Original Article

Serum Fibroblast Growth Factor-23 Levels and Progression of Aortic Arch Calcification in Non-Diabetic Patients on Chronic Hemodialysis

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***Aim*: Vascular calcification is a cause of cardiovascular death in hemodialysis (HD) patients. The aim of the present study was to evaluate the relationship between the progression of aortic arch calcification (AoAC) and serum fibroblast growth factor (FGF)-23.**
***Methods*: The enrolled study subjects were 127 (83 men and 44 women) HD patients. Calcification of the aortic arch was semiquantitatively estimated with a score (AoACS) on plain chest radiology. Change in AoACS (△AoACS) was obtained by subtracting the baseline AoACS value from the follow-up AoACS value. The second assessment was performed from 5 years after the first determination.**
***Results*: The percentage of male gender in non-progressors (58.5%) was lesser than in regressors (60.0%) and progressors (74.6%). In addition, the dialysis duration in regressors (14.1 ± 5.1 years) was shorter than in non-progressors (19.5 ± 7.0 years) and progressors (16.8 ± 7.5 years). Interestingly, the serum FGF-23 level in regressors (39225.5 ± 9247.9 pg/mL) was significantly higher than in non-progressors (12896.5 ± 26323.5 pg/mL) and progressors (14062.4 ± 18456.8 pg/mL). Multiple regression analyses showed male gender (β value = 0.969, F = 5.092, p = 0.0192), serum levels of albumin (β value = −1.395, F = 4.541, p = 0.0296) and log FGF-23 (β value = −0.001, F = 7.273, p = 0.0115) to be significant independent determinants of △AoACS.**
***Conclusion*: Changes in AoAC evaluated by using a simple chest radiograph are associated with serum FGF-23 levels. Excess accumulation of FGF-23 in serum may enable to inhibit the calcification process in vessel walls in chronic HD patients.**

J Atheroscler Thromb, 2011; 18:217-223.

Key words; Aortic arch calcification, Chest radiography, Fibroblast growth factor-23, Hemodialysis, Cardiovascular disease

Introduction

Cardiovascular disease is the major cause of death in patients with chronic kidney disease (CKD)[1]. In patients with end-stage renal disease (ESRD), atherosclerosis is more advanced[2, 3]. Recently, vascular calcification in the coronary arteries and aorta has been recognized as an important risk factor for cardiovascular disease in hemodialysis (HD) patients[4]. Vascular calcification is very common in ESRD, especially in HD patients[5]. The mechanisms of vascular calcification are hyperphosphatemia and elevated calcium (Ca) x phosphate (P) products[6-8]. Vascular calcification induces stiffening of the vessel wall and reduces vascular compliance, which has been found to be predictive of cardiovascular mortality[9-11].

Fibroblast growth factor-23 (FGF-23), a phosphorus-regulating factor, decreases serum P concentrations by directly reducing renal P reabsorption and by suppressing 1-25(OH)$_2$D formation through the inhibition of 1α-hydroxylase[12]. Furthermore, FGF-23 is one of the major phosphatonins and is elevated in chronic HD patients[11]. FGF-23 has an important role in the regulation of Ca and P metabolism and the function of parathyroid hormone (PTH)[13]. In most HD patients, the serum concentration of FGF-23 is

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Received: April 9, 2010
Accepted for publication: October 8, 2010

more than 100-fold higher than in patients not undergoing HD therapy[14, 15].

The aim of the present study was to examine whether serum FGF-23 levels affect the progression of aortic arch calcification (AoAC) assessed by chest radiography in HD patients.

Methods

Of the HD patients treated at the Dialysis Unit of Hidaka Hospital, 127 patients (83 men and 44 women) without diabetes gave their written informed consent to enroll in this study. The underlying diseases were chronic glomerulonephritis in 104 patients (81.3%), nephrosclerosis in 10, polycystic kidney disease in 8, chronic pyelonephritis in 3 and lupus nephritis in 2. HD was performed three times weekly (4 hours/day). Blood was drawn before starting each dialysis session after an overnight fast to measure the various markers, including Ca, P, albumin, C-reactive protein (CRP), total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride. The mean values of three measurements taken during the three months before chest radiology were used in the analysis. Blood pressure (BP) was recorded three times after the subject had rested in the supine position for at least 10 min, and the average value of the three measurements was adopted. Serum intact-PTH was measured once at the time of chest radiology using an Allegro Intact PTH IRMA assay (Nichol's Institute, San Juan Capistrano, CA, USA). Serum FGF-23 was measured once at the time of first chest radiography using a two-step FGF-23 enzyme immunoassay (ELISA) kit (Kainos Laboratories Inc., Tokyo, Japan) as previously described[16]. The intra-assay and inter-assay variations of FGF-23 measurements were 3% and 4% to 6%, respectively. With regard to urea kinetics, we measured a blood-based dialysis parameter, Kt/V[17]. Medical records were carefully checked for prescriptions of vitamin D and P binders. The monthly prescribed doses of 1α-hydroxy vitamin D_3 and $CaCO_3$ at the time of chest radiography were calculated and used for statistical analyses. This study complies with the Declaration of Helsinki and is in agreement with the guidelines approved by the ethics committee of our institution.

We performed a prospective assessment of all patients from the start of dialysis therapy from January, 2005. Two radiologists (one specializing in chest radiography) independently assessed all chest radiographs obtained from HD patients studied. Each radiologist was blinded to the readings of the other and to the patient's results. Radiographs were assessed for the presence of AoAC using a specific scale. As previously described[18], the scale, which was divided into 16 circumferences, was attached to the aortic arch on chest radiography and then the number of sectors with calcification was divided by 16. The aortic arch calcification score (AoACS) was calculated after multipliying by 100 to express the results as a percentage. This value was an indicator of the AoAC. The change in AoACS ($\triangle$AoACS) was obtained by subtracting the baseline AoACS value from the follow-up AoACS value. The second assessment was performed from 5 years after the first determination. The patients were divided into three groups according to $\triangle$AoACS, progressors with an increased $\triangle$AoACS, non-progressors with no changes in $\triangle$AoACS, and regressors with a reduced $\triangle$AoACS compared with baseline values.

All results are presented as the means ± SD. The values are the means of data during the follow-up period. Differences between mean values of the groups were tested by analysis of variance. In terms of data with a skewed distribution, including FGF-23, groups were compared by the Kruskal Wallis H-test. Univariate associations between $\triangle$AoACS and clinical parameters were assessed using linear regression. Correlations between non-parametric factors were evaluated by Spearman's correlation analysis. All variables with a *p*-value less than 0.1 in univariate analysis were included in the multivariate regression model. Multivariate linear regression was performed to determine the factors related to the progression of AoAC using variables such as age, gender, duration of dialysis, albumin, CRP, Ca, P, Ca x P product, intact PTH, FGF-23, prescribed doses of active vitamin D_3 and $CaCO_3$ and sevelamer. Stepwise backward elimination was used, beginning with the variable with the highest *p*-value. All analyses were conducted using SPSS software, version 9.51 (Comworks, Japan) and *p*-values less than 0.05 were considered significant.

Results

The clinical characteristics of patients at the start of HD are shown in **Table 1**. A total of 127 HD patients were studied. All measurements listed below were performed before the dialysis session. Mean age was 62.1 ± 12.5 years, ranging from 36 to 85 years, and the mean duration of dialysis was 17.5 ± 7.2 years, ranging from 9.0 to 32.8 years. Fifty-four patients (42.5%) had no AoAC at the first determination. The mean AoACS was 3.0 ± 3.4%, ranging from 0 to 12%. P binders included vitamin D (79.5%), $CaCO_3$ (98.4%) and sevelamer (54.3%). The mean erythropoietin dose was 120 U/kg/week, achieving a mean

Table 1. Baseline characteristics of hemodialysis patients

Characteristics	Total (rate or range)
Gender (male/female)	127 (83/44)
Age (years)	62.1 ± 12.5 (36.0-85.0)
BMI (kg/m^2)	21.3 ± 4.0 (14.1-29.8)
Dialysis duration (years)	17.5 ± 7.2 (9.0-32.8)
Kt/V	1.2 ± 0.2 (0.8-1.9)
SBP (mmHg)	144.7 ± 16.3 (95.8-173.3)
DBP (mmHg)	76.1 ± 7.3 (55.5-93.9)
Albumin (g/dL)	3.7 ± 0.6 (3.0-4.2)
Total cholesterol (mg/dL)	146.3 ± 28.8 (94.8-229.2)
HDL cholesterol (mg/dL)	46.2 ± 13.8 (25.8-71.3)
Triglyceride (mg/dL)	105.9 ± 59.0 (33.2-379.3)
Ca (mg/dL)	9.1 ± 0.2 (6.2-10.4)
P (mg/dL)	5.6 ± 1.1 (2.8-10.1)
Ca x P products (mg/dL)^2	49.6 ± 11.8 (17.9-72.7)
Intact PTH (pg/mL)	314.1 ± 245.5 (27.3-605.2)
C-reactive protein (mg/dL)	0.4 ± 0.8 (0-0.7)
FGF-23 (pg/mL)	17511.3 ± 42381.9 (76.0-94794.0)
AoACS (%)	3.0 ± 3.4 (0-12)
Medications (%)	
Vitamin D	101 (79.5)
$CaCO_3$	125 (98.4)
Sevelamer	69 (54.3)

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; Ca, calcium; P, phosphorus; PTH, parathyroid hormone; FGF, fibroblast growth factor; AoACS, aortic arch calcification score.

hemoglobin level of 10.8 g/dL.

To investigate the reliability of AoACS evaluated by plain chest radiology, two radiologists independently evaluated chest radiographs during the study period. Each radiologist was blinded to the readings of the other. The coefficient of intra-observer variation was 2.1% and 2.2% in two radiologists. As previously described[18], AoACS was highly correlated with AoAC volume estimated by multi-detector computed tomography (MDCT) ($r=0.635$, $p<0.001$). Any differences between radiologist interpretations were resolved by consensus of a committee of three additional investigators who also were blinded to the study protocol.

At baseline assessment, the AoACS was significantly correlated with age ($r=0.358$, $p<0.0001$) and dialysis duration ($r=0.234$, $p=0.0078$). The log FGF-23 in males (3.8 ± 0.7) was significantly higher than in females (3.4 ± 0.9) ($p=0.0157$). No significant correlation was detected between log FGF-23 and AoACS at the baseline assessment ($p=0.0825$) (**Fig. 1**). On the other hand, univariate linear regression analyses showed a significant correlation between log FGF-23 and dialysis duration ($r=-0.275$, $p=0.0016$), Kt/V ($r=-0.253$, $p=0.0038$), systolic BP ($r=0.189$, $p=0.0329$), diastolic BP ($r=0.229$, $p=0.0091$), serum levels of Ca ($r=0.591$, $p<0.0001$), P ($r=0.659$, $p<0.0001$), Ca x P product ($r=0.772$, $p<0.0001$), intact PTH ($r=0.337$, $p<0.0001$) and prescribed dose of vitamin D3 ($r=-0.215$, $p=0.0146$) at the baseline assessment.

Changes in AoACS were from 5.70 ± 2.90% to 3.25 ± 3.29% in regressors and from 3.67 ± 3.16% to 6.26 ± 3.21% in progressors. In non-progressors, the AoACS did not change (1.25 ± 2.77%). The mean value of △AoACS was 0.7 ± 2.3%, ranging from −7% to 12% during 5-year follow-up. In order to examine the relationships between AoACS and clinical parameters, univariate analysis was performed. The △AoACS value correlated positively with prescribed male gender ($r=0.197$, $p=0.0258$), and negatively with serum levels of albumin ($r=-0.187$, $p=0.0350$) and FGF-23 ($r=-0.234$, $p=0.0080$). No significant correlation was detected between △AoACS and other clinical parameters.

Table 2 shows the comparison of clinical profiles among regressors ($n=20$), non-progressors ($n=53$) and progressors ($n=54$). The percentage of male gender in non-progressors (58.5%) was lesser than in regressors (60.0%) and progressors (74.6%). No significant difference in the percentage of male gender was detected among the 3 groups ($p=0.3176$). In addition, the dialysis duration in regressors (14.1 ± 5.1 years) was shorter than in non-progressors (19.5 ± 7.0 years) and progressors (16.8 ± 7.5 years). The serum TC level in regressors (149.1 ± 28.7 mg/dL) tended to be higher than in progressors (144.8 ± 27.3 mg/dL), but did not reach statistical significance. Serum FGF-23 level in regressors (39225.5 ± 9247.9 pg/mL) also tended to be higher than in non-progressors (12896.5 ± 26323.5 pg/mL) and progressors (14062.4 ± 18456.8 pg/mL), but did not reach statistical significance.

In an attempt to investigate the independent variables for the progression of AoAC, multiple regression analysis was performed. The results showed male gender (β value = 0.969, F = 5.092, $p=0.0192$), serum levels of albumin (β value = −1.395, F = 4.541, $p=0.0296$) and log FGF-23 (β value = −0.001, F = 7.273, $p=0.0115$) to be significant independent determinants of △AoACS (**Table 3**).

Discussion

We developed new indices of the severity of AoAC. AoACS determination by plain chest radiography was performed at the start of HD therapy. As pre-

Table 2. Comparison of clinical and biochemical profiles according to changes in the aortic arch calcification score (AoACS) during the 5-year follow-up period

(△AoACS)	Changes in AoACS			*p*-value
	Regressors (−2.5 ± 1.9)	Non-progressors (0)	Progressors (2.6 ± 2.0)	
Number	20	53	54	
Age (years)	65.0 ± 12.8	56.9 ± 10.5	65.7 ± 7.1	0.0003
Men (%)	60.0	58.5	74.6	0.3176
Duration (years)	14.1 ± 5.1	19.5 ± 7.0	16.8 ± 7.5	0.0095
BMI (kg/m^2)	20.5 ± 3.5	20.1 ± 3.0	20.4 ± 2.8	0.9674
SBP (mmHg)	142.7 ± 16.2	142.4 ± 16.6	145.3 ± 16.2	0.5440
DBP (mmHg)	73.7 ± 5.7	75.5 ± 7.7	75.2 ± 1.0	0.3825
Kt/V	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	0.9593
Ca (mg/dL)	9.2 ± 0.5	8.9 ± 0.7	9.2 ± 0.5	0.3786
P (mg/dL)	5.7 ± 1.1	5.5 ± 1.3	5.5 ± 0.9	0.8962
Ca x P (mg/dL)^2	52.6 ± 11.6	48.5 ± 13.0	49.6 ± 10.6	0.6027
iPTH (pg/mL)	327.4 ± 209.2	311.2 ± 270.1	311.9 ± 236.5	0.7773
CRP (mg/dL)	0.5 ± 0.8	0.3 ± 0.9	0.4 ± 0.4	0.0283
Albumin (g/dL)	3.7 ± 0.2	3.8 ± 0.3	3.6 ± 0.3	0.1103
TC (mg/dL)	149.1 ± 28.7	146.9 ± 30.7	144.8 ± 27.3	0.9366
HDL (mg/dL)	46.0 ± 13.8	48.3 ± 12.2	43.2 ± 14.7	0.0265
TG(mg/dL)	116.7 ± 60.4	100.7 ± 41.8	106.9 ± 71.7	0.5983
FGF-23 (pg/mL)	39225.5 ± 9247.9	12896.4 ± 26323.5	14062.4 ± 18456.8	0.0842
Vitamin D (μg/mo)	137.4 ± 163.0	137.4 ± 153.1	125.1 ± 142.8	0.8237
$CaCO_3$ (g/mo)	42.8 ± 22.5	46.4 ± 19.0	45.1 ± 20.6	0.7266
Sevelamer (g/mo)	132.0 ± 219.0	141.6 ± 22.9	133.4 ± 24.0	0.9695

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Ca, calcium; P, phosphorus; iPTH, intact parathyroid hormone; CRP, C-reactive protein; TC, total cholesterol; HDL, high-density lipoprotein; TG, triglyceride; FGF, fibroblast growth factor.

Table 3. Multiple regression analysis of factors associated with changes in aortic arch calcification score in hemodialysis patients

Independent variable	F-value (β-value)	*p*-value
Male gender (male-1)	5.092 (0.969)	0.0192
Albumin (g/dL)	4.541 (−1.395)	0.0296
Log FGF-23 (pg/mL)	7.273 (−0.001)	0.0115

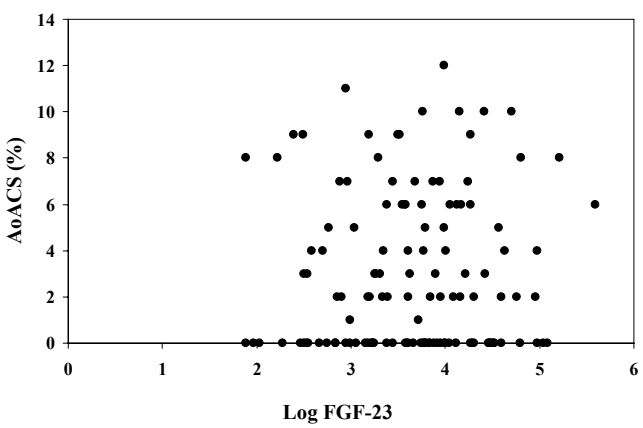

Fig. 1. Correlation between log fibroblast growth factor (FGF)-23 levels and aortic arch calcification score (AoACS) at the baseline assessment.

viously described[18, 19], the validity of AoACS was confirmed. The results from the present study suggest that the AoACS value at baseline correlated positively with age and dialysis duration, suggesting that they followed the common progress of aortic atherosclerosis[20]. Obviously, AoAC detected by chest radiography is a nonspecific finding; yet, it is not possible to rule out whether calcium deposits involve the intima or media layer of the arteries, however, it is related to widespread atherosclerosis[21]. On the other hand, AoAC could be a benchmark able to distinguish patients with calcification of the intimal layer of the arteries and therefore, patients possibly affected by widespread atherosclerosis from those with calcification of the medial wall.

In the general population, AoAC assessed by

chest radiography is associated with an increased risk of vascular events[22]. AoAC was associated with a six-fold increased risk of cardiovascular death in men aged over 45 years[23]. Li *et al.*[24] calculated AoAC sensitivity and specificity for coronary disease, which were 0.58 and 0.63, respectively: 91% of patients involved in their study with AoAC and 82% of those without AoAC had coronary artery disease on angiography. Salgueira *et al.*[25] analyzed the influence of vascular calcifications detected by a radiologic series on cardiac morbidity and mortality in 79 HD patients. Vascular calcifications were observed in 55.7% of patients and related to left ventricular hypertrophy, diastolic dysfunction, cardiac valve calcification, ischemic heart disease and cardiac failure episodes. We also reported that the extent of AoAC and arterial stiffness were independent determinants of left ventricular diastolic function in chronic HD patients[26].

In the present study, the serum FGF-23 level was shown to be a significant predictor of Ca and P metabolism in chronic HD patients without diabetes. Ca×P products in HD patients were of particular interest, since this parameter raises the possibility of a specific effect of FGF-23 on medial calcification of the aorta, distinct from its effect on serum Ca and P levels. It is noteworthy that there was no significant correlation between serum levels of FGF-23 and intact PTH, despite serum P and the Ca×P product value. Since 79.5% of patients were taking active vitamin D_3, it may have affected serum PTH while increasing serum P. We also found that the serum FGF-23 level was negatively associated with the △AoACS during the 5-year follow-up period, suggesting that FGF-23 is a negative regulator of the progression of AoAC in non-diabetic HD patients. Non-progressors were significantly younger than regressors and progressors in the present study; therefore, AoAC could be regulated with active vitamin D_3 and sevelamer in young HD patients according to the KDO KDOQI clinical practice guidelines for bone metabolism and disease in CKD[27].

FGF-23 was recently identified as one of the key molecules involved in the regulation of P homeostasis, without directly affecting Ca homeostasis. Inactivating mutations in the FGF-23 gene have been shown to be associated with familial tumoral calcinosis, an autosomal recessive disorder characterized by hyperphosphatemia and ectopic calcification due to reduced biological activity of FGF-23[28]. FGF-23 is a mostly bone-derived circulating factor, and mice without FGF-23 (fgf-23$^{-/-}$ mice) have extensive vascular calcification with high serum 1,25-$(OH)_2$D levels induced by increased renal expression of 1α-hydroxylase[29]. In double-knockout mice produced by ablation of the 1 α-hydroxylase gene in FGF-null mice, hyperphosphatemia is reversed and serum Ca levels are lowered. Furthermore, soft tissue calcification is eliminated, suggesting that vascular calcification in FGF-null mice develops through increased 1,25 $(OH)_2$D synthesis[30]. However, Imanishi *et al.*[12] reported previously that the mean plasma levels of FGF-23 in HD patients increased, reaching approximately 24 times the normal upper limit, due to impaired urinary excretion. Nasrallah *et al.* have recently reported that FGF-23 is independently associated with aortic calcification in HD patients[31]; therefore, extraordinary accumulation of FGF-23 in serum may enable inhibition of the calcification process at vessel walls.

At the present time, the exact mechanisms of AoAC regression with elevated FGF-23 levels remain uncertain. Firstly, elevated FGF-23 levels, which are closely associated with serum P levels, have been suggested to reflect a higher time-averaged P burden in HD patients. Secondly, elevated FGF-23 lower calcitriol levels via the inhibition of 25-hydroxyvitamin D_3 1-α-hydroxylase. Given the strong evidence for vitamin D deficiency as a non-traditional cardiovascular risk factor in HD patients[32], increased FGF-23 levels may indirectly exert a harmful effect on AoAC by inducing hypovitaminosis D. Thirdly, a previous report suggests that oral P binders might affect FGF-23 levels[33]. Even though there was no significant difference in serum FGF-23 levels among the three groups, most patients in the three groups had taken vitamin D and sevelamer, which could have affected serum FGF-23 levels in this study. However, it is possible that an elevated FGF-23 level has an effect on bone metabolism or vascular calcification both directly and indirectly, but the exact mechanism is not known yet.

There were a few limitations of our study, which was observational but a 5-year follow-up study from the start of HD therapy. Our analysis was limited by the study population, since it included only those patients preselected by their acceptance of this study protocol. However, until technology improves so that coronary disease can be diagnosed with noninvasive imaging, it will be ethically impossible to carry out a prospective randomized trial to further quantify the risk for coronary disease in HD patients with AoAC. Consequently, when combined with previous work, this study presents better evidence that patients with AoAC seen on plain chest radiography are at an increased risk for AoAC. In addition, we could not measure changes in serum FGF-23 levels in most HD patients who were treated with vitamin D_3 and P binders; therefore, we could not rule out the possibil-

ity of serum FGF-23 fluctuation during the follow-up period.

In conclusion, we found that changes in AoAC evaluated using a simple chest radiograph are associated with serum FGF-23 levels. Extraordinary accumulation of FGF-23 in serum may enable inhibition of the calcification process at vessel walls in chronic HD patients.

Acknowledgments

We thank the medical staff of the dialysis unit at Hidaka Hospital for collecting medical records. This work was supported by grants from the Japan Research Promotion Society for Cardiovascular Diseases and International Research.

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