ELSEVIER

Archives of Medical Research 44 (2013) 638–644

Archives of Medical Research

ORIGINAL ARTICLE

De novo Development of Heart Valve Calcification in Incident Peritoneal Dialysis Patients

Marcela Ávila-Díaz,[a] Carmen Mora-Villalpando,[a] Ma. del Carmen Prado-Uribe,[a] Oscar Orihuela-Rodriguez,[b] Edgar Villegas-Antelo,[c] Ana Ma. Gómez-Noriega,[d] Diana Villanueva-Noches,[e] Héctor Hinojosa-Hernandez,[f] Juan Serrato-Avila,[a] Begoña Ilabaca,[g] and Ramón Paniagua[a]

[a]*Medical Research Unit in Nephrology Diseases,* [b]*Department of Cardiology,*
[c]*Department of Internal Medicine, Specialty Hospital, CMNS XXI, Instituto Mexicano del Seguro Social (IMSS), Mexico, D.F., Mexico*
[d]*General Hospital of Zone No.32,* [e]*General Hospital of Zone No.25,* [f]*General Hospital of Zone No.47,* [g]*General Hospital of Zone A1, IMSS, Mexico, D.F., Mexico*

Received for publication October 23, 2013; accepted October 24, 2013 (ARCMED-D-13-00585).

Background and Aims. Cardiac valve calcification (VC) is a frequent complication in chronic kidney disease and is considered a risk factor for all-cause and cardiovascular mortality. However, little is known about the pathophysiology mechanisms that originate it and the factors associated with its development. We undertook this study to analyze the frequency and factors related to de novo development of mitral valve calcification (MVC) and aortic valve calcifications (AVC) in incident peritoneal dialysis (PD) patients.

Methods. A prospective cohort of 124 incident PD patients was studied. Demographic and clinical data were recorded and blood assayed at baseline and after 1 year of follow-up for calcium, phosphorus, glucose, urea, creatinine, cholesterol, triglycerides by spectrophotometry assay; high-sensitivity C-reactive protein (CRP) by immunoturbidimetric ultrasensitive assay, intact parathormone (iPTH) and osteocalcin by electrochemiluminescence, fetuin-A and osteoprotegerin by EDI-ELISA. Valve calcification was evaluated by M-mode bidimensional echocardiogram.

Results. Sixty eight percent of patients were male, ages 43 ± 13 years; 51% were diabetic with 1.4 ± 1 months on PD. After 12.3 ± 1 months, 57 patients (46%) developed VC: AVC in 33 (57.8%), MVC in 15 (26.3%) and 9 (15.8%) patients in both valves. There was no correlation between AVC and MCV. In univariate logistic regression analysis, age, diabetes and elevated concentrations of OPG, iPTH and CRP were risk factors for development MVC. In multivariate analysis, only iPTH remained an independent risk factor as was also the case in AVC.

Conclusions. Age, diabetes, osteoprotegerin, parathormone and C-reactive protein are risk factors related to de novo development of MVC and iPTH for AVC in incident dialysis patients. © 2013 IMSS. Published by Elsevier Inc.

Key Words: Heart valve calcification, Peritoneal dialysis, Diabetes, Cardiovascular disease, Chronic kidney disease, Mineral metabolism.

Introduction

Presence of heart valve calcification is associated with chronic kidney disease (CKD); more advanced stages have higher rates of valve calcification (1,2). Valve calcification may be 5 to 10 times more frequent in patients with end-stage renal disease (ESRD) in comparison with a non-

Address reprint requests to: Marcela Ávila-Díaz, Unidad de Investigación Médica en Enfermedades Nefrológicas, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Cuauhtemoc No. 330, Col. Doctores, Mexico, D.F. 06720, Mexico; Phone: (+52) (55) 5627-6900 ext. 21371; FAX: (+52) (55) 56060991; E-mail: cramav@gmail.com

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2013.10.015

renal population (3). Prevalence of 35−44.5% has been reported for mitral valve calcification (MVC) and 25−52.0% for aortic valve calcification (AVC) in hemodialysis (HD) patients (4,5). Similar data were also reported in peritoneal dialysis (PD) patients (6).

Heart valve calcifications are associated with other vascular pathological conditions such as atherosclerosis and vascular calcifications (7) and have also been identified as risk factors for cardiovascular morbidity and mortality. MVC was associated with atrial fibrillation, stroke, and increased morbidity and mortality of cardiovascular origin in both the general and the CKD populations (8−10). On the other hand, AVC was reported as a risk factor for cardiovascular morbidity and mortality (11).

In spite of its high frequency and importance as a risk factor for cardiovascular mortality in CKD patients, little is known about the mechanisms and risk factors for their development. In cross-sectional studies, MVC was associated with inflammation (12) and hyperphosphatemia (4), and AVC seems to be associated with duration of HD treatment and some markers of mineral metabolism (13,14). However, studies about the development of new valve calcifications are not available. The aim of this study was to analyze the frequency and factors related to de novo development of MVC and AVC in incident PD patients.

Materials and Methods

Design

A prospective cohort study was performed in ESRD patients from six dialysis units in the metropolitan area of Mexico City affiliated with the national network of the Instituto Mexicano del Seguro Social. The protocol was approved by the Human Research and Ethics Committees of each of the participating hospitals. Patients gave their signed informed consent before enrollment in the study.

Patient Population

Two hundred forty-eight patients initiated PD in six hospitals participating in the study in the period between October 2009 and August 2010. Of these patients, 133 (54%) met the inclusion criteria. Of those accepted, three died, one was lost to follow-up and five had valve calcification at baseline and were excluded; 124 patients (50%) of the total population were included in the final analysis.

The patients were considered eligible for inclusion if they were incident (<3 months) on continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD). All were adults (18 years or older) without selection by gender, cause of renal disease or dialysis modality. Patients were excluded if they had pre-existing heart valve calcifications, heart failure, infections, malignancy, chronic liver disease, seropositivity for hepatitis or HIV or if they received immunosuppressive treatment. Patients with incomplete data were also excluded. All patients were dialyzed using conventional lactate-buffered glucose-based PD solutions. The patients received medications such as antihypertensives, calcium based-phosphate binders ($CaCO_3$ average 2.5 g/day) and $1\alpha25\ (OH)_2\ D_3$, (calcitriol, 0.25−0.75 μg/day) as indicated by their attending physicians.

Data Collection

After enrollment, basal clinical, biochemical and echocardiographic evaluations were performed. Second (final) similar evaluations took place at 12 months of follow-up. In the meantime, patients were followed by their health care team with bimonthly visits for their regular treatment and unscheduled visits and treatment as needed.

Demographic and Clinical Data

Demographic and clinical data were obtained from clinical files or directly from patient during scheduled visits. They included age, gender, smoking status, systolic and diastolic blood pressure (BP), body mass index, diabetes mellitus status, evolution time of kidney disease, and PD and pharmacology prescriptions.

Biochemical Parameters

Fasting venous blood samples were drawn for biochemical analyses. Glucose, urea, creatinine, albumin, cholesterol, triglycerides, total calcium (tCa), and phosphorous (PO_4) were performed by conventional spectrophotometry assay. High-sensitivity C-reactive protein (hs-CRP) was measured using the immunoturbidimetric ultrasensitive assay (Tina-quant CRP, Latex, Roche Diagnostics GmbH, Mannheim, Germany) (Hitachi 902 Automatic Analyser, Tokyo, Japan). The %CV of the CRP between run of assay was 5.8% at concentration for 5.5 mg/L and 1.5% in run with 4.0 mg/L. Intact parathormone (iPTH, 1−84) and MID-osteocalcin were analyzed by electrochemiluminescence immunoassay (Elecsys Modular Analytics 2010 Roche, Hitachi, Tokyo, Japan). Osteoprotegerin (OPG) and fetuin-A were determined by ELISA (MicroVue Eia Kit. Quidel Corp. Specialty Products, San Diego, CA and Epitope Diagnostic Inc., San Diego, CA, respectively). The intra-assay precision was 4.8−5.5% and inter-assay precision was 5.7−6.8%. Residual glomerular filtration rate (GFR) was measured as the average of 24 h urine urea and creatinine clearance.

Echocardiographic Measurements

Heart valve calcification was defined as bright echoes of >1 mm on one or more cusps of the aortic valve or mitral valve or mitral annulus or both and were measured using two-dimensional echocardiography using a digital commercial harmonic imaging ultrasound system with

an 3.3 mHz phased-array transducer (Philips Mod IE33, Philips Medical Systems, Service Hardware Rev D.0, Bothell, WA) with subjects lying in left decubitus position. Echocardiography was performed according to the recommendations of the American Society of Echocardiography (15) by a single observer and images were analyzed by a single experienced cardiologist who was blinded to all clinical details. Sensitivity and specificity for echocardiographic detection of calcium in the mitral valve and aortic valve were reported to be 76% and 89–94%, respectively (16).

Statistical Analysis

Data are expressed as mean ± SD or median and interquartile range, or frequencies according variable characteristics and distributions. In non-normally distributed variables, logarithmic transformations were applied. Changes between the final and initial evaluations are indicated as delta (Δ). Differences between groups were analyzed by Student t-test or Mann-Whitney U test for independent samples, according to variable characteristics and distributions. χ^2 tests were used for differences in proportion. To analyze differences between basal and final evaluations, paired-samples t test was used. Rho Spearman and Pearson's correlation coefficient was used to test associations between two types of parameters. Uni- and multivariate logistic regression was used to identify risk factors for the development of MVC or AVC; $p \leq 0.05$ was considered to be significant in all analyses. SPSS Windows v.15 was used for all statistical analyses.

Results

A total of 124 patients from the total incident dialysis population were included in the final analysis.

Baseline Characteristics

Demographic, clinical and biochemical baseline characteristics of the 124 patients are shown in Table 1. Time on dialysis at baseline evaluation was 1.4 ± 1.0 months. No patient had evidence of valve calcification in the initial echocardiographic evaluation. Male gender was overrepresented with 68% of the cases, half of the patients were diabetic, 16 (12.9%) had urinary volume <100 mL/day and the proportions in CAPD and APD were similar. Assignment to a dialysis modality was according to patient preference with orientation by the healthcare team and without the intervention of the researchers.

Follow-up

All 124 patients completed the follow-up period, and the final evaluation was done 12.35 ± 1.02 months after the baseline evaluation. At the end of the follow-up period, valve calcifications were detected in 57 (46%) patients. The aortic valve was calcified in 33 cases (57.8%), the mitral valve in 15 cases (26.3%) and in nine cases (15.8%) both valves were calcified. There was no correlation in the presence or magnitude of calcifications between valves; therefore, for the purposes of analysis, they were considered independently: MVC (42 cases) and AVC (24 cases). Table 2 and Table 3 show the baseline and final values for clinical and biochemical values of patients who developed new MVC or AVC, and they were compared with the 67 patients who did not develop valve calcifications (non-VC).

In the baseline evaluation, patients who developed MVC were older, a greater proportion had diabetes, and they had higher values of OPG when compared with patients who did not develop calcifications. After 1 year, this group showed increased values of values of hs-CRP, iPTH and OPG when compared with the patients who did not develop calcifications. In this group, we also observed significant increments in creatinine, albumin, and phosphorus and decreases in GFR between baseline and final values. All other characteristics were similar between baseline and final evaluations and in groups.

At baseline, patients who developed AVC had higher values of cholesterol than patients who did not develop calcifications. No differences were found in the other

Table 1. Baseline clinical and biochemical characteristics of 124 patients on dialysis

Age (years)	46 (30–54)
Gender *n* (male %)	85 (68)
Diabetes *n* (%)	63 (51)
Smoking status *n* (%)	68 (55)
BMI (kg/m^2)	25 ± 4.43
Systolic BP (mmHg)	134 ± 27
Diastolic BP (mmHg)	83.8 ± 15.6
Time on PD (month)	1.4 ± 1
Dialysis *n* (CAPD/APD)	53/58
GFR (ml/min/1.73 m^2)	2.3 (1.18–5.7)
Glucose (mg/dL)	96 (87–130)
Urea (mg/dL)	126 ± 41
sCrea (mg/dL)	9.1 (6.5–11.4)
TC (mg/dL)	186 (164–221)
Albumin (g/dL)	3.48 (3.1–3.8)
cCa (mg/dL)	8.9 (8.3–9.6)
PO_4 (mg/dL)	4.6 (3.8–5.9)
iPTH (pg/mL)	104 (85–186)
Fetuin (ng/mL)	48 ± 16
Osteocalcin (ng/mL)	176 (104–300)
hs-CRP (mg/L)	1.6 (0.46–4.8)
Phosphatase alkaline (IU/L)	92 (71–128)
Osteoprotegerin (pmol/L)	11.6 (7.5–15.5)

BMI, body mass index; PD, peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; APD, automated peritoneal dialysis; GFR, glomerular filtration rate; BP, blood pressure; TC, total cholesterol; hs-CRP, high-sensitivity C-reactive protein; iPTH, intact parathormone.
Continuous data are expressed as mean ± SD; unless specified otherwise median (interquartile range).

Table 2. Clinical characteristics of all patients with and without new development of mitral and aortic valve calcification at baseline and 1 year later

	Non-valve calcification (n = 67)		Mitral valve calcification (n = 24)		Aortic valve calcificación (n = 42)	
Variable	Baseline	1 year	Baseline	1 year	Baseline	1 year
Age	42 ± 13	43 ± 13	9 ± 10[a]*	50 ± 11[b]*	39 ± 13	40 ± 13
Gender m/f	47/20	47/20	8/7	9/15	23/10	23/10
DM y/n	31/36	31/36	18/6[a]*	18/6[b]*	14/19	14/19
Smokers (y/n)	39/28	39/28	16/8	16/8	15/18	15/18
BMI	25.2 ± 4.5	25.9 ± 4.1[c]*	27.1 ± 1.4	27.5 ± 2.6[c]*	24.9 ± 4.4	26.8 ± 5[c]*
SBP (mmHg)	135 ± 26	141 ± 27	139 ± 26	144 ± 26	128 ± 26	143 ± 29[c]*
DBP (mmHg)	85 ± 14	89 ± 13	85 ± 14	89 ± 10	83 ± 17	89 ± 17[c]*
GFR (ml/min/1.73 m^2)	4.8 ± 4	2.9 ± 2[c]*	4.1 ± 2.7	1.7 ± 1.6[c]**	4.2 ± 2	2.6 ± 3

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GFR, glomerular filtration rate.
Statistical analysis was done with valves.
[a]Significant difference between mitral VC and non-mitral VC at baseline.
[b]Significant difference between mitral VC and non-mitral VC after 1 year.
[c]Differences between the baseline and final evaluations.
*Significant difference, p <0.01.
**Significant difference, p <0.05.

biochemical variables between AVC and non-AVC groups. After 1 year, the AVC group had incremental values of iPTH, which were higher when compared with the patients who did not develop calcifications, and significant increments were observed in BMI, SBP, DBP, creatinine, albumin, cCa, triglycerides and hs-CRP and decreases were observed in cholesterol, fetuin and osteocalcin between baseline and final evaluations. All other characteristics were similar between baseline and final evaluations and groups.

Logistic regression was performed to analyze risk factors for developing CV. In the case of MVC, in univariate analysis, age, diabetes, baseline and final concentrations of OPG and iPTH (log), the incremented trend between initial and final values of hs-CRP (Δhs-CRP), and iPTH (ΔiPTH) were risk factors. Nevertheless, in multivariate analysis (Model I), only iPTH was a risk factor for MVC. Regarding changes of biochemical variables, Model II showed that ΔiPTH remained an independent risk factor as was also the case in AVC (RR = 2.002, p <0.034 95% CI 1.052−3.81). Results are shown in Table 4.

To determine the association between the magnitude of valve calcification (total mm^2 of both valves) and the changes

Table 3. Biochemical characteristics of all patients with and without development of mitral and aortic valve calcification at baseline and 1 year later

	Non-valve calcification (n = 67)		Mitral valve calcification (n = 24)		Aortic valve calcification (n = 42)	
Variable	Baseline	1 year	Baseline	1 year	Baseline	1 year
Glucose (mg/dL)	113 ± 44	117 ± 70	135 ± 107	129 ± 61	119 ± 65	119 ± 63
Urea (mg/dL)	122 ± 43	119 ± 3	132 ± 31	138 ± 3[b]**	130 ± 42	115 ± 36
sCrea (mg/dL)	9 ± 3.5	11.4 ± 4.5[c]*	8.4 ± 2.4	12.2 ± 3[c]*	9 ± 3	11.6 ± 3.5[c]*
Alb (g/dL)	3.4 ± 0.5	3.8 ± 0.4[c]*	3.3 ± 0.5	3.6 ± 0.5[c]*	3.5 ± 0.5	3.7 ± 0.4[c]*
cCa (mg/dL)	8.9 ± 1.5	9.2 ± 0.6	8.6 ± 0.9	9 ± 0.8	8.6 ± 0.8	9.0 ± 0.7[c]*
iPTH (pg/mL)	96 (48−179)	34 (9−144)[c]*	107 (71−133)	208 (79−296)[b]*	106 (64−195)	153 (22−305)[b]*
PO_4 (mg/dL)	4.6 (3.7−5.9)	4.8 (3.8−6.2)	5 (3.8−5.8)	5.2 (4.8−6.8)[c]*	4.6 (3.8−6)	5.1 (4.6−6.1)[c]**
TC (mg/dL)	187 ± 45	189 ± 48	199 ± 11	181 ± 9	202 ± 43[a]*	187 ± 41[c]*
TG (mg/dL)	189 ± 144	194 ± 101	170 ± 57	201 ± 101	186 ± 92	214 ± 111[c]*
Fetuin-A (ng/mL)	48 ± 16	39 ± 16[c]*	52 ± 17	41 ± 16	49 ± 16	45 ± 16[c]**
OT (ng/mL)	172 ± 99	138 ± 98[c]*	189 ± 89	178 ± 107	202 ± 92	180 ± 101[c]*
hs-CRP(mg/L)	2 (0.6−4.9)	2.3 (0.8−6.3)	1.1 (0.6−2.6)	4.4 (1.5−8.7)[bc]*	1.1 (0.6−4.6)	2.9 (0.8−7.4)[c]*
ALP (IU/L)	97 ± 44	117 ± 97	125 ± 68	108 ± 58	110 ± 60	103 ± 47
OPG (pmol/L)	12 ± 6	12 ± 6	15 ± 7[a]**	17 ± 7[bc]*	13.1 ± 6	14.6 ± 7[c]*

cCa, albumin corrected calcium; TC, total cholesterol; TG, triglycerides; iPTH, intact parathormone; OT, osteocalcin; hs-CRP, high-sensitivity C-reactive protein; ALP, alkaline phosphatase; OPG, osteoprotegerin.
Statistical analysis was done with valves.
[a]Significant difference between mitral VC and non-mitral VC at baseline.
[b]Significant difference between mitral VC and non-mitral VC after 1 year.
[c]Differences between the baseline and final evaluations.
*Significant differences, p <0.01.
**Significant differences, p <0.05.

Table 4. Risk factors for mitral valve calcification

	Univariate			Multivariate					
				Model I			Model II		
	RR	*p*	95% CI	RR	*p*	95% CI	RR	*p*	95% CI
Age (years)	1.051	0.02	1.06–1.09	1.044	0.27	0.96–1.12	1.05	0.22	0.97–1.13
DM (yes)	0.287	0.01	0.10–0.8	0.618	0.66	0.07–5.38	0.81	0.83	0.12–5.42
OPG	1.15	0.008	1.03–1.21	1.032	0.73	0.86–1.23	-	-	-
PTH (log)	7.41	0.04	1.08–50.5	8.93	0.03	1.12–71.71	-	-	-
ΔCRP (mg/L)	1.09	0.04	1.04–1.19	-	-	-	0.92	0.49	0.73–1.16
ΔPTH (log)	3.45	0.021	1.20–9.96	-	-	-	4.6	0.03	1.14–18.4
ΔOPG	1.08	0.17	0.96–1.2	-	-	-	1.10	0.21	0.94–1.28

DM, diabetes mellitus; OPG, osteoprotegerin; CRP, C-reactive protein; PTH, parathormone.
Logistic regression.
Significant difference, $p < 0.05$.

of biochemical variables, we made correlations and results with VC were with ΔCRP ($r = 0.20$, $p < 0.03$), ΔOPG ($r = 0.23$, $p < 0.01$) and ΔiPTH($r = 0.22$, $p < 0.05$) throughout the study. The correlation between ΔOPG and ΔhsCRP was ($r = 0.25$, $p < 0.009$), ΔiPTH with Δserum albumin ($r = 0.24$, $p < 0.04$), Δalbumin with Δhs-PCR ($r = -0.20$, $p < 0.03$) and Δhs-PCR with Δphosphorus ($r = 0.22$, $p < 0.02$). There were no significant correlations between valve calcification and gender, time on dialysis and the other biochemical factor of osteoblastic activity.

An additional analysis was performed to study factors related with faster development of valve calcifications. Patients were divided into two categories: slow calcifications in any valve ($n = 103$) and fast calcifications in any valve ($n = 21$). The cutoff point was 30 mm^2 in total.

Patients with fast progression of VC were older, had DM, and had high levels of OPG and low levels of albumin and GFR (Table 5).

Discussion

Data herein reported show a frequent and rapid de novo development calcification of mitral and aortic valves in patients starting treatment with PD. Data also show lack of correlation between mitral and aortic valve calcification as well as different risk factors for calcification in

Table 5. Clinical and biochemical characteristics of 124 patients on dialysis with slow and rapid development of valve calcification

	Slow development of valvular calcification	Rapid development of valvular calcification	*p*
Patients *n* (%)	103 (83%)	21 (17%)	
Age (years)	42 ± 13	48 ± 11	0.02
Gender *n* (m/f)	71/34	14/7	NS
DM (*n*)	49/56	16/5	0.012
BMI (kg/m^2)	24 ± 4	26 ± 4	NS
Smokers (*n*)	57/48	12/9	NS
Systolic BP (mmHg)	130 (113–150)	133 (117–152)	NS
Diastolic BP (mmHg)	84 (73–95)	79 (74–90)	NS
Glucose (mg/dL)	96.2 (87–130.1)	103.3 (88.2–138.2)	NS
Urea (mg/dL)	124.1 ± 41.9	132.8 ± 38.3	NS
sCreatinine (mg/dL)	9.16 ± 3.4	8.73 ± 2.10	NS
GFR (ml/min/1.73 m^2)	3.9 3.8	2.5 1.4	0.04
Alb (g/dL)	3.7 0.3	3.4 0.3	0.04
cCa (mg/dL)	8.5 (8–9.1)	8.5 (7.7–9.4)	NS
iPTH (pg/mL)	99 (56–187)	106 (71–194)	NS
PO_4 (mg/dL)	4.9 ± 1.5	5 ± 1.5	NS
TC (mg/dL)	184.7 (164–225)	197.7 (169–234.4)	NS
Fetuin (ng/mL)	49.01 ± 16.61	49.39 (38.41–64.86)	NS
OT (ng/mL)	175 (94–300)	138.3 (106.4–300)	NS
hs-CRP (mg/L)	1.9 (0.5–5)	1.2 (0.6–3.8)	NS
ALP (IU/L)	92 (71–127)	94 (82–139)	NS
OPG (pmol/L)	9.9 (7.2–15.9)	16.8 (11.8–22.4)	0.009

DM, diabetes mellitus; cCa, albumin corrected calcium; TC, total cholesterol; iPTH, intact parathormone; OT, osteocalcin; hs-CRP, high-sensitivity C-reactive protein; ALP, alkaline phosphatase; OPG, osteoprotegerin.
Continuous data are expressed as mean ± SD unless otherwise specified.
Median (interquartile range).

each valve. These findings suggest the presence of different mechanisms underlying the damage in different valves.

A significant number of patients developed new valve calcification in the relatively short period of 1 year of follow-up: 26.3% in the mitral valve and 57.8% in the aortic valve. These are not unexpected rates of damage because similar or even higher rates of calcification have been reported in several studies in prevalent HD and PD patients (4–6).

In spite of general similarities of this study with other previous studies, it is necessary to underline differences. Most previous reports were oriented to the analysis of consequences or impact of valve calcification on clinical outcomes such as morbidity and mortality of cardiovascular origin (17,18). However, regarding valve calcification process, they are cross-sectional analyses on prevalent HD or PD populations where an adequate analysis of risk factors for valve calcification was lacking; this is particularly important for biochemical data because it was obtained late, just at the time of valve calcification detection (19).

We did not find correlation of presence or magnitude of calcifications between mitral and aortic valves, which suggests different mechanism and risk factors for its development.

The aortic valve was more frequently affected than the mitral valve, which has been previously noted (5,20), but no special considerations were made in those reports.

On the other hand, in the mitral valve, calcification is associated with certain traditional risk factors and biochemical changes, as discussed below.

As expected, traditional cardiovascular risk factors such as age and diabetes were found to be risk factors for MVC in the univariate logistic regression analysis. Inflammation represented by increased levels of hs-CRP was also significant. Patients who developed MVC had an incremental trend of hs-CRP serum concentration from initial to final stage, emphasizing the role of inflammation in the calcification process. This is in line with what has previously been reported (21,22). Mineral metabolism-related variables were also important; serum phosphorus increased between the first and last evaluation. In most of the patients studied, iPTH was $<$150 pg/mL, the suggested minimal value in clinical practice guidelines (150–300 pg/mL) (23). Although patients with MVC were not outside the range (median: 208 pg/mL), they differed with non-VC, showing higher values of iPTH and a trend to increase iPTH levels from baseline to final evaluation. Previous studies mentioned the role of mineral environment in calcification process where hyperphosphatemia seems to be particularly important (24,25). Our data are congruent with that concept (median: 5.2 mg/dL). Whether iPTH has a role in calcification is a matter of discussion. In this study, iPTH remained essentially low, and the small increment observed may be secondary to increment in serum phosphorus concentration more than a direct effect on calcification.

OPG levels at baseline and final evaluation were significant risk factors for MVC. The same picture has been found in vascular calcification (26). Experimental studies have demonstrated the OPG inhibitory effect on calcification (27,28); therefore, high OPG levels as a risk factor for MVC may sound contradictory. However, in the clinical context (29), OPG levels should be interpreted as a consequence of active calcification because it is synthesized and secreted by cells with osteoblast activity. The amount secreted may not only be derived from valves being calcified, but also from arteries being calcified. Correlation between MVC and arterial calcification has been previously reported (30–32). Data suggest that the inflammatory state may induce overexpression of OPG as has been previously demonstrated in experimental studies (33) and that valvular endothelial cells unleash the pathway for osteogenic differentiation and calcification of the same. This is supported by the observation that a correlation is found between ΔOPG and Δhs-CRP ($r = 0.25$, $p < 0.009$).

In the multivariate logistic regression, only PTH and ΔPTH remained as independent risk factors, probably due to the strong correlation between variables, as was the case with ΔiPTH with Δserum albumin, (inverse) Δalbumin and Δhs-PCR and Δhs-PCR with Δserum phosphorus.

Calcification of the aortic valve is associated with cyclic mechanical stress derived from hemodynamic overwork as well as biochemical alterations.

Regarding development of AVC, patients in this group had only small but significantly higher values of serum cholesterol than non-VC group as in another study of non-renal patients (34) and showed significant increments from baseline to final evaluation in BMI, SBP, DBP, sCr, cCa, triglycerides and hs-CRP and decreases were observed in fetuin. In spite of these differences, only PTH was an independent risk factor for AVC, similar to another study for AVC (10).

Patients with rapid progression (>30 mm^2) during 1 year of VC were older, had DM and had high levels of OPG and low levels of albumin and GFR, as reported in others studies (19,35,36). It is interesting to note that elevated concentrations of OPG persist in our patients with VC.

Our study has some limitations. It has a small sample derived from stringent selection criteria, as the decision was to include only patients free of detectable valve calcification. However, it should be noted that restrictions allowed us to clarify the beginning of the calcification process. Another limitation refers to the relatively short follow-up time. We should mention that other studies report periods of 16 months, very similar to this study (13).

In summary, heart valve calcification is a frequent and rapid phenomenon that seems to affect mitral and aortic valves in different ways and to different magnitudes. Age, diabetes, osteoprotegerin, parathormone and C-reactive protein are risk factors for mitral calcification and iPTH for aortic valve in incident dialysis patients.

The results offer a new perspective on knowledge about the pathophysiology of VC in patients on dialysis that may orient towards new prevention and treatment strategies for the cardiovascular complications of chronic kidney disease.

Acknowledgments

The authors want to thank to Monica Ericsson for OPG measurement, Ma. Isabel Sambrano for PTH measurement, and Susan Drier for manuscript preparation. This work was sponsored by Consejo Nacional de Ciencia y Tecnología, México (CONACYT) No. 111941 and Genzyme Corp (now Sanofi).

References

1. Fox CS, Larson MG, Vasan RS, et al. Cross-sectional association of kidney function with valvular and annular calcification: the Framingham Hear Study. J Am Soc Nephrol 2006;17:521–527.
2. Maher ER, Young G, Smyth-Walsh B, et al. Aortic and mitral valve calcification in patients with end stage renal disease. Lancet 1987;2:875–877.
3. Bellasi A, Ferramosca E, Ratti C, et al. Cardiac valve calcification is a marker of vascular disease in prevalent hemodialysis patients. J Nephrol 2012;25:211–218.
4. Straumann E, Meyer B, Misteli M, et al. Aortic and mitral valve disease in patients with end stage renal failure on long-term haemodialysis. Br Heart J 1992;67:236–239.
5. Ribeiro S, Ramos A, Brandao A, et al. Cardiac valve calcification in haemodialysis patients: role of calcium-phosphate metabolism. Nephrol Dial Transplant 1998;13:2037–2040.
6. Wang AY, Wang M, Woo J, et al. Cardiac valve calcification as an important predictor for all-cause mortality and cardiovascular mortality in long-term peritoneal dialysis patients: a prospective study. J Am Soc Nephrol 2003;14:159–168.
7. Leskinen Y, Paana T, Saha H, et al. Valvular calcification and its relationship to atherosclerosis in chronic kidney disease. J Heart Valve Dis 2009;18:429–438.
8. Fox CS, Parise H, Vasan RS, et al. Mitral annular calcification is a predictor for incident atrial fibrillation. Atherosclerosis 2004;173:291–294.
9. Benjamin EJ, Plehn JF, D'Agostino RB, et al. Mitral annular calcification and the risk of stroke in an elderly cohort. N Engl J Med 1992; 327:374–379.
10. Fox CS, Vasan RS, Parise H, et al. Mitral annular calcification predicts cardiovascular morbidity and mortality: The Framingham Heart Study. Circulation 2003;107:1492–1496.
11. Otto CM, Lind BK, Kitzman DW, et al. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. N Engl J Med 1999;341:142–147.
12. Rao AK, Djamali A, Korcarz CE, et al. Mitral annular calcification is associated with reduced left ventricular function and inflammation in patients with chronic kidney disease. J Am Soc Echocardiogr 2008;21:747–750.
13. Ureña P, Malergue MC, Goldfarb B, et al. Evolutive aortic stenosis in hemodialysis patients: analysis of risk factors. Nephrologie 1999;20: 217–225.
14. Petrović D, Obrenović R, Stojimirović B. Risk factors for aortic valve calcification in patients on regular hemodialysis. Int J Artif Organs 2009;32:173–179.
15. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1989;2:358–367.
16. Wong M, Tei C, Shah PM. Sensitivity and specificity of two dimensional echocardiography in the detection of valvular calcification. Chest 1983;84:423–427.
17. Takahashi H, Ishii H, Aoyama T, et al. Predictors of mortality in end-stage renal disease patients with mitral annulus calcification. Am J Kidney Dis 2013;61:254–261.
18. Sharma R, Pellerin D, Gaze DC, et al. Mitral annular calcification predicts mortality and coronary artery disease in end stage renal disease. Atherosclerosis 2007;191:348–354.
19. Wang Angela YM, Woo J, Wai-Kei Lam C, et al. Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients. Nephrol Dial Transplant 2005;20:1676–1685.
20. Raggi P, Bellasi A, Gamboa C, et al. All-cause mortality in hemodialysis patients with heart valve calcification. Clin J Am Soc Nephrol 2011;6:1990–1995.
21. Kajbaf S, Veinot JP, Ha A, et al. Comparison of surgically removed cardiac valves of patients with ESRD with those of the general population. Am J Kidney Dis 2005;46:86–93.
22. Miller JD, Weiss RM, Heistad DD. Calcific aortic valve stenosis: methods, models and mechanisms. Circulation Res 2011;108: 1392–1412.
23. National Kidney Foundation. K/DOQUI. Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease. Am J Kidney Dis 2003;42:SI–S202.
24. Adeney KL, Siscovick DS, Ix JH, et al. Association of serum phosphate with vascular and valvular calcification in moderate CKD. J Am Soc Nephrol 2009;20:381–387.
25. Ketteler M, Biggar PH. Use of phosphate binders in chronic kidney disease. Curr Opin Nephrol Hypertens 2013;22:413–420.
26. Nitta K, Akiba T, Uchida K, et al. The progression of vascular calcification and serum oesteoprotegerin levels in patients in long-term hemodialysis. Am J Kidney Dis 2003;42:303–309.
27. Bucay N, Sarosi I, Dunstand CR, et al. Osteoprotegerin deficient mice developed early onset osteoporosis and arterial calcification. Gene Dev 1998;12:1260–1268.
28. Orita Y, Yamamoto H, Kohno N, et al. Role of osteoprotegerin in arterial calcification: development of animal model. Arteroscler Thromb Vasc Biol 2007;27:2058–2064.
29. Morena M, Terrier N, Jaussent I, et al. Plasma osteoprotegerin is associated with mortality in hemodialysis patients. JAm Soc Nephrol 2006; 17:262–270.
30. Piers LH, Touw HR, Gansevoort R, et al. Relation of aortic valve and coronary artery calcium in patients with chronic kidney disease to the stage and etiology of the renal disease. Am J Cardiol 2009;103: 1473–1477.
31. Bellasi A, Ferramosca E, Muntner P, et al. Correlation of simple imaging tests and coronary artery calcium measured by computed tomography in hemodialysis patients. Kidney Int 2006;70:1823–1828.
32. Tenenbaum A, Shemesh J, Fisman EZ, et al. Advanced mitral annular calcification is associated with severe coronary calcification on fast computed tomography. Invest Radiol 2000;35:193–198.
33. Collin-Osdoby P. Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. Circ Res 2004;95: 1046–1057.
34. Pohle K, Maffert R, Ropers D, et al. Progression of aortic valve calcification. Association with coronary atherosclerosis and cardiovascular risk factors. Circulation 2001;104:1927–1932.
35. Di Lullo L, Floccari F, Santoboni A, et al. Progression of cardiac valve calcification and decline of renal function in CKD patients. J Nephrol 2013;26:739–744.
36. Mazzaferro S, Coen G, Bandini S, et al. Role of ageing, chronic renal failure and dialysis in the calcification of mitral annulus. Nephrol Dial Transpl 1993;8:335–340.