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ORIGINAL ARTICLE

The balance of fetuin-A and osteoprotegerin is independently associated with diastolic dysfunction in hemodialysis patients

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Fetuin-A and osteoprotegerin (OPG) are arterial calcification regulators, which are related to cardiovascular survival in hemodialysis patients. We hypothesized that a balance of these calcification regulators might mediate the progression of left ventricular (LV) diastolic dysfunction in hemodialysis patients. We recruited 63 hemodialysis patients and measured their serum fetuin-A, OPG, arterial stiffness, aortic calcification and echocardiographic parameters, including the transmitral early diastolic velocity/tissue Doppler mitral annular early diastolic velocity ratio (*E*/*E*′), and analyzed the relationships between these variables. Fetuin-A levels were significantly and negatively correlated with the ankle–brachial pulse wave velocity (baPWV), aortic calcification score (AOCS), left atrial volume index (LAVI), LV mass index (LVMI) and *E*/*E*′. OPG levels and the ratio of OPG to fetuin-A levels were significantly and positively correlated with the baPWV, AOCS, LAVI and *E*/*E*′. A stepwise multiple regression analysis revealed that *E*/*E*′ was independently correlated with fetuin-A levels (β=−0.334, *P*=0.02), OPG levels (β=0.367, *P*=0.01) and the ratio of OPG to fetuin-A (β=0.295, *P*=0.04). Categorizing the patients according to their serum fetuin-A and OPG levels revealed that patients with low fetuin-A and high OPG levels had the highest LAVI, LVMI and *E*/*E*′ values after adjusting for potential confounders. Serum fetuin-A levels negatively reflected, whereas OPG levels and the ratio of OPG to fetuin-A positively reflected an increase in vascular and ventricular stiffness, leading to the aggravation of diastolic dysfunction. Therefore, based on our results, the balance of the tissue calcification regulators fetuin-A and OPG could mediate the progression of LV diastolic dysfunction in hemodialysis patients.
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INTRODUCTION

Approximately 40% of hemodialysis patients have clinical evidence of ischemic heart disease or heart failure.[1,2] Vascular calcification, which results in increased vascular stiffness, is a common pathological feature of hemodialysis patients[1,3] and is accompanied by left ventricular hypertrophy (LVH) and diastolic dysfunction.[4,5] Vascular calcification is an active process controlled by a balance of serum calcification inhibitors and enhancers. Fetuin-A (α2-Heremans Schmid glycoprotein) is a 62-kDa liver-derived glycoprotein and potent circulatory inhibitor of vascular calcification, which is downregulated by inflammation.[6,7] Chemically, fetuin-A acts as an inhibitor of spontaneous calcium phosphate precipitation by forming soluble colloidal calciprotein particles.[7] Fetuin-A-deficient mice experience enhanced myocardial fibrosis and stiffness,[8] and several studies have documented an inverse relationship between serum fetuin-A levels and survival in dialysis patients.[9,10]

Osteoprotegerin (OPG), a soluble decoy receptor of the osteoclast receptor activator of the nuclear factor κB ligand (RANKL), acts as an important regulator of vascular calcification.[11] OPG-deficient mice develop severe osteoporosis and vascular calcification.[12] In contrast, elevated serum levels of OPG are associated with the progression of vascular stiffness and cardiovascular events in hemodialysis patients.[13,14]

We therefore hypothesized that an imbalance between fetuin-A and OPG may be associated with increased cardiovascular stiffness and LV diastolic dysfunction in hemodialysis patients.

METHODS

The eligibility criteria for our study were as follows: inadequacy of dialysis with a urea Kt/V level of <1.2, active malignancy, active respiratory infection, acute heart failure, atrial fibrillation at the time of the study, significant valvular heart disease, peripheral arterial disease (ankle–brachial index <0.9) and chronic use

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of glucocorticoids and immunosuppressive agents. We performed echocardiographies in 108 consecutive hemodialysis patients who underwent 4 h of dialysis three times per week from February 2009 until November 2010 in the dialysis departments of Kitasaito Hospital and Hokkaido Prefectural Kitami Hospital. Among these 108 consecutive patients, 14 with significant valvular heart disease, 12 with peripheral arterial disease, 9 with atrial fibrillation, 6 with inadequacy of dialysis and 4 with acute heart failure were excluded from the study. Finally, 63 hemodialysis patients were recruited for the study. Before entry into the study, written informed consent was obtained from all patients in accordance with the ethical standards laid down in the Declaration of Helsinki.

Before the hemodialysis session, the weight, height and blood pressure (BP) of each patient were measured. Hypertension was defined as a predialysis brachial BP of $>140/90$ mm Hg and/or a current anti-hypertensive treatment. The ankle–brachial pulse wave velocity (baPWV) was measured using a waveform analyzer (VP-1000; Colin Co., Komaki, Japan) after at least 5 min of rest, as previously reported.[15] The body surface area was calculated according to the following formula: body surface area=0.6×height (m)+0.0128×weight (kg)–0.1529. Diabetes mellitus was defined based on the World Health Organization criteria.[16] Fasting predialysis blood samples were taken, and the following laboratory data were measured using standard laboratory methods. To measure serum fetuin-A, OPG and procollagen type III N-terminal amino peptide (PIIIP) levels, the samples were centrifuged immediately, and the serum was stored at $-80\,^{\circ}C$ until analysis. Serum fetuin-A and OPG levels were measured using a human fetuin-A enzyme-linked immunosorbent assay kit (Epitope Diagnostics Inc., San Diego, CA, USA) and enzyme-linked immunosorbent assay system (Biomedica, Vienna, Austria). Serum PIIIP levels were measured by immunoradiometry using an IRMA kit (Riagnost P-III-P; CIS Bio International, Ceze, France). All samples were measured in duplicate.

To estimate the aortic calcification score (AOCS), we performed abdominal computed tomography scans with a 3-mm collimation, 5-mm slice thickness and a 35-cm field of view. A standard phantom was used to adjust the scan score brightness, and the calcium levels in a 10-cm segment of the distal abdominal aorta above the aortic bifurcation were used for the analysis. Calcification was defined as a value $>1\,mm^2$ with a density of >130 Hounsfield units and was quantified using an Agatston scoring method with an offline software analysis program (Aqualion, Toshiba, Japan), as previously reported.[17]

Before the hemodialysis session, standard echocardiographic measurements were obtained from the left parasternal and apical views according to the recommendations of the American Society of Echocardiography,[18] using an ALOKA alpha 10 ultrasound system (Aloka, Tokyo, Japan). The LV end-diastolic dimension, LV end-systolic dimension, interventricular septum thickness and posterior wall thickness were determined using standard echocardiographic 2D measurements. The left atrial volume (LAV) was measured using the biplane method of discs incorporating both apical 4- and 2-chamber views. The LV mass (LVM) was determined using the 2D area-length formula. LAV and LVM were indexed to the body surface area as the LAV index (LAVI) and LVM index (LVMI), respectively. The ejection fraction was calculated by the modified Simpson method. The transmitral inflow was recorded using pulsed wave Doppler recordings at the mitral valve leaflet tips in the apical 3-chamber view. The peak early diastolic filling velocity (E), peak late diastolic filling velocity (A), E/A ratio and deceleration time of the E wave were measured. The tissue Doppler image program was set to the pulsed wave Doppler mode, and sample volumes were positioned at the septal corner of the mitral annulus.[19] Then, we measured the early diastolic mitral inflow velocity and calculated the ratio of E over E' (E/E') to represent LV filling pressure.[20]

All values are presented as the mean ± s.e.m. A univariate linear regression was used for the continuous variables, including the ratio of OPG to fetuin-A. A stepwise multiple regression analysis was performed to determine the correlation and independent variables for E/E'. We selected the classical cardiovascular risk factors and factors associated with LV diastolic function as the variables. In Model 1, serum fetuin-A levels, age, sex, duration of dialysis, mean BP, heart rate, body mass index, hemoglobin, brain natriuretic peptide level, smoking, medication for hypertension, medication for diabetes, medication for dyslipidemia and prior coronary artery disease (CAD) were included as independent variables with or without the baPWV. In models 2 and 3, serum OPG levels and the ratio of OPG to fetuin-A, respectively, were included instead of the serum fetuin-A level. Furthermore, we divided the patients into four groups according to the median serum fetuin-A ($225\,ng\,ml^{-1}$) and OPG ($235\,ng\,ml^{-1}$) levels as follows: high fetuin-A ($\geqslant 225\,ng\,ml^{-1}$) plus low OPG ($\leqslant 234\,ng\,ml^{-1}$); high fetuin-A ($\geqslant 225\,ng\,ml^{-1}$) plus high OPG ($\geqslant 235\,ng\,ml^{-1}$); low fetuin-A ($\leqslant 224\,ng\,ml^{-1}$) plus low OPG ($\leqslant 234\,ng\,ml^{-1}$); and low fetuin-A ($\leqslant 224\,ng\,ml^{-1}$) plus high OPG ($\geqslant 235\,ng\,ml^{-1}$). Their associations with the baPWV, AOCS, LAVI, LVMI and E/E' were evaluated with an analysis of covariance for age, sex, duration of dialysis, mean BP, heart rate, body mass index, hemoglobin, brain natriuretic peptide levels, smoking, medication for hypertension, medication for diabetes, medication for dyslipidemia and prior CAD. P-values <0.05 were considered to be statistically significant. All statistical analyses were performed with the SPSS (Statistical Package for the Social Sciences) software package Version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The clinical parameters of the hemodialysis patients in the present study are summarized in Table 1. The etiology of renal failure was associated with diabetes mellitus in 47.6% (30 of 63), hypertensive nephrosclerosis in 25.4% (16 of 63), chronic glomerular disease in 17.4% (11 of 63), polycystic kidney disease in 4.7% (3 of 63), post-nephrectomy due to renal cell carcinoma in 3.2% (2 of 63) and unknown in 1.6% (1 of 63) of the patients. A total of 19 patients (30.2%) had CAD diagnosed by coronary angiography; 3 patients had one vessel disease without intervention, 5 had one vessel disease with a percutaneous coronary intervention, 7 had two vessel diseases with a percutaneous coronary intervention and four had three vessel diseases with coronary artery bypass graft surgery. The E/E' was significantly and positively correlated with the baPWV ($r=0.348$, $P<0.01$), but not with prior CAD.

As shown in Table 2, the serum fetuin-A levels were inversely related to systolic BP, pulse pressure, serum phosphorus, Ca×P product, LAVI (Figure 1), LVMI (Figure 1), PIIIP levels (Figure 2), E/E' (Figure 2), baPWV and AOCS, and were significantly and positively related to HDL-C, albumin and 25-hydroxyvitamin D levels. A stepwise multiple regression analysis of model 1 revealed that E/E' showed an independent correlation only with fetuin-A levels (β coefficient=−0.334, $P=0.02$), suggesting that serum fetuin-A levels were negatively correlated with E/E', independent of the traditional risk factors (Table 3).

As also shown in Table 2, serum OPG levels were significantly and positively related to the duration of dialysis, plasma human atrial natriuretic peptide, LAVI (Figure 1), PIIIP (Figure 2), E/E' (Figure 2), baPWV and AOCS. There was a positive, but non-significant, correlation with the LVMI (Figure 1). Furthermore, a stepwise multiple regression analysis of model 2 revealed that E/E' correlated independently with OPG levels (β coefficient=0.367, $P=0.01$), suggesting that OPG levels were positively correlated with E/E', independent of the traditional risk factors (Table 3).

We categorized the patients into four groups according to their serum fetuin-A and OPG levels (Group 1: those with high fetuin-A and low OPG, Group 2: those with high fetuin-A and high OPG, Group 3: those with low fetuin-A and low OPG, Group 4: those with low fetuin-A and high OPG) and examined the correlation between each group and the baPWV, AOCS, LAVI, LVMI and E/E' after adjusting for potential confounders. We found that the group with low fetuin-A and high OPG levels had the highest baPWV, AOCS (Figure 3), LAVI, LVMI and E/E' (all $P<0.05$ for the trend) (Figure 4). Furthermore, the ratio of OPG to fetuin-A was also significantly and positively related to the duration of dialysis, plasma human atrial natriuretic peptide, PIIIP levels, baPWV (Figure 5a), AOCS (Figure 5b), LAVI (Figure 5c), LVMI and E/E' (Figure 5d) (all

Table 1 Characteristics of hemodialysis patients

N, M/F (%)	44/19 (70/30)
Age (years)	64.8 ± 1.6
Duration of dialysis (months)	94.5 ± 13.9
Smokers, *N* (%)	38 (60.3%)
Systolic BP (mm Hg)	143.3 ± 3.1
Diastolic BP (mm Hg)	75.1 ± 2.0
Mean BP (mm Hg)	96.0 ± 2.5
Pulse pressure (mm Hg)	68.1 ± 2.6
Heart rate (min^{-1})	73.4 ± 1.4
Body mass index ($kg\,m^{-2}$)	21.8 ± 0.5
Hypertension, *N* (%)	39 (61.9%)
Diabetes mellitus, *N* (%)	30 (47.6%)
Coronary artery disease, *N* (%)	19 (30.2%)
Hemoglobin ($g\,dl^{-1}$)	10.4 ± 0.2
Albumin ($g\,dl^{-1}$)	3.7 ± 0.1
Creatinine ($mg\,dl^{-1}$)	8.9 ± 0.1
Total cholesterol ($mg\,dl^{-1}$)	151.5 ± 4.8
HDL-C ($mg\,dl^{-1}$)	44.2 ± 1.9
LDL-C ($mg\,dl^{-1}$)	78.8 ± 3.7
Calcium ($mg\,dl^{-1}$)	8.9 ± 0.1
Phosphorus ($mg\,dl^{-1}$)	5.0 ± 0.2
Ca×P	44.1 ± 1.5
Intact PTH ($pg\,ml^{-1}$)	144.8 ± 18.3
25-Hydroxyvitamin D ($pg\,ml^{-1}$)	11.4 ± 0.8
Hemoglobin A1c (%)	5.6 ± 0.2
C-reactive protein ($mg\,dl^{-1}$)	0.6 ± 0.2
hANP ($pg\,ml^{-1}$)	103.9 ± 12.0
BNP ($pg\,ml^{-1}$)	308.0 ± 73.1
PIIIP ($ng\,ml^{-1}$)	2.4 ± 0.2
Fetuin A ($ng\,ml^{-1}$)	226.8 ± 6.9
Osteoprotegerin ($ng\,ml^{-1}$)	260.5 ± 17.8
baPWV ($cm\,s^{-1}$)	2232.0 ± 95.6
Ankle–brachial index	1.03 ± 0.2
AOCS (Agatston score)	10898 ± 1248
Echocardiographic parameters	
LVEF (%)	61.1 ± 1.3
Fractional shortening (%)	40.1 ± 6.2
IVSd (mm)	11.4 ± 0.2
PWd (mm)	11.2 ± 0.2
LVDd (mm)	48.5 ± 0.9
LVDs (mm)	32.9 ± 1.3
DcT (ms)	228.1 ± 8.3
E/*A*	0.80 ± 0.04
E/*E*′	12.6 ± 0.6
LAVI ($ml\,m^{-2}$)	41.4 ± 2.6
LVMI ($g\,m^{-3}$)	126.0 ± 5.7
Oral medication, N *(%)*	
Calcium-channel blockers	31 (49.2)
ACEI/ARB	30 (47.6)
Beta blockers	27 (42.9)
Statins	12 (19.1)
Calcium carbonate	32 (50.8)
Lanthanum carbonate	27 (42.9)
1α-Hydroxyvitamin D3	34 (53.9)
Sevelamer	10 (15.9)
Cinacalcet	4 (6.3)

Abbreviations: *A*, late mitral inflow; ACE, angiotensin-converting enzyme; AOCS, aorta calcification score; ARB, angiotensin II receptor blocker; baPWV, brachial–ankle pulse wave velocity; BNP, brain natriuretic peptide; BP, blood pressure; DcT, deceleration time; *E*, early mitral inflow; *E*′, early diastolic mitral annulus motion; hANP, human atrial natriuretic peptide; HDL-C, high-density lipoprotein cholesterol; IVSd, interventricular septum thickness in diastole; LAVI, left atrial volume index; LDL-C; low-density lipoprotein cholesterol; LVDd, left ventricular diameter in diastole; LVDs, left ventricular diameter in systole; LVEF, left ventricular ejection fraction; LVMI, left ventricular volume index; PIIIP, procollagen type III N-terminal amino peptide; PTH, parathyroid hormone; PWd, posterior wall thickness in diastole.
Variables are presented as mean ± s.e.m., or percentage.

Table 2 Correlation of the serum fetuin-A, osteoprotegerin, ratio of the osteoprotegerin to fetuin-A and the clinical parameters

	Fetuin-A		*OPG*		*OPG/fetuin A*	
Variable	r	P	r	P	r	P
Age	−0.171	0.185	0.195	0.13	0.199	0.122
Sex (M=1, F=0)	−0.097	0.455	0.138	0.115	0.143	0.269
Duration of dialysis	−0.069	0.549	0.543	<0.001	0.466	<0.001
Systolic BP	−0.425	0.001	0.078	0.58	0.145	0.299
Mean BP	−0.082	0.565	0.083	0.558	0.057	0.686
Pulse pressure	−0.535	0.001	0.187	0.179	0.291	0.035
Body mass index	0.116	0.373	−0.251	0.051	−0.245	0.057
Hemoglobin	0.169	0.169	−0.05	0.705	−0.097	0.463
Albumin	0.525	0.001	−0.209	0.106	−0.353	0.005
Creatinine	−0.018	0.888	−0.041	0.752	−0.023	0.858
Total cholesterol	0.175	0.176	−0.211	0.103	−0.190	0.143
HDL-C	0.271	0.033	0.161	0.212	−0.201	0.118
LDL-C	0.081	0.532	−0.232	0.069	−0.179	0.164
Calcium	−0.074	0.572	0.121	0.353	0.085	0.514
Phosphorus	−0.325	0.01	0.193	0.133	0.243	0.057
Ca×P	−0.296	0.019	0.174	0.176	0.226	0.078
Intact PTH	0.202	0.125	−0.183	0.165	−0.183	0.165
25-Hydroxyvitamin D	0.441	0.001	0.007	0.96	−0.138	0.296
C-reactive protein	−0.212	0.101	0.002	0.988	0.193	0.135
HANP	−0.211	0.103	0.298	0.02	0.252	0.049
BNP	−0.119	0.361	0.21	0.104	0.195	0.132
PIIIP	−0.317	0.013	0.545	<0.001	0.589	<0.001
LVEF	0.237	0.237	−0.055	0.68	−0.175	0.188
E/*E*′	−0.343	0.007	0.345	0.007	0.334	0.009
LAVI	−0.357	0.016	0.498	0.004	0.457	0.002
LVMI	−0.313	0.034	0.118	0.435	0.166	0.271
baPWV	−0.277	0.029	0.622	<0.001	0.594	<0.001
AOCS (Agatston score)	−0.362	0.004	0.257	0.046	0.326	0.010

Abbreviations: AOCS, aorta calcification score; baPWV, brachial-ankle pulse wave velocity; BNP, brain natriuretic peptide; BP, blood pressure; *E*, early mitral inflow; *E*′, early diastolic mitral annulus motion; hANP, human atrial natriuretic peptide; HDL-C, high-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; LAVI, left atrial volume index; LDL-C; low-density lipoprotein cholesterol; LVMI, left ventricular volume index; OPG, osteoprotegerin; PIIIP, procollagen type III N-terminal amino peptide; PTH, parathyroid hormone.

$P<0.05$). A stepwise multiple regression analysis of model 3 revealed that *E*/*E*′ correlated independently with the ratio of OPG to fetuin-A ($\beta=0.295$, $P=0.04$), thus indicating that the balance of fetuin-A and OPG levels could be useful for identifying increased vascular stiffness and LV diastolic dysfunction in hemodialysis patients.

DISCUSSION

To the best of our knowledge, this study is the first to describe the circulating serum vascular calcification regulators fetuin-A and OPG, which are closely related to vascular stiffness, and thereby help to elucidate cardiac function in hemodialysis patients. The major findings of our study are that fetuin-A and OPG serum levels are significant modulators for identifying vascular stiffness, ventricular stiffness and diastolic dysfunction in hemodialysis patients.

Vascular stiffness is associated with LV diastolic dysfunction.[4] Previous reports have demonstrated that marked vascular calcification and stiffness predisposed hemodialysis patients to LVH and cardiac diastolic dysfunction.[21] We investigated the impact of fetuin-A and OPG serum levels, which are representative of potent vascular calcification regulators, on vascular stiffness and ventricular function. In hemodialysis patients, the levels of serum fetuin-A have been shown to be consistently low, probably due to its enhanced consumption[7]

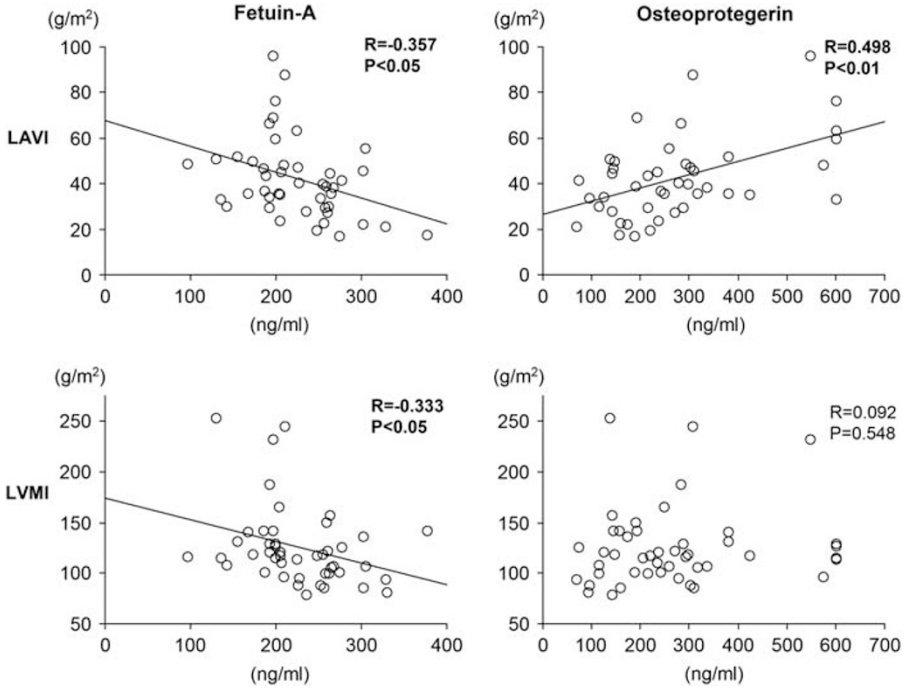

Figure 1 The relationship between fetuin-A levels, osteoprotegerin levels, LAVI and LVMI in all 63 subjects. The LAVI and LVMI correlated significantly and negatively with fetuin-A levels. The correlation between the LVMI and OPG levels was positive, but not significant. LAVI, left atrial volume index; LVMI, left ventricular mass index.

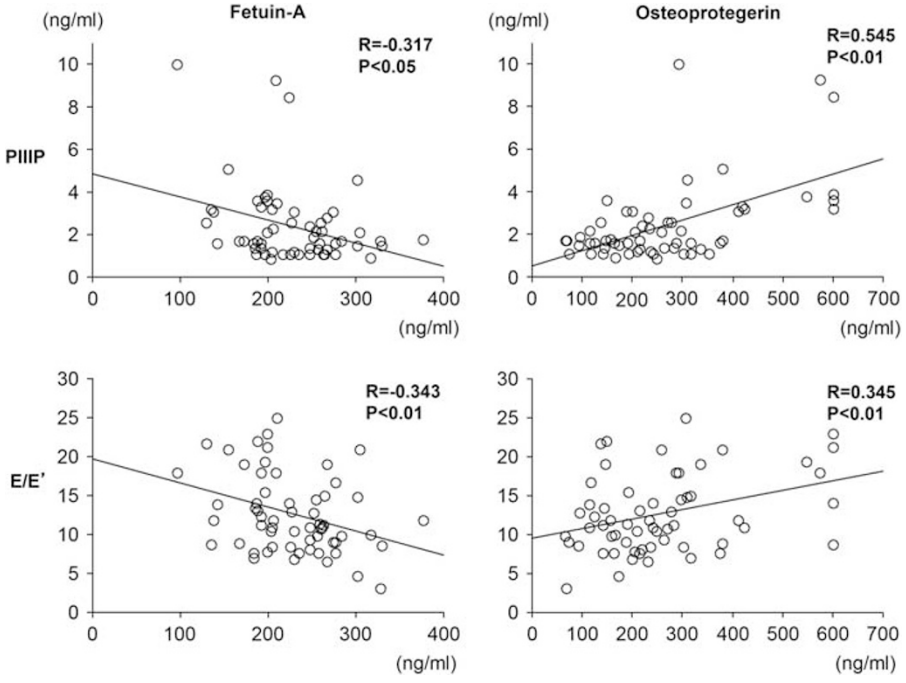

Figure 2 The relationship between fetuin-A levels, osteoprotegerin levels, PIIIP levels and *E*/*E*′ in all 63 subjects. PIIIP levels and *E*/*E*′ correlated significantly and negatively with fetuin-A levels and positively with osteoprotegerin levels. PIIIP, procollagen type III N-terminal amino peptide.

caused by the calcium phosphate load in addition to a chronic microinflammatory status. In our study, the serum fetuin-A levels were inversely related to the AOCS and PWV. Furthermore, we showed for the first time that serum fetuin-A levels were significantly inversely correlated with *E*/*E*′, LAVI and LVMI, thus suggesting that low fetuin-A levels indicate not only an increased vascular stiffness but also an enhanced ventricular stiffness and diastolic dysfunction.

Table 3 Multiple regression analysis for *E*/*E*′ and related parameters

	With baPWV			*Without baPWV*		
	β	t	P	β	t	P
Model 1						
Fetuin-A	−0.325	−2.308	0.026	−0.298	−2.230	0.031
Age	0.168	1.181	0.244	0.096	1.181	0.244
Sex (M=1)	−0.268	−1.953	0.057	−0.261	−1.953	0.057
Duration of dialysis	0.067	0.468	0.642	0.089	0.468	0.642
Mean BP	−0.151	−1.062	0.294	−0.098	−1.062	0.294
Heart rate	−0.990	−0.638	0.527	−0.015	−0.638	0.527
BMI	−0.121	−0.852	0.399	−0.055	−0.852	0.399
Hemoglobin	−0.020	−0.135	0.894	−0.005	−0.135	0.894
Smoking	0.037	0.260	0.796	−0.004	0.260	0.796
Medication for hypertension	0.171	1.211	0.232	0.148	1.211	0.232
Medication for diabetes	0.140	0.969	0.338	0.098	0.969	0.338
Medication for dyslipidemia	0.192	1.160	0.080	0.251	1.160	0.080
Prior coronary artery disease	0.062	0.468	0.642	0.038	0.468	0.642
BNP	0.252	1.892	0.059	0.339	2.537	0.015
baPWV	0.344	2.551	0.015	—	—	—
Model 2						
Osteoprotegerin	0.367	2.563	0.010	0.404	2.995	0.004
Age	0.148	1.043	0.303	0.080	1.043	0.573
Sex (M=1)	−0.258	−2.088	0.063	−0.263	−2.005	0.068
Duration of dialysis	−0.204	−1.151	0.256	−0.178	−1.151	0.303
Mean BP	−0.153	−1.092	0.281	−0.071	−1.092	0.620
Heart rate	−0.151	−1.068	0.291	−0.124	−1.068	0.369
BMI	−0.053	−0.373	0.711	−0.019	−0.373	0.891
Hemoglobin	−0.050	−0.346	0.731	−0.005	−0.346	0.973
Smoking	0.052	0.368	0.715	0.122	0.368	0.380
Medication for hypertension	0.207	1.508	0.139	0.184	1.508	0.171
Medication for diabetes	0.244	1.789	0.080	0.186	1.789	0.178
Medication for dyslipidemia	0.204	1.484	0.145	0.138	1.484	0.321
Prior coronary artery disease	0.153	1.098	0.278	0.104	1.098	0.450
BNP	0.282	2.000	0.052	0.263	2.000	0.059
baPWV	0.273	1.432	0.159	—	—	—
Model 3						
OPG/fetuin-A	0.295	2.071	0.044	0.275	1.934	0.048
Age	0.162	1.114	0.303	0.105	0.737	0.465
Sex (M=1)	−0.243	−1.810	0.068	−0.258	−1.888	0.058
Duration of dialysis	−0.094	−0.547	0.256	−0.012	−0.073	0.942
Mean BP	−0.138	−0.960	0.281	−0.086	−0.608	0.546
Heart rate	−0.143	−0.966	0.291	−0.086	−0.592	0.557
BMI	−0.068	−0.470	0.711	−0.016	−0.112	0.911
Hemoglobin	−0.053	−0.353	0.731	−0.050	−0.352	0.727
Smoking	0.067	0.462	0.715	0.020	0.141	0.888
Medication for hypertension	0.199	1.413	0.139	0.178	1.307	0.198
Medication for diabetes	0.205	1.459	0.120	0.165	1.200	0.237
Medication for dyslipidemia	0.243	1.742	0.145	0.207	1.520	0.136
Prior coronary artery disease	0.127	0.891	0.278	0.101	0.728	0.470
BNP	0.297	2.189	0.034	0.311	2.213	0.032
baPWV	0.284	1.905	0.063	—	—	—

Abbreviations: β, β coefficient; baPWV, brachial–ankle pulse wave velocity; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; OPG, osteoprotegerin.
Model 1, *F* ratio=6.243; r^2=0.221 (*P*=0.004) with baPWV, *F* ratio=6.203; r^2=0.220 (*P*=0.004) without baPWV.
Model 2, *F* ratio=6.571; r^2=0.219 (*P*=0.004) with baPWV, *F* ratio=6.176; r^2=0.210 (*P*=0.008) without baPWV.
Model 3, *F* ratio=4.780; r^2=0.178 (*P*=0.013) with baPWV, *F* ratio=4.782; r^2=0.188 (*P*=0.010) without baPWV.

Because fetuin-A binds to calcium and phosphorus to form a soluble complex (calciprotein particles)[7] and prevents $Ca \times PO_4$ precipitation, low serum fetuin-A levels would be expected to predispose the patient to vascular calcification and would subsequently lead to increased vascular stiffness, as evidenced by an increase in the PWV. The mechanisms of diastolic dysfunction caused by vascular calcifica-

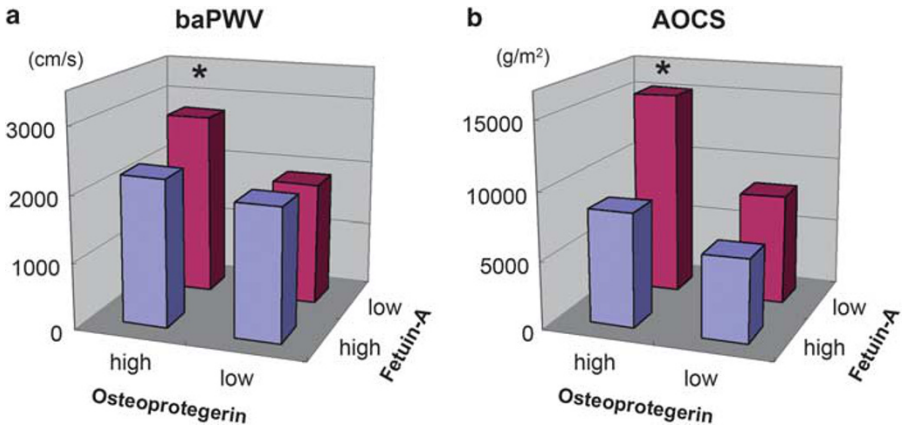

Figure 3 The association between baPWV and AOCS in patients based on the balance between fetuin-A and OPG. After adjusting for age, sex, duration of dialysis, mean BP, heart rate, body mass index, hemoglobin, smoking, medication for hypertension, medication for diabetes, medication for dyslipidemia and prior CAD, significant differences in the baPWV (**a**) and AOCS (**b**) were observed across the four groups ($P<0.05$). The combination of low fetuin-A and high OPG levels was significantly associated with a high baPWV and AOCS. *$P<0.05$ *vs.* the high fetuin-A and low OPG group. AOCS, aortic calcification score; baPWV, ankle–brachial pulse wave velocity.

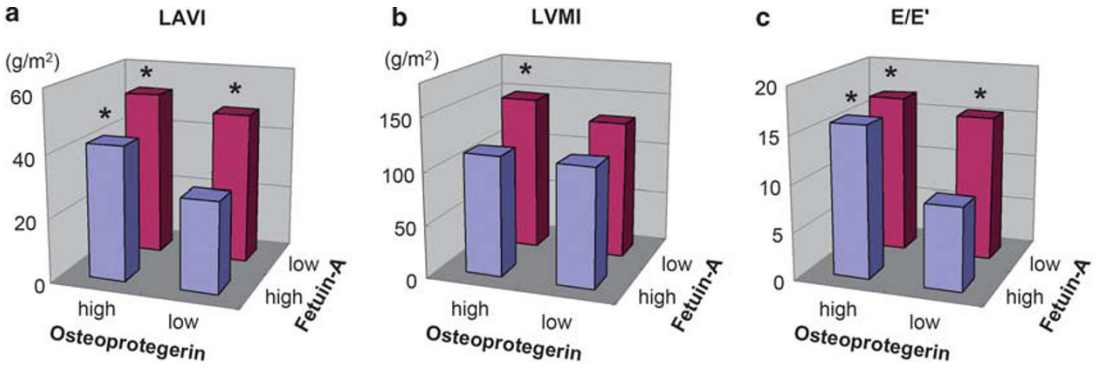

Figure 4 The association of the LAVI, LVMI and *E*/*E*′ in patients based on a combination of fetuin-A and OPG. After adjusting for age, sex, duration of dialysis, mean BP, heart rate, body mass index, hemoglobin, smoking, medication for hypertension, medication for diabetes, medication for dyslipidemia and prior CAD, significant differences in the LAVI (**a**), LVMI (**b**), and *E*/*E*′ (**c**) were observed across the four groups ($P<0.05$). The combination of low fetuin-A and high OPG levels was significantly associated with a high LAVI, LVMI and *E*/*E*′. *$P<0.05$ *vs.* the high fetuin-A and low OPG group.

tion may be ascribed to the earlier arrival of the reflected wave during end systole in stiff and heavily calcified arteries. The reflected wave during end systole would thus merge with the incident wave, which is generated by the ventricular systolic ejection, leading to higher systolic pressures and lower diastolic pressures.[22] The resultant increase in the afterload during LV systole and reduction in the coronary perfusion pressure during LV diastole may lead to LV hypertrophy and slowing of the LV relaxation.[23,24] However, hyperphosphatemia in uremic animal models has also been found to aggravate myocardial fibrosis and cause LVH.[8] As a chelating agent of calcium phosphate, fetuin-A might protect the myocardium against the harmful effects of hyperphosphatemia. Furthermore, fetuin-A also acts as a soluble receptor-like antagonist of TGF-β,[7] resulting in persistent low serum fetuin-A levels, which might directly contribute to increased LV stiffness and hypertrophy. Our novel finding that a significant inverse correlation exists between levels of fetuin-A and PIIIP, which is a marker of myocardial fibrosis, also supports this mechanism.

We found a significant positive relationship between OPG levels and AOCS, as well as the PWV, as previously reported. OPG could act as a survival factor for serum-deprived smooth muscle cells.[11] Hence, high OPG levels in patients with a high AOCS may reflect an incomplete defense mechanism against factors that promote heavy arterial calcification,[25] which ultimately results in increased ventricular stiffness, as explained above.

Another new finding in this study was the significant positive correlation between OPG levels, plasma PIIIP levels and LV *E*/*E*′, which reflects an increased LV stiffness and aggravated diastolic dysfunction, as was observed in the hemodialysis patients. Ueland *et al.*[26] showed increased expression of OPG, RANK and RANKL in the failed human myocardium, and they hypothesized that high OPG expression levels could be protective against the harmful effects of RANKL on the myocardium in patients with heart failure, thus suggesting that OPG has a cardiac-protective role. However, another possibility should also be considered. Namely, high serum OPG levels have been associated with LVH in hypertensive African-Americans.[27] OPG also induces an anti-apoptotic effect by binding to tumor necrosis factor-related apoptosis-inducing ligands, which reduces the number of apoptotic bodies.[11] Although we could not demonstrate a significant positive relationship between

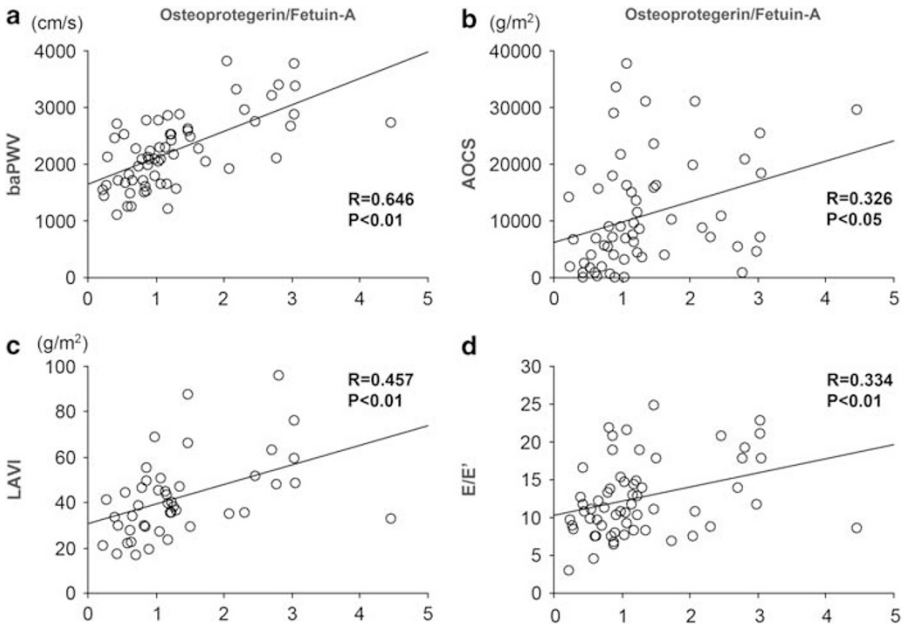

Figure 5 The relationship between the ratio of OPG to fetuin-A, baPWV (**a**), AOCS (**b**), LAVI (**c**) and *E*/*E*′ (**d**) in all 63 subjects. The ratio of OPG to fetuin-A correlated significantly and positively with the baPWV, AOCS, LAVI and *E*/*E*′. A full color version of this figure is available at the *Hypertension Research* journal online.

OPG levels and LVMI in the present study, a significant positive relationship between OPG and PIIIP levels, and between OPG levels and the LV diastolic dysfunction parameter may indicate that OPG has a potential role in promoting diastolic dysfunction in hemodialysis patients.

When we categorized the hemodialysis patients according to the median level of serum fetuin-A levels, which served as a negative marker of diastolic dysfunction, and serum OPG levels, which served as a positive marker of diastolic dysfunction, we found that low serum fetuin-A and high serum OPG levels were present in patients with the worst spectrum of LA enlargement, LVH and diastolic dysfunction. The combination of low fetuin-A and high OPG is a significant biomarker of LV diastolic dysfunction, thereby potentially indicating a high risk of complications with atrial fibrillation in hemodialysis patients. Hence, measuring the serum levels of both markers and the ratio of OPG to fetuin-A could be useful for identifying patients with imminent diastolic heart failure. Furthermore, the ratio of OPG to fetuin-A remained significant after adjusting for vascular stiffness in Table 3, suggesting that fetuin-A and OPG might be associated with the progression of LV diastolic dysfunction directly and indirectly through an increase in the afterload owing to elevating vascular stiffness.

This study has several limitations. First, this study sampled a relatively small size of the population and used a cross-sectional design. Second, we might not have completely excluded asymptomatic CAD because we diagnosed CAD by coronary angiography. Third, peripheral arterial disease is commonly observed in hemodialysis patients, but we excluded those patients defined by an ankle–brachial index of <0.9 because of the lack of baPWV measurements. Thus, the subgroup of patients included in this study might not be representative of hemodialysis patients as a whole because of the restrictive eligibility criteria. Larger prospective studies will therefore be needed to confirm our results.

In conclusion, a balance of the tissue calcification regulators fetuin-A and OPG might mediate the progression of LV diastolic dysfunction by increasing the afterload by elevating vascular stiffness in hemodialysis patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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