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Serum Paraoxonase 1 (PON1) Concentration as a Marker of Left Ventricular Mass Index (LVMI) and Atherosclerosis in Hemodialysis Patients

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Abstract: Cardiovascular disease (CVD) is the most common cause of morbidity and mortality among patients on hemodialysis (HD). Increasing evidence suggests that paraoxonase (PON) 1 is an important risk factor for CVD. In this study, we evaluated the correlations between PON1 protein concentration and cardiac function and atherosclerosis in patients on HD. A cohort of 119 patients (59 males) on maintenance HD participated in the study in which we measured common clinical parameters and serum PON1 concentrations. We also evaluated cardiac function by echocardiography after maintenance dialysis sessions. The median serum PON1 concentration was 40.8 μg / mL (range, 11.8–81.1 μg / mL) in patients on HD and is significantly associated with a history of CVD and peripheral artery disease. The serum PON1 level is positively correlated with the concentration of albumin ($r = 0.26$, $P < 0.01$), high-density lipoprotein cholesterol (HDL, $r = 0.19$, $P < 0.05$), calcium ($r = 0.23$, $P < 0.05$), urea nitrogen ($r = 0.20$, $P < 0.05$) and creatinine ($r = 0.22$, $P < 0.05$), and negatively correlated with pulse pressure ($r = -0.20$, $P < 0.05$). Among the echocardiographic parameters, the PON1 concentration is significantly and negatively correlated with left atrial dimension (LAD, $r = -0.31$, $P < 0.05$) and left ventricular mass index (LVMI, $r = -0.35$, $P < 0.005$). Stepwise multivariate regression analysis showed that PON1 is an independent predictor of LVMI (adjusted $r^2 = 0.34$). Therefore the serum PON1 concentration could contribute to the development of LVH and it could be an independent predictor of CVD in patients on maintenance HD.

Key words: cardiovascular disease (CVD), echocardiography, hemodialysis, left ventricular mass index (LVMI), paraoxonase 1 (PON1)

Introduction

Cardiovascular disease (CVD) is the most common cause of morbidity and mortality among patients on hemodialysis (HD). Several risk factors, including aging, hypertension,

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inflammation, malnutrition and dyslipidemia, are associated with the accelerated development of atherosclerosis. The prevalence of dyslipidemia is approximately 67% in patients on dialysis[1], and it is characterized by reduced high-density lipoprotein cholesterol (HDL) and modestly increased triglyceride, but not by increased low-density lipoprotein cholesterol (LDL)[2]. Epidemiological studies have demonstrated an inverse correlation between plasma levels of HDL and the risk of CVD among patients with chronic renal failure. Not only is HDL a key player in reverse cholesterol transport, but it also protects LDL against oxidation. The essential mechanism of the inhibition of LDL oxidation by HDL is partly enzymatic, and paraoxonase 1 (PON1) has been implicated in this process[3,4].

Human serum PON1 (aryldialkylphosphatase, EC 3.1.8.1) is a calcium-dependent esterase associated with apolipoprotein A1 in HDL. PON1 hydrolyzes organophosphates (*e.g.*, paraoxon, diazoxon) and arylesters (*e.g.*, phenylacetate), and it also possesses antioxidative activity[4]. PON1 hydrolyzes phospholipid peroxides in both HDL and LDL *in vitro*, and through this activity HDL prevents the oxidative modification of LDL[4]. Polymorphisms in PON1 at amino acid 55 that produces a leucine (L) to methionine (M) transition (L55M), and at position 192 that produces a glutamine (Q) to arginine transition (Q192R) influence PON activity[5]. The arylesterase activity of PON1 is not polymorphic[6].

Serum PON1 activity is low in patients with myocardial infarction[7], hypercholesterolemia, diabetes mellitus[8] and those on HD[9,10], and PON1 has recently been considered a risk factor for coronary heart disease (CHD). Kujiraoka *et al* reported that Japanese patients with CHD had significantly lower serum PON1 concentrations than healthy controls, and this association was independent of the polymorphism at position 192[6]. These findings indicate that PON1 plays an important role in CVD not only among healthy individuals, but also among patients with diabetes and chronic kidney disease (CKD). The association between PON1 and cardiac function, however, is not fully understood, particularly in patients with HD.

The concentration of PON in serum has a strong and positive association with both serum arylesterase activity and serum paraoxonase activity[6]. The present study used an enzyme-linked immunosorbent assay (ELISA) to investigate the correlation between serum PON1 concentration and both cardiac function and CVD risk factors in patients with HD.

Patients and Methods

Study Design and Subjects

Stable patients on maintenance HD were solicited to participate in this study. Only those patients who had been on regular dialysis three times each week for 3–4 h for at least three months were selected. The exclusion criteria were as follows: current smokers, presence of acute or chronic liver disease, onset of cardiovascular disease during the previous six months, presence of overt infectious complications, statin administration and unwillingness to participate in the study. Using these criteria, a cohort of 119 patients (59 males; median age, 68

years) undergoing maintenance HD (median duration of HD, 64 months; range, three - 368 months) were enrolled. The Showa University Medical Faculty Ethics Committee approved the study, and written informed consent was obtained from all of the participants.

The causes of CKD were diabetic nephropathy in 35 patients (29%), glomerulonephritis in 16 patients (13%), polycystic kidney disease in five patients (4%), nephrosclerosis in 15 patients (13%), other causes in eight patients (7%), and unknown causes in 40 patients (34%). Fifty patients (42%) had a history of cerebrovascular, cardiovascular, and/or peripheral arterial disease (PAD) at the start of the study. Twenty-four patients had PAD (14 patients at Fontaine stage II-IV), 11 patients had a history of stroke or cerebral bleeding, and three patients had a history of aortic aneurysms.

Erythropoietin was administered to 102 (88%) of the patients. Most of the patients were undergoing double or triple therapy, including angiotensin-converting enzyme inhibitors (ACEI, 11%) or angiotensin receptor blockers (ARB, 24%), calcium channel blockers, and/or β-blockers in various combinations, as well as other drugs that are generally used to treat CKD, such as phosphate, potassium binders and diuretics.

Samples and Laboratory Methods

Venous blood was collected from the arteriovenous fistula of each patient into one EDTA-containing tube and one serum sample tube before the first dialysis session of the week. Samples were immediately centrifuged at 1500 × g for 10 min at 4℃ and stored frozen at −70℃ if not analyzed immediately. Serum/blood levels of leukocytes, hemoglobin, platelets, albumin, urea nitrogen, creatinine, uric acid, calcium, phosphorus, intact parathyroid hormone (PTH), cholesterol, triglyceride, HDL, LDL, blood sugar, C-reactive protein and brain natriuretic peptide (BNP) were determined using routine methods at a clinical laboratory.

Sandwich ELISA for Determination of Serum PON Concentration

Microtiter plate wells were coated with 0.25 μg of the monoclonal antibody, 5-10D, and were incubated at 4℃ overnight. Non-specific binding was blocked with Block AceTM (Dainihon Pharmacy, Osaka, Japan) for 2 h at room temperature. Standard and serum samples [diluted in PBS containing 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-propanesulfonic acid (CHAPS)] were added to the wells, and the plates were incubated for 2 h at room temperature. The plates were washed with PBS containing 0.1% Tween 20, whereupon 100 μL of biotinylated monoclonal antibody 4C-1 (0.5 μg/mL) was added to each well and they were incubated for 2 h at room temperature. The plates were similarly washed again and 100 μL of avidin-horseradish peroxidase conjugate (0.2 μg/mL) in PBS containing 0.1% Tween 20 was added to the wells. After incubation for 1 h at room temperature, the plates were washed and 100 μL of o-phenylenediamine dihydrochloride (0.4 mg) in 50 mM citrate buffer (pH 5.0) containing 0.012% H_2O_2, was added to each well and the plates

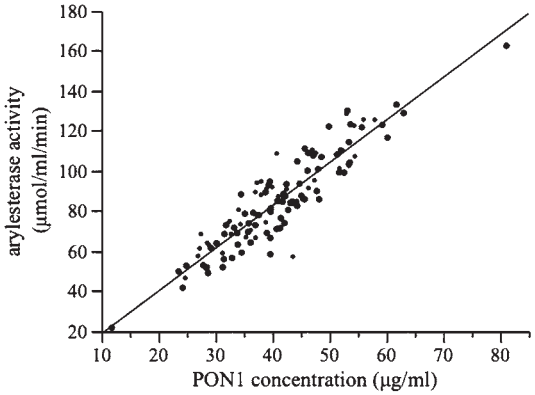

Fig. 1. Association between PON1 concentration and arylesterase activity in dialysis patients (adjusted $r^2 = 0.83$, $p < 0.0001$)

were incubated for 30 min at room temperature. The reaction was terminated by the addition of 20 μL of 1 M H_2SO_4, and absorbance was determined at 492 nm using a microplate reader. Serum concentrations of PON were determined by reference to a standard curve constructed with purified PON[6].

We compared the PON concentration and arylesterase activity that had been previously determined[11] and established that the PON concentration can be used as marker for PON hydrolytic activity in dialysis patients. The PON concentration was strongly associated with arylesterase activity in HD patients (Fig. 1).

Echocardiography

Ultrasonographic scans of cardiac function were evaluated with high-resolution echo color Doppler ultrasonography, SSA370A (Toshiba Medical Systems, Tokyo, Japan) after dialysis sessions. The same physician examined all of the patients by two-dimensional M-mode pulsed-wave and color Doppler sonography.

Statistical Analysis

All values are shown as medians and ranges unless indicated otherwise. A P value of $<$ 0.05 was considered statistically significant. Comparisons between two groups were assessed using the Wilcoxon rank-sum and Fisher's exact tests for continuous and nominal variables, respectively. Correlations between the PON1 concentration and other variables were determined using Spearman's rank correlation. The multivariate stepwise regression analysis for the left ventricular mass index (LVMI) employed a model that included the following parameters : age, gender, history of CVD, diabetes, hemoglobin, albumin, creatinine, C-reactive protein, LDL and PON1 concentration. All data were statistically analyzed using JMP, version 7.0.1 for Macintosh (SAS Institute Inc., Cary, NC, USA).

Table 1. Association between PON1 concentrations and characteristics of HD patients

	N	PON1 concentration (μg / mL)	p value
Gender (Male / Female)	59 / 60	39.6 / 41.2	0.9
CVD (yes / no)	50 / 69	38.9 / 42.8	<0.05
Diabetes (yes / no)	35 / 84	41.3 / 39.7	0.4
Peripheral artery disease (yes / no)	24 / 95	36.0 / 42.0	<0.005
Erythropoietin (yes / no)	102 / 16	39.9 / 44.2	0.8
ACEIs or ARBs (yes / no)	39 / 78	39.6 / 41.9	0.4
Phosphate binder (yes / no)	78 / 39	41.7 / 38.0	0.3

All values are shown as medians.

Results

The median serum PON1 concentration was 40.8 μg / mL (range, 11.8 - 81.1 μg / mL) in patients with HD. Table 1 shows associations between patients' characteristics and serum PON1 levels. The PON1 concentration is significantly lower in patients with a history of CVD ($P<0.05$). The PON1 concentration is also significantly lower in patients on HD who also had peripheral artery disease (PAD) compared with those without PAD ($P<0.005$). Serum PON1 concentration did not significantly differ with gender, diabetes, and the use of erythropoietin, ACEIs, ARBs and phosphate binders.

Table 2 shows associations between PON1 concentrations and laboratory parameters in patients with HD. Serum PON1 levels are positively correlated with albumin ($r=0.26$, $P<0.01$), HDL ($r=0.19$, $P<0.05$), calcium ($r=0.23$, $P<0.05$), urea nitrogen ($r=0.20$, $P<0.05$) and creatinine ($r=0.22$, $P<0.05$), but negatively correlated with pulse pressure ($r=-0.20$, $P<0.05$). The PON1 concentration was not associated with age, body mass index, leukocyte, hemoglobin, platelets, total cholesterol, triglyceride, LDL, phosphorus, C-reactive protein, intact PTH and BNP.

Table 3 shows the association between the PON1 concentration and echocardiographic parameters in patients on HD. These data indicate that the PON1 concentration is significantly and negatively correlated with left atrial dimension (LAD, $r=-0.31$, $P<0.05$) and LVMI ($r=-0.35$, $P<0.005$, Fig. 2). We detected a trend towards negative correlations between PON1 and left ventricular diameter at end diastole (LVDd) and left ventricular diameter at end systole (LVDs), but the differences did not reach significance ($P=0.05$). In addition, PON1 was not related to cardiac output (CO) and left ventricular ejection fraction (LVEF).

We established the factors that were independently associated with LVMI in patients on dialysis using stepwise multivariate regression analysis (Table 4). This model explained the 34% variation in the LVMI of this population. Our results show that PON1 is one of the

Table 2. Spearman rank correlation coefficients for serum PON1 concentrations relative to variables in patients on HD.

	Median (range)	Spearman rho	p value
Age (years)	68 (30-93)	−0.14	0.1
Body Mass Index	20.3 (12.3-30.0)	−0.02	0.8
Systolic blood pressure (mmHg)	140 (90-187)	−0.09	0.38
Diastolic blood pressure (mmHg)	80 (55-109)	−0.046	0.63
Pulse pressure (mmHg)	60 (20-100)	−0.20	<0.05
Leukocytes ($\times 10^3$ / mm^3)	5.3 (2.0-11.8)	−0.06	0.5
Hemoglobin (g / dL)	9.7 (7.2-12.5)	−0.05	0.6
Platelets ($\times 10^4$ / μL)	17.7 (5.6-34.5)	0.09	0.2
Albumin (g / dL)	3.9 (3.1-4.7)	0.26	<0.01
Total cholesterol (mg / dL)	157 (91-279)	0.1	0.2
Triglyceride (mg / dL)	95 (29-489)	−0.05	0.6
HDL cholesterol (mg / dL)	45 (9-105)	0.19	<0.05
LDL cholesterol (mg / dL)	84 (45-162)	0.06	0.5
Calcium (mg / dL)	9.4 (7.6-11.0)	0.23	<0.05
Phosphorus (mg / dL)	5.6 (2.6-8.4)	−0.07	0.5
C-reactive protein (mg / dL)	0.08 (0.007-7.04)	−0.17	0.06
Urea nitrogen (mg / dL)	73.8 (38-119.5)	0.20	<0.05
Creatinine (mg / dL)	10.8 (3.1-23.3)	0.22	<0.05
Intact parathyroid hormone (pg / dL)	120 (5-650)	0.002	0.9
Blood sugar (mg / dL)	120 (72-326)	−0.14	0.2
Brain natriuretic peptide (BNP) (pg / dL)	167 (10-1273)	−0.14	0.3

Table 3. Spearman rank correlation coefficients for PON1 concentrations relative to echocardiographic parameters in patients on HD

	Median (range)	Spearman rho	p value
CO (L / min)	4.6 (2.2-8.8)	−0.17	0.2
LVEF	0.64 (0.34-0.94)	0.12	0.3
LAD (mm)	32 (16-52)	−0.31	<0.05
LVDd (mm)	46 (31-63)	−0.23	0.05
LVDs (mm)	30 (19-50)	−0.24	0.05
LVMI (g / m^2)	119 (53-252)	−0.35	<0.005

CO, cardiac output ; LVEF, left ventricular ejection fraction ; LAD, left atrial dimension ; LVDd, left ventricular diameter at end diastole ; LVDs, left ventricular diameter at end systole ; LVMI, left ventricular mass index.

most important independent predictors of LVMI ($\beta = -0.34$).

Discussion

Left ventricular hypertrophy (LVH) is associated with high mortality rates in the general

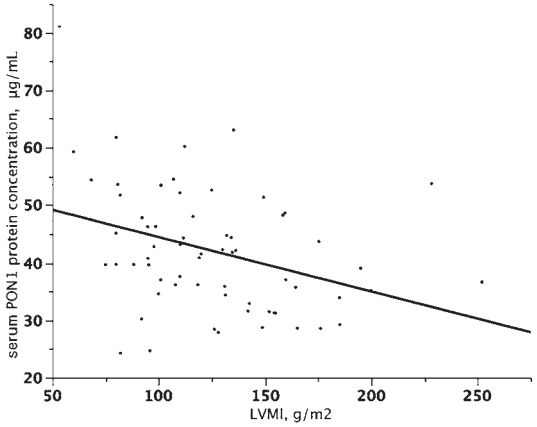

Fig. 2. Relationship between serum PON1 and LVMI in patients on HD (adjusted $r^2 = -0.35$, $p < 0.005$).

Table 4. Stepwise multivariate regression models of predictors of LVMI

	Estimate	Standard error	β	p value
Intercept	253.1	50.8		< 0.0001
PON1	−1.3	0.4	−0.34	< 0.005
History of CVD	8.2	4.1	0.21	< 0.05
Hemoglobin	−13.4	5.0	−0.28	< 0.01
Creatinine	4.4	1.4	0.32	< 0.005
C-reactive protein	22.2	10.7	0.22	< 0.05

Multivariate stepwise regression analysis employed a model that included the following parameters: Age, gender, history of CVD, diabetes, hemoglobin, albumin, creatinine, C-reactive protein, LDL, HDL and PON1. Adjusted $r^2 = 0.34$.

population[12]. Patients with LVH frequently develop coronary artery disease, heart failure, stroke and other cardiovascular complications[13]. Cardiac structural abnormalities such as LVH cannot be accounted for by hemodynamic factors alone. Despite growing awareness of the need to clinically identify individuals with LVH for cardiovascular risk stratification, the pathophysiological basis of left ventricular structural and functional abnormalities in patients with hypertension remains unclear. One important contributor to the pathogenesis of cardiovascular disease is oxidative stress. In patients starting dialysis, LVH is the most common cardiac anomaly (75% of patients)[14], and it is a powerful predictor of cardiovascular outcomes among patients on HD[15]. Although LVH is likely to be a multifactorial process in CKD with a number of causes including anemia, pressure loading through hypertension and reduced vascular compliance, its mechanisms and suitable markers remain uncertain. The present study shows that the serum concentration of PON1 is significantly

associated with LVMI in patients on HD. PON and arylesterase activity decreases in patients on HD independent of the L55M and Q192R PON1 gene polymorphisms[16, 17], and the human serum PON1 concentration predicts cardiovascular mortality in these patients[18]. Oxidative stress markers, especially oxidized LDL, are associated with echocardiographic parameters such as LVMI and EF[19], therefore the serum PON1 concentration might predict the development of LVH in such patients. This study is the first to identify correlations between PON1 and LVH in patients on HD.

Early epidemiological studies have recognized that a decrease in HDL, which is a common feature of type 2 diabetes mellitus and metabolic syndrome, is an independent predictor of cardiovascular disease. The beneficial effects of HDL on the cardiovascular system have been attributed to its anti-inflammatory, antioxidant and antithrombotic properties as well as its ability to reverse cholesterol transport. These properties of HDL act together to improve endothelial function and to prevent atherosclerosis. This study found that the serum concentration of PON1 is correlated with that of HDL, essentially because HDL is a carrier for PON1, which provides one explanation for the decreased PON1 concentration that is associated with changes in HDL concentrations. This association may provide one explanation for the accelerated CVD in patients on dialysis. A relationship between LVH and HDL has been established in both hypertensive patients and in the general population[20]. HDL is an independent predictor of LVH among Japanese patients with essential hypertension, and it is inversely related to the risk of cardiovascular events. There is a negative relationship between HDL levels and LVMI[21]. These studies suggest that HDL has direct cardioprotective effects and since paraoxonase is a central contributor to the antioxidant capacity of HDL, this provides an explanation for the association of serum PON1 concentration with LVMI in patients on HD.

Multivariate analysis revealed that the serum PON1 concentration is significantly correlated with LVMI after adjusting for the HDL concentration. Impaired HDL metabolism, such as a reduction in the HDL2 subfraction and a decreased ratio of HDL2 to HDL3 cholesterol, has been recognized in patients who are on dialysis and in uremic patients[22]. The HDL concentration might also be regulated independently by its constituent fractions other than PON1. Hence, HDL might be qualitatively and quantitatively changed in patients on dialysis. The role of HDL in CVD complications remains to be determined and this may be difficult because HDL is multifunctional, especially in patients on dialysis. We propose that PON1 is associated with the cardioprotective function of HDL. Although hypertension is a leading risk factor for LVH, the systolic pressure prior to dialysis represents no more than 16% of the variance in LV mass among patients with ESRD[23]. Thus, the high prognostic value of LVH in patients with CKD must depend on the fact that LV mass integrates not only the long-term effects of hypertension, but also the combined effect of other risk factors. Our findings suggest that PON1 has direct cardioprotective effects in patients on dialysis.

Several mechanisms have been proposed to explain the role of low PON1 activity in

causing LVH. One possible effect of PON1 on LVMI might be the direct inhibition of oxidative stress and inflammation. Free radicals, especially of the superoxide family, increase during the development of pressure-overload myocardial hypertrophy[24]. Reactive oxygen species (ROS) can induce myocardial contractile dysfunction and structural damage[25]. Moreover, inflammation is an independent risk factor for high blood pressure and therefore inflammatory cytokines could be associated with LVH[26]. Oxidative stress and inflammation promotes several key events in the development of cardiovascular disease. Complexes of PON1 with HDL are implicated in the anti-inflammatory and antioxidant activities of HDL[27] and genetic polymorphisms of PON1 are associated with systemic oxidative stress and cardiovascular risk[28]. Thus, the association between LVH and PON1 could be related to its antioxidant and anti-inflammatory effects, but in this study, we found that the serum PON1 level did not correlate with C-reactive protein ($r = -0.17$, $P = 0.06$).

Several limitations of this study need to be considered, including the fact that there was not a control group, and the sample size was relatively small. In addition, our findings cannot explain the mechanisms of the cardioprotective effect of PON1 due to the cross-sectional design of the study. A further limitation was that the patients' registrations were obtained from only two dialysis facilities. Treatment with ACEIs and ARBs can influence lipid profiles and oxidative stress, but these parameters were not affected in patients who were taking these drugs. We defined atherosclerosis based only on a history of CVD and echocardiographic parameters.

Conclusions

In summary, our findings indicate that the serum PON1 concentration could contribute to the development of LVH and could be an independent predictor of CVD in patients on maintenance HD. In addition, a decrease in PON1 might be one cause of atherosclerotic progression in patients on HD. Prospective long-term follow up studies should clarify the influence of PON1 and cardiovascular morbidity and mortality among patients on HD.

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Contributions :
Omori M ; Study design, data analysis, manuscript preparation.
Watanabe M ; Study design, data analysis, manuscript preparation.
Honda H ; Experimental design, data collection.
Hattori H ; Analysis of serum PON1 concentration, manuscript preparation.
Akizawa T ; Experimental design, data collection, manuscript preparation.
Conflict of Interest :
Hattori H is a member of staff at BML, Inc.
Akizawa T is a member of the advisory board of Kirin, Chugai and Astellas.
None of the other authors have any personal or financial conflict of interest.
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