from atherogenic mixed dyslipidemia of metabolic syndrome [27].

Plasmalogen levels in blood plasma and erythrocytes have been proposed as surrogate markers of oxidative stress in CKD patients [28–30]. However, few studies have examined the relationship between plasmalogen and clinical and biochemical parameters of CKD, especially with regards to atherosclerosis. Collaborative meta-analysis of general population cohorts indicated that end-stage renal disease (ESRD) patients have a nearly two-fold more accelerated cardiovascular mortality than mild-to-moderate CKD [31]. The atherogenic diathesis associated with ESRD is driven by inflammation, oxidative stress and dyslipidemia. Particularly, reduced HDL-C level and HDL dysfunction are the hallmarks of ESRD-related dyslipidemia [32]. Thus, we measured plasmalogens levels of blood plasma, HDL fractions, and erythrocytes in mild to moderate-stage (CKD-3-4) and ESRD patients (CKD-5), and examined their relationship with clinical and biochemical parameters, including the cholesterol efflux capacity of HDL.

## 2. Materials and methods

### 2.1. Study subjects and design

From 2010 to 2012, we enrolled 24 mild to moderate degree CKD patients (CKD-3-4: mean eGFR, $56.4 \pm 24.3$ mL/min/1.73 $m^2$) and 33 ESRD patients just prior to hemodialysis initiation (CKD-5: mean eGFR, $5.2 \pm 1.9$ mL/min/1.73 $m^2$), who were referred to the outpatient department of the Teikyo University Hospital and the affiliated clinics. Patients with evidence of cancer were excluded from this study. Demographic data (including age, gender, body mass index, blood pressure), cardiovascular history, risk factors (hypertension, diabetes, hyperlipidemia, cigarette smoking), and medications were recorded. This study was approved by the ethics committees on human research at the Teikyo University School of Medicine (# 09–126) and all of the patients provided informed consent.

### 2.2. Markers of atherosclerosis

Ankle-brachial index (ABI) and brachial-ankle pulse wave velocity (baPWV) were measured using a volume-plethysmographic apparatus (BP-203RPE III, Omron Colin Co., Ltd., Tokyo, Japan). The recorded ABI and baPWV values were the means of the right-side and left-side measurements. The max intima media thickness (IMT) and plaque score (PS) were assessed using carotid ultrasonography (Aplio XG, Toshiba Medical Systems Co., Tokyo, Japan) to identify atherosclerotic changes in the carotid artery. The IMT was the distance between the leading edge of the lumen-intima echo and the media-adventitia echo in the plaque-free area. At least three measurements were taken over a 1-cm length of each wall segment on both sides. The PS was calculated by adding the maximal thickness in millimeters of plaques in each segment on both sides. The length of individual plaques was not taken into consideration in the present study. The methods used have been reported elsewhere in more detail [33].

### 2.3. Blood testing

Two to 4 h postprandial blood was drawn into a plastic tube containing EDTA-2Na, sodium fluoride, or heparin sodium as anticoagulant. The blood samples were tested using the hematology auto-analyzer (Sysmex XE-5000, Kobe, Japan) and blood chemistry parameters were measured by routine measurements using the auto-analyzer (LABOSPECT 008, Hitachi High-Technologies Corporation, Tokyo, Japan). Serum and urine creatinine concentrations were measured by an enzymatic method. Estimated GFR (eGFR) was calculated according to the equation for Japanese subjects using the measured serum creatinine levels [34].

Oxidized low-density lipoprotein cholesterol (Ox-LDL; JaICA Co., Shizuoka, Japan), insulin (Mercodia AB, Uppsala, Sweden), total adiponectin (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), interleukin-6 (IL-6; GE Healthcare UK Ltd., Buckinghamshire, England), and asymmetric dimethylarginine (ADMA; Immundiagnostik, Bensheim, Germany) were determined using commercial ELISA kits. Vitamin E (V.E) was quantified by an HPLC equipped with fluorescent detector (excitation 297 nm, emission 327 nm; RF-20A, Shimadzu) [35].

### 2.4. Plasmalogens and phospholipids analyses of plasma and erythrocytes

The blood samples were separated by centrifugation at 1500*g* for 10 min at 4 °C to obtain a plasma sample to which EDTA and butylated hydroxytoluene (BHT; Sigma-Aldrich, St. Louis, MO) were added as antioxidants at the final concentration of 1 mM and 10 μM, respectively. The plasma samples were immediately frozen at −80 °C and stored until analysis. The blood cells separated by centrifugation were washed three times with phosphate buffered saline (PBS) containing 1 mM EDTA and 10 μM BHT. The obtained erythrocytes were diluted two-fold with the same washing buffer, frozen at −80 °C, and stored until use.