ORIGINAL ARTICLE

Differences in associated factors between aortic and mitral valve calcification in hemodialysis

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Increased prevalence of aortic and mitral valve calcification has been reported in patients on hemodialysis, but it remains unknown whether aortic and mitral valve calcification arise from similar pathogenesis. We detected heart valve calcification using two-dimensional echocardiography, and we related valve calcification to various clinical parameters in patients treated with hemodialysis three times a week for more than 1 year. In 112 patients (77 men and 35 women, age 67 ± 10 years, duration on hemodialysis 95 ± 67 months), aortic and mitral valve calcification were observed in 84 (75.0%) and 58 (51.7%) patients, respectively. Aortic valve calcification was associated with increased age, higher serum calcium, lower serum albumin, lower total cholesterol and higher high-sensitivity C-reactive protein. Multivariate analysis showed that increased age and higher serum calcium were independently associated with aortic valve calcification. Conversely, mitral valve calcification was associated with increased age, higher high-sensitivity C-reactive protein and higher serum β_2-microglobulin, but not with higher serum calcium. In multivariate analysis, increased age and higher serum β_2-microglobulin were independently associated with mitral valve calcification. Serum β_2-microglobulin was associated with longer duration on hemodialysis, malnutrition inflammation (lower serum albumin and higher high-sensitivity C-reactive protein) and dyslipidemia. Considering the results in previous studies showing that the distribution of β_2-microglobulin amyloid deposition was consistent with that of tissue calcification in patients on hemodialysis, β_2-microglobulin may have pathogenic roles in valve calcification.

INTRODUCTION

The mechanisms leading to valve calcification were traditionally believed to be due to passive accumulation of hydroxyapatite mineral, but recent studies have shown active processes similar to those that occur in atherosclerosis, including inflammation and lipid deposition.[1,2]

Patients with end-stage renal disease have an increased cardiovascular morbidity and mortality.[3] In patients on hemodialysis (HD), heart valve calcification is frequently observed in association with systemic atherosclerosis and cardiovascular complication.[4–6] Previously, we detected calcification of the aortic and mitral valves by echocardiography, and related the number of calcified valves (0=no calcification, 1=calcification of the aortic or mitral valve, 2=calcification of both valves) with various clinical parameters.[6] Age, serum calcium, high-sensitivity C-reactive protein (hs-CRP) and serum β_2-microglobulin (β_2M) showed significant positive correlation, and serum albumin showed negative correlation with the number of calcified valves. Among these parameters, age and serum calcium were independently associated with valve calcification. Of note, serum β_2M showed marginally significant association with valve calcification in multivariate analysis. Because an increase in serum β_2M is peculiar in HD, our results suggested HD-related mechanisms of heart valve calcification. However, there remains a question whether calcification of the aortic and mitral valves arises from similar pathogenesis. In this study, we investigated the associated factors of aortic valve calcification (AVC) and mitral valve calcification (MVC) separately.

METHODS

Patients

The patients treated with HD three times a week for more than 1 year were included in this cross-sectional study. Dialysate containing a calcium concentration of $1.25 \, \text{mmol} \, \text{l}^{-1}$ was used in all patients. Those with a history of surgery or catheter therapy for heart valve disease were excluded. We recorded patient's age, sex, duration on HD, HD adequacy (Kt/V urea), the presence of diabetes mellitus, systolic and diastolic blood pressure, serum albumin, uric acid, calcium, phosphorus, calcium-phosphorus product ($\text{Ca} \times \text{P}$), intact parathyroid hormone, total cholesterol, triglyceride, high-density lipoprotein

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cholesterol (HDL-C), low-density lipoprotein cholesterol, hs-CRP, serum β_2M, and administration of angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, statin and calcium bicarbonate. Blood sampling was performed before the first dialysis session in the week after an overnight fast. Diabetes mellitus was diagnosed if the patient showed fasting plasma glucose more than or equal to 126 mg per 100 ml, or had a history of diabetic medication. Regarding calcium bicarbonate, we recorded the cumulative doses during 1 year immediately preceding the inclusion to this study, and calculated the averaged doses shown in g per day. In addition, we recorded carotid intima-media thickness (IMT) measured using a 7.5 MHz linear array transducer with high-resolution B-mode echography, as previously described.[7]

This study was a *post hoc* analysis of our previous study.[6] Of 115 patients in the previous study, 3 were excluded in this study, because adequate data were not obtained. All data were obtained between July 2005 and June 2006. All patients provided their informed consent, and the ethical committee of our hospital approved this study.

Echocardiography

Two-dimensional echocardiography was performed by single well-trained laboratory staff without prior knowledge of any information concerning the patients. We recorded the presence of calcification of the aortic and mitral valve, defined as the presence of bright echoes of more than 1 mm on one or more cusps of the aortic valve, mitral valve or mitral annulus.[8] In 20 patients, echocardiography was performed twice after an interval of 1 week, and the results of valve calcification were completely consistent. Therefore, there was no intra-observer variability.

Statistical analysis

Data are shown as mean ± s.d. In comparisons between the two groups, Mann–Whitney's *U*-test or χ^2-test was used as appropriate. Stepwise logistic regression analysis was used to select independent determinants among parameters with *P*-value <0.10 in univariate analyses. A *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS 11.0 software (SPSS, Chicago, IL, USA).

RESULTS

Clinical characteristics of the patients are shown in Table 1. The patients included 77 men and 35 women aged 67 ± 10 years. They were treated with HD for 95 ± 67 months (range 12–368) with mean Kt/V urea of 1.42. The mean values of systolic and diastolic blood pressure were 146 and 77 mm Hg, respectively. Thirty-eight patients (33.9%) had diabetes mellitus and 10 were treated with insulin. Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, statin and vitamin D analogue were administered in 47 (41.9%), 5 (4.4%) and 63 (56.2%) patients, respectively. Calcium bicarbonate was administered in 96 patients (85.7%), and the averaged dose was 3.69 ± 2.84 g per day. AVC and MVC were observed in 84 (75.0%) and 58 (51.7%) patients, respectively. Forty-eight patients (42.8%) had calcification of both valves, and 18 (16.0%) had no calcification. The mean value of carotid IMT was 0.93 ± 0.22 mm (range 0.5–2.0).

Table 2 shows the comparison of clinical parameters between the patients with and without AVC. There were significant differences in age, serum albumin, calcium, total cholesterol and hs-CRP. Stepwise logistic regression analysis showed that age and serum calcium were independent determinants for AVC (Table 3). In addition, carotid IMT was significantly increased in those with AVC compared with those without AVC (0.96 ± 0.23 vs. 0.84 ± 0.16 mm, *P*=0.01).

Table 1 Clinical characteristics of 112 patients

Sex (M/F)	77:35	
Age (years)	67 ± 10	(37–90)
Duration on HD (months)	95 ± 67	(12–368)
Kt/V urea	1.42 ± 0.21	(0.93–1.99)
Diabetes mellitus	38	(33.9%)
Systolic blood pressure (mm Hg)	146 ± 20	(94–190)
Diastolic blood pressure (mm Hg)	77 ± 13	(40–107)
Albumin (g per 100 ml)	3.7 ± 0.2	(2.9–4.7)
Uric acid (mg per 100 ml)	7.5 ± 1.4	(4.5–11.2)
Calcium (mg per 100 ml)	9.0 ± 0.7	(7.0–11.3)
Phosphorus (mg per 100 ml)	5.6 ± 1.1	(3.4–8.4)
Ca×P (mg^2 per (100 ml)^2)	50.9 ± 10.6	(30.4–82.1)
iPTH (pg ml^{-1})	218 ± 152	(11–898)
Total cholesterol (mg per 100 ml)	152 ± 34	(86–247)
Triglyceride (mg per 100 ml)	90 ± 58	(26–493)
HDL-C (mg per 100 ml)	47.8 ± 15.2	(20.3–88.3)
LDL-C (mg per 100 ml)	75 ± 25	(26–142)
hs-CRP (mg per 100 ml)	0.25 ± 0.36	(0.01–2.30)
β_2-Microglobulin (µg ml^{-1})	28.7 ± 5.5	(16.9–45.2)
ACE-I/ARB	47	(41.9%)
Statin	5	(4.4%)
Calcium bicarbonate (g per day)	3.69 ± 2.84	(0–14.0)
Vitamin D analogue	63	(56.2%)
Valve calcification		
Aortic valve	84	(75.0%)
Mitral valve	58	(51.7%)
Both valves	48	(42.8%)
None	18	(16.0%)

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca×P, calcium-phosphorus product; HD, hemodialysis; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; iPTH, intact parathyroid hormone; LDL-C, low-density lipoprotein cholesterol.

Table 2 Comparison of clinical parameters between patients with and without aortic valve calcification

	Aortic valve calcification		
	+	−	P-value
N	84	28	
Male patients (n, %)	55 (65.4)	22 (78.5)	0.19
Age (years)	69 ± 8	62 ± 13	0.01
Duration on HD (months)	98 ± 69	89 ± 64	0.61
Kt/V urea	1.42 ± 0.22	1.42 ± 0.16	0.53
Diabetes mellitus (n, %)	29 (34.5)	9 (32.1)	0.81
Systolic blood pressure (mm Hg)	144 ± 19	151 ± 22	0.17
Diastolic blood pressure (mm Hg)	77 ± 14	76 ± 13	0.78
Albumin (g per 100 ml)	3.6 ± 0.2	3.8 ± 0.2	0.009
Uric acid (mg per 100 ml)	7.6 ± 1.4	7.4 ± 1.2	0.34
Calcium (mg per 100 ml)	9.1 ± 0.8	8.7 ± 0.6	0.03
Phosphorus (mg per 100 ml)	5.6 ± 1.1	5.6 ± 0.9	0.97
Ca×P (mg^2 per (100 ml)^2)	51.4 ± 11.1	49.2 ± 9.0	0.42
iPTH (pg ml^{-1})	217 ± 162	222 ± 118	0.49
Total cholesterol (mg per 100 ml)	148 ± 33	162 ± 34	0.03
Triglyceride (mg per 100 ml)	86 ± 40	103 ± 93	0.85
HDL-C (mg per 100 ml)	46.7 ± 14.9	51.0 ± 15.8	0.12
LDL-C (mg per 100 ml)	73 ± 24	80 ± 27	0.25
hs-CRP (mg per 100 ml)	0.29 ± 0.38	0.13 ± 0.22	0.003
β_2-Microglobulin (µg ml^{-1})	29.0 ± 5.2	27.7 ± 6.4	0.24
ACE-I/ARB (n, %)	34 (40.4)	13 (46.4)	0.58
Statin (n, %)	5 (5.9)	0 (0)	0.32
Calcium bicarbonate (g per day)	3.70 ± 3.00	3.65 ± 2.31	0.80
Vitamin D analogue (n, %)	48 (57.1)	15 (53.5)	0.74

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca×P, calcium-phosphorus product; HD, hemodialysis; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; iPTH, intact parathyroid hormone; LDL-C, low-density lipoprotein cholesterol.

Table 3 Stepwise logistic regression analysis for independent determinants of aortic valve calcification

	Odds ratio (95% CI)	P-value
Age (years)	1.06 (1.01–1.11)	0.01
Calcium (mg per 100 ml)	2.16 (1.05–4.44)	0.03
hs-CRP (mg per 100 ml)	5.47 (0.59–50.13)	0.13
Total cholesterol (mg per 100 ml)	0.99 (0.97–1.00)	0.29
Albumin (g per 100 ml)	0.41 (0.06–2.52)	0.34

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein.

Table 5 Stepwise logistic regression analysis for independent determinants of mitral valve calcification

	Odds ratio (95% CI)	P-value
β_2-Microglobulin (μg ml^{-1})	1.10 (1.01–1.20)	0.01
Age (years)	1.04 (1.01–1.09)	0.04
iPTH (pg ml^{-1})	0.99 (0.99–1.00)	0.13
hs-CRP (mg per 100 ml)	1.70 (0.52–5.54)	0.37
Albumin (g per 100 ml)	0.57 (0.12–2.64)	0.47
HDL-C (mg per 100 ml)	1.00 (0.97–1.03)	0.83

Abbreviations: CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; iPTH, intact parathyroid hormone.

Table 4 Comparison of clinical parameters between patients with and without mitral valve calcification

	Mitral valve calcification		
	+	−	P-value
N	58	54	
Male patients (n, %)	41 (70.6)	36 (66.6)	0.64
Age (years)	70 ± 9	65 ± 11	0.03
Duration on HD (months)	101 ± 71	90 ± 63	0.41
Kt/V urea	1.44 ± 0.22	1.40 ± 0.18	0.45
Diabetes mellitus (n, %)	21 (36.2)	17 (31.4)	0.59
Systolic blood pressure (mm Hg)	145 ± 22	147 ± 18	0.45
Diastolic blood pressure (mm Hg)	77 ± 14	77 ± 13	0.97
Albumin (g per 100 ml)	3.6 ± 0.2	3.7 ± 0.2	0.10
Uric acid (mg per 100 ml)	7.4 ± 1.3	7.6 ± 1.5	0.78
Calcium (mg per 100 ml)	9.0 ± 0.8	9.0 ± 0.6	0.71
Phosphorus (mg per 100 ml)	5.6 ± 1.1	5.6 ± 1.0	0.88
Ca×P (mg per 100 ml)^2	51.2 ± 11.1	50.2 ± 9.1	0.93
iPTH (pg ml^{-1})	201 ± 169	235 ± 129	0.06
Total cholesterol (mg per 100 ml)	149 ± 35	154 ± 32	0.42
Triglyceride (mg per 100 ml)	88 ± 42	91 ± 72	0.48
HDL-C (mg per 100 ml)	46.1 ± 16.3	49.6 ± 13.8	0.09
LDL-C (mg per 100 ml)	73 ± 24	76 ± 25	0.62
hs-CRP (mg per 100 ml)	0.30 ± 0.33	0.19 ± 0.38	0.01
β_2-Microglobulin (μg ml^{-1})	30.1 ± 5.6	27.2 ± 5.1	0.007
ACE-I/ARB (n, %)	25 (43.1)	22 (40.7)	0.80
Statin (n, %)	4 (6.8)	1 (1.8)	0.36
Calcium bicarbonate (g per day)	3.50 ± 2.91	3.89 ± 2.76	0.42
Vitamin D analogue (n, %)	33 (56.8)	30 (55.5)	0.88

Abbreviations: Ca×P, calcium-phosphorus product; HD, hemodialysis; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; iPTH, intact parathyroid hormone; LDL-C, low-density lipoprotein cholesterol.

The clinical factors associated with MVC were different from those associated with AVC (Table 4). The patients with MVC were more elderly and showed higher hs-CRP and serum β_2M compared with those without MVC. Serum albumin, intact parathyroid hormone and HDL-C showed marginal significance, although serum calcium was comparable. In multivariate analysis, serum β_2M and age were independent determinants for MVC (Table 5). Carotid IMT was higher in those with MVC, but the difference did not reach statistical significance (0.97 ± 0.24 vs. 0.88 ± 0.20 mm, P=0.06).

Finally, serum β_2M correlated with duration on HD (r=0.273, P=0.004), serum albumin (r=−0.209, P=0.02), total cholesterol (r=−0.243, P=0.01), triglyceride (r=0.189, P=0.04), HDL-C (r=−0.337, P<0.001), hs-CRP (r=0.246, P=0.009), but not with Kt/V urea (r=−0.024, P=0.80).

DISCUSSION

Valve calcification is an important finding, because it can predict all-cause and cardiovascular mortality[9] or re-stenosis of drug-eluting stents[10] in patients with chronic kidney disease. Previous studies in a general population have reported the similarities in the risk factors for heart valve calcification and atherosclerosis, including age, diabetes mellitus, hyperlipidemia and hypertension.[1,2] These data suggested that heart valve calcification may be a manifestation of systemic atherosclerosis. In patients on HD, clinical factors peculiar to HD have been reported to be associated with valve calcification: duration on HD,[4,11,12] calcium-phosphate metabolism,[4,11–13] malnutrition inflammation[6] and serum β_2M.[6]

In this study, we investigated whether the associated factors of AVC were consistent with those of MVC in patients on HD. Boon et al.[14] investigated the aortic and mitral valves separately in a general population, and showed that AVC and MVC had similar risk factors. However, this issue has not been confirmed in patients on HD. Our results showed that the associated factors of AVC were not same as those of MVC. AVC was independently associated with increased age and higher serum calcium, which are known as important risk factors for systemic vascular calcification.[15] Therefore, AVC seemed to be more strongly associated with atherosclerosis/vascular calcification compared with MVC. Of interest, MVC showed a unique characteristic: no association with calcium metabolism. Considering a pivotal role of calcium in tissue calcification, this was an unexpected result. The reason explaining our result remains unknown, but serum level of calcium may not correlate with calcium accumulation in the tissue, or unknown mechanisms may exist. For example, Shroff et al.[16] have recently suggested the contribution of not only calcium accumulation in the vessels but also apoptosis of vascular smooth muscle cells to vascular calcification in patients on HD.

Another notable finding in MVC was an independent association with serum β_2M. Recent studies have shown that in a general population, serum β_2M correlates with pulse wave velocity[17] and the severity of peripheral arterial disease.[18] In patients on HD, serum β_2M is markedly increased and predicts all-cause mortality,[19,20] but studies on the relationship between serum β_2M and cardiovascular disease are limited. It has been reported that serum β_2M correlates with carotid IMT[21] and the number of calcified valves.[6]

Serum β_2M indicates the clearance of middle molecules by HD, or may be related to inflammation, as suggested by some authorities.[22,23] Accumulation of β_2M leads to dialysis-related amyloidosis, which usually becomes apparent after several years of HD. However, a postmortem study showed that β_2M amyloid deposition occurs in 21% of cases within 2 years and in 33% within 4 years after initiation of HD.[24] Although the significance of pathologically evident but

clinically silent amyloid deposition is unclear, it is notable that in patients on HD, pathological distribution of β_2M amyloid deposition was consistent with that of tissue calcification in previous reports.[25,26] There may be pathogenic interactions between amyloid deposition and calcification.

Another possibility is that serum β_2M may reflect various harmful effects of HD on the cardiovascular system. In our study, an increase in serum β_2M was associated with long-term HD, dyslipidemia (low total cholesterol, high triglyceride and low HDL-C) and malnutrition inflammation (low serum albumin and high hs-CRP), all of which are recognized as 'notorious' members in cardiovascular disease in HD. In the clinical course of chronic kidney disease, heart valve calcification can be seen even before initiation of HD.[27,28] The results in Multi-Ethnic Study of Atherosclerosis showed the prevalence of AVC was higher than that of MVC in patients with chronic kidney disease not requiring HD.[28] In our patients with mean HD duration of 95 months, the prevalence of AVC and MVC was 75.0 and 51.7%, respectively. Also in some studies on valve calcification in chronic HD, the prevalence of AVC was higher than that of MVC.[12,13] Therefore, we consider that a substantial proportion of the patients had already had AVC at initiation of HD, and developed MVC after HD. In our study, the prevalence of valve calcification was higher in patients with longer HD duration, although this association was not statistically significant. Regarding AVC, the prevalence was 61.9% in those with HD duration less than 3 years, 69.0% in those with HD duration less than 5 years and 76.7% in those with HD duration less than 7 years. The prevalence of MVC was 42.8% in those with HD duration less than 3 years, 45.2% in those with HD duration less than 5 years and 50.0% in those with HD duration less than 7 years. In the course of HD, heart valve calcification may progress under the influences of aging, calcium-phosphorus metabolism or serum β_2M.

Heart valve interstitial cells includes heterogeneous cell types, which express molecular markers similar to those of skeletal, cardiac and smooth muscle cells, including α-smooth muscle actin, a marker of myofibroblasts.[29] To elucidate pathogenic mechanisms of valve calcification, pathological and histochemical studies should be performed more extensively, but such studies are quite limited in HD. Kajbaf et al.[30] reported that heart valves surgically removed from HD patients showed more enhanced inflammation characterized by macrophage infiltration compared with non-HD patients. In addition to inflammation, cellular and molecular mechanisms characteristic of HD may exist. On the basis of our results, there may be pathogenic interactions between amyloid and calcification.

Some risk factors for cardiovascular disease in patients on HD are consistent with those in a general population, but others are paradoxical. We showed the association between AVC and lower total cholesterol. This result may seem curious, but some studies have shown the association between lower total cholesterol and increased mortality.[31] Cardiovascular disease in HD seems complicated because of peculiar factors such as malnutrition inflammation.

There are some limitations in our study: a cross-sectional study design, relatively small sample size and a single-center study design, which make it difficult to draw general conclusions and causal relationships. In addition, we did not evaluate valve calcification quantitatively, and computed tomography can detect calcification with higher sensitivity. Studies resolving these limitations are required. The distribution or number of calcified cusps is another issue of interest. Evaluation of calcified cusps may lead to more profound understanding the pathogenesis of valve calcification.

In conclusion, we showed differences in the associated factors between AVC and MVC in patients on HD. In multivariate analysis, AVC was associated with age and calcium metabolism, but MVC was associated with age and serum β_2M. There remains a question whether AVC and MVC arise from different pathogenesis, but β_2M may have pathogenic roles in heart valve calcification.

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