Cardiac Valvular Calcification as a Marker of Atherosclerosis and Arterial Calcification in End-stage Renal Disease

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Background: Patients with end-stage renal disease (ESRD) are at increased risk for tissue calcifications as a result of deranged mineral metabolism. We tested the hypothesis that valvular calcification is a marker of atherosclerosis in patients with ESRD.

Methods: Echocardiography was performed in 92 patients undergoing peritoneal dialysis with no background atherosclerotic vascular complications to detect valvular calcification. We used B-mode ultrasonography to determine carotid artery intima-media thickness and the presence of plaque and calcification.

Results: Compared with patients without valvular calcification (n=66), those with valvular calcification (n=26) had higher C-reactive protein levels ($P=.01$) and greater mean±SE carotid intima-media thickness (1.12±0.06 vs 0.88±0.04 mm; $P=.003$). Carotid artery calcification was present unilaterally and bilaterally in 11 patients (17%) and 17 patients (24%) without, respectively ($P=.001$). Carotid artery plaque was present unilaterally and bilaterally in 11 patients (12%) and 16 patients (24%) without, respectively ($P=.001$). Using multiple logistic regression analysis, every 1-mm increase in carotid intima-media thickness was independently associated with a 6.51-fold (95% confidence interval, 1.58-26.73; $P=.009$) increased risk of valvular calcification, and calcification and plaque in the carotid arteries were associated with a 7.18-fold (95% confidence interval, 2.39-21.51; $P<.001$) and a 5.00-fold (95% confidence interval, 1.77-14.13; $P=.002$) increased risk of valvular calcification, respectively.

Conclusion: The associations among valvular calcification, inflammation, carotid atherosclerosis, and arterial calcification suggest that valvular calcification is a marker of atherosclerosis and arterial calcification in patients with ESRD.

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Calcification of the aortic valve or mitral annulus has been considered to be a chronic, noninflammatory degenerative process that occurs predominantly in elderly patients.1-3 Several recent studies4,5 reported a high prevalence of valvular and vascular calcification in patients with end-stage renal disease (ESRD). Other than aging, dysregulation of mineral metabolism with a high calcium load and the resulting poor calcium-phosphorus balance are suggested to be largely responsible for the excessive valvular and vascular calcification in this population.4,5 The recent demonstration of a significant association between C-reactive protein and valvular calcification4 suggests that calcification in patients with ESRD may not simply be a passive degenerative process but rather may involve active inflammation.

In large non-ESRD population cohort studies,6,7 aortic valve and mitral annulus calcification were associated with increased cardiovascular mortality and morbidity. Pathological studies8 showed collections of foam cells, which represent early atherosclerotic lesions, on the ventricular surface of the posterior mitral leaflet and on the aortic aspects of each of the aortic valve cusps in patients who developed coronary atherosclerosis. Furthermore, many studies9-11 in the non-ESRD population demonstrated similarities in the risk factors for aortic valve calcification, mitral annulus calcification, and atherosclerosis, including age, hypertension, hyperlipidemia, and diabetes mellitus. These data suggest that calcification of the aortic valve or mitral annulus may represent a form of atherosclerosis. In a recent prospective study,12 our group demonstrated the importance of valvular calcification in predicting all-
cause mortality and cardiovascular death in patients with ESRD. The exact mechanism was not clear, but it was unlikely to be explained by valvular obstruction. The mortality and cardiovascular death rates of patients with valvular calcification were no different from those of patients with clinical atherosclerotic vascular complications, suggesting that valvular calcification may also be a form of atherosclerosis. Because the mechanisms of valvular and vascular calcification are considered inequivalent in patients with and without ESRD, an important question is whether valvular calcification represents part of the atherosclerotic process in patients with ESRD. We tested the hypothesis that calcification of the aortic valve or mitral annulus in patients with ESRD not only reflects poor calcium-phosphorus balance but is also a marker of atherosclerosis and arterial calcification.

METHODS

The study protocol was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. Informed consent was obtained from all patients before study entry.

STUDY POPULATION

Altogether, 92 patients (38 men and 54 women) with ESRD receiving continuous ambulatory peritoneal dialysis treatment for at least 6 months were consecutively recruited into the study from the Prince of Wales Hospital. All patients underwent dialysis using low-calcium glucose–based lactate-buffered peritoneal dialysis solution. Phosphorus binders used in this study included calcium carbonate and aluminum hydroxide. Oral alfalcacidol was the only form of vitamin D used in our study patients. The type of phosphorus binders used, daily calcium and vitamin D dose, and statin use were recorded at study entry. Patients with background atherosclerotic vascular complications, that is, those with ischemic heart disease, a history of myocardial infarction with or without coronary artery bypass surgery or stenting, cerebrovascular disease, or peripheral vascular disease, were excluded because the presence of atherosclerotic vascular disease may confound the relationship between valvular calcification and carotid intima-media thickness (IMT). Other exclusion criteria were underlying malignancy, liver cirrhosis, chronic obstructive pulmonary disease, systemic inflammatory disease (such as systemic lupus erythematosus), and tuberculosis while still undergoing treatment.

ECHOCARDIOGRAPHY

Two-dimensional echocardiography was performed using a GE-VingMed System 5 echocardiographic machine (GE-VingMed Sound AB, Horten, Norway) with a 3.5-MHz multiphase array probe in patients lying in the left decubitus position. All echocardiographies were performed according to the recommendations of the American Society of Echocardiography and were analyzed by a single experienced cardiologist (M.W.) who was masked to all clinical and echocardiographic data. Carotid IMT was defined as a low-level echo gray band that does not project into the arterial lumen and was the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far edge. Three segments were measured bilaterally: the 1-cm section of the common carotid artery immediately proximal to the beginning of the dilatation of the bifurcation, the 1-cm section of the bifurcation immediately proximal to the tip of the flow divider, and the 1-cm section of the internal carotid artery immediately distal to the tip of the flow divider. The mean of the 6 measurements was used as the carotid artery IMT. All measurements were performed using the Philips SD800 sonographic system (Philips Medical Systems, Best, the Netherlands) with a 7.5-MHz high-resolution linear transducer. A plaque was defined as a local thickening relative to the adjacent segment with a distinct area of hyperechogenicity or protrusion into the lumen of the vessel that was at least 50% thicker than the surrounding area. Arterial calcification was identified if acoustic shadowing was seen arising from a lesion from the arterial wall. Using the same 6-segment scoring system (the 1-cm section of the common carotid artery immediately proximal to the beginning of the dilatation of the bifurcation, the 1-cm section of the bifurcation immediately proximal to the tip of the flow divider, and the 1-cm section of the internal carotid artery immediately distal to the tip of the flow divider), the number of segments with plaque or calcification present were counted.

The intraobserver reproducibility of the carotid artery IMT measurement was assessed by a second examination in 10 patients by the same observer. The interobserver reproducibility of the carotid artery IMT measurement was assessed by repeated carotid artery IMT measurements in 10 patients by the second independent observer. The intraobserver and interobserver intraclass correlation coefficients for carotid artery IMT measurement were 0.94 and 0.83, respectively.

BLOOD PRESSURE AND LABORATORY MEASUREMENTS

Systolic and diastolic blood pressure values were calculated as the mean of the systolic and diastolic blood pressures measured at 8-week intervals for the 12 months preceding echocardiography and carotid assessments.

Serum albumin levels were measured using the bromocresol purple method (Roche Diagnostics GmbH, Mannheim, Germany). Total fasting cholesterol and triglyceride levels were measured using the Hitachi 911 analyzer (Roche Diagnostics GmbH). High-density lipoprotein cholesterol levels were measured using the precipitation of apolipoprotein B–containing lipoproteins with phosphotungstate, whereas low-density lipoprotein cholesterol levels were calculated using the Friedwald formula. We measured C-reactive protein levels using the Tinaquant (latex) ultrasensitive assay (Roche Diagnostics GmbH). Serum calcium and phosphorus levels were measured in the standard chemical pathology laboratory.
STATISTICAL ANALYSIS

Continuous data are expressed as either mean±SE or median (interquartile range), depending on the data distribution, whereas categorical data are expressed as number (percentage). Between-group comparisons were tested using analysis of variance or the Mann-Whitney U test for continuous data and the χ² test for categorical data. Multiple logistic regression analysis was used to test for the associations between valvular calcification and carotid atherosclerosis, as denoted by carotid artery IMT, calcifications, and plaques, with adjustments for confounding covariates, including age, sex, smoking, diabetes mellitus, systolic blood pressure, low-density lipoprotein cholesterol, calcium × phosphorus product, high-sensitivity C-reactive protein, use of statins, vitamin D, and daily calcium supplement dose. Statistical analysis was performed using a statistical software package (SPSS for Windows version 10.0; SPSS Inc, Chicago, Ill). P<.05 is considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS

Our study patients had a mean±SD age of 54±11 years and underwent dialysis for a mean±SD duration of 40±33 months before being recruited into the study. The underlying causes of ESRD were chronic glomerulonephritis in 34 patients (37%), diabetic nephropathy in 16 (17%), hypertensive nephrosclerosis in 7 (8%), obstructive uropathy in 8 (9%), tubulointerstitial disease in 5 (5%), and polycystic kidney disease in 5 (5%). The cause of ESRD was unknown in 17 patients (19%). Of 28 patients (30%) with a history of smoking, 9 were current smokers and 19 were ex-smokers. Twenty patients (22%) had underlying diabetes mellitus.

Of the 92 patients, 26 (28%) had valvular calcification (9 patients had isolated aortic valve calcification, 13 had isolated mitral valve calcification, and 4 had calcification of both valves). Carotid artery IMT in our patients was 0.95±0.04 mm. Forty-six patients (50%) had carotid artery calcification; 15 in 1 carotid artery and 31 in both carotid arteries. Using the 6-segment scoring system (with 3 segments in each carotid artery), 13 patients were noted to have calcification in a single segment, whereas 33 patients had calcification in 2 or more segments of the carotid artery. Forty-seven patients had plaque present in the carotid artery (14 patients had plaque in 1 carotid artery and 33 had plaque in both carotid arteries). Using the 6-segment scoring system, 12 patients had plaque in 1 segment, and 35 had plaque in 2 or more segments of the carotid arteries.

CHARACTERISTICS OF PATIENTS IN RELATION TO VALVULAR CALCIFICATION

The clinical and biochemical characteristics of patients with and without valvular calcification are compared in Table. The causes of ESRD did not differ between patients with and without valvular calcification (P = .64). Carotid artery IMT was higher in patients with valvular calcification than in those with no valvular calcification (1.12±0.06 vs 0.88±0.04 mm; P = .003). Twenty-one (81%) of the 26 patients with valvular calcification vs 25 (38%) of the 66 patients without valvular calcification had carotid artery calcification (P = .001). Carotid artery calcification was present unilaterally and bilaterally in 4 patients (15%) and 17 patients (65%) with valvular calcification vs 11 patients (17%) and 14 patients (21%) with no valvular calcification, respectively (P < .001). Eighteen patients (69%) with valvular calcification vs 15 patients (23%) with no valvular calcification had calcifications present in 2 or more segments of the carotid arteries (P < .001). Carotid artery plaque was present in 20 patients (77%) with valvular calcification and in 27 (41%) without valvular calcification (P = .002). Plaque was present in 1 or both of the carotid arteries in 11 patients (12%) and 16 patients (65%) with valvular calcification vs 3 patients (17%) and 17 patients (24%) with no valvular calcification, respectively (P = .001). Eighteen patients (69%) with valvular calcification vs 17 (26%) without valvular calcification had plaque present in 2 or more segments of the carotid arteries (P = .001). The degree of valvular calcification was graded as mild, moderate, and severe in 5, 7, and 14 patients, respectively. Carotid artery IMT, however, did not differ among the 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Valvular Calcification (n = 26)</th>
<th>No Valvular Calcification (n = 66)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F, No.</td>
<td>8/18</td>
<td>30/06</td>
<td>.20</td>
</tr>
<tr>
<td>Age, y</td>
<td>57 ± 2</td>
<td>53 ± 1</td>
<td>.20</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.5 ± 0.5</td>
<td>23.0 ± 0.4</td>
<td>.51</td>
</tr>
<tr>
<td>Duration of dialysis, mo</td>
<td>41.4 ± 7.4</td>
<td>38.9 ± 3.9</td>
<td>.75</td>
</tr>
<tr>
<td>Smoking history, No. (%)</td>
<td>10 (38)</td>
<td>18 (27)</td>
<td>.29</td>
</tr>
<tr>
<td>Diabetes mellitus, No. (%)</td>
<td>9 (35)</td>
<td>11 (17)</td>
<td>.06</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>151 ± 4</td>
<td>146 ± 2</td>
<td>.22</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84 ± 2</td>
<td>84 ± 1</td>
<td>.97</td>
</tr>
<tr>
<td>Calcium × phosphorus product, mg/dL²</td>
<td>55.9 ± 3.8</td>
<td>49.8 ± 1.9</td>
<td>.11</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>Total: 203 ± 8</td>
<td>217 ± 6</td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td>HDL: 45 ± 2</td>
<td>45 ± 2</td>
<td>.89</td>
</tr>
<tr>
<td></td>
<td>LDL: 122 ± 7</td>
<td>136 ± 5</td>
<td>.11</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>179 ± 17</td>
<td>188 ± 14</td>
<td>.72</td>
</tr>
<tr>
<td>Serum albumin, g/dL</td>
<td>2.8 ± 0.06</td>
<td>2.9 ± 0.05</td>
<td>.41</td>
</tr>
<tr>
<td>C-reactive protein, median (IQR), mg/L</td>
<td>4.2 (1.5-14.2)</td>
<td>2.1 (0.8-4.4)</td>
<td>.01</td>
</tr>
<tr>
<td>Use of angiotensin-converting enzyme inhibitors, No. (%)</td>
<td>6 (23)</td>
<td>14 (21)</td>
<td>.85</td>
</tr>
<tr>
<td>Use of statins, No. (%)</td>
<td>4 (15)</td>
<td>6 (9)</td>
<td>.38</td>
</tr>
<tr>
<td>Daily calcium supplement dose, mg</td>
<td>1708 ± 196</td>
<td>1603 ± 117</td>
<td>.64</td>
</tr>
<tr>
<td>Use of aluminum-based phosphorus binders, No. (%)</td>
<td>7 (27)</td>
<td>11 (17)</td>
<td>.26</td>
</tr>
<tr>
<td>Average daily vitamin D dose, median (IQR), µg</td>
<td>0.18 (0.0-0.43)</td>
<td>0 (0-0.29)</td>
<td>.09</td>
</tr>
</tbody>
</table>

Abbreviations: HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein.

*Continuous data are expressed as mean±SE, except where otherwise specified.
groups of patients with increasing severity of valvular calcification (1.10±0.39 vs 1.16±0.38 vs 1.11±0.32 mm, respectively; \( P = .95 \)).

**RELATIONSHIPS BETWEEN VALVULAR CALCIFICATION AND CAROTID ATHEROSCLEROSIS, CALCIFICATION, AND PLAQUE**

Controlling for age, sex, smoking, diabetes mellitus, systolic blood pressure, low-density lipoprotein cholesterol, calcium \( \times \) phosphorus product, C-reactive protein, use of statins, vitamin D dose, and calcium supplement dose, every 1-mm increase in carotid artery IMT was independently associated with a 6.51-fold (95% confidence interval, 1.58-26.73; \( P = .009 \)) increased risk of valvular calcification. Another significant factor in patients undergoing dialysis was C-reactive protein, with a 1.07-fold (95% confidence interval, 1.01-1.14; \( P = .03 \)) increased risk of valvular calcification. Adjusting for the same confounding covariates, carotid artery calcification was associated with a 7.18-fold (95% confidence interval, 2.39-21.51; \( P < .001 \)) increased risk of valvular calcification. Having carotid artery plaque was associated with a 5.00-fold (95% confidence interval, 1.77-14.13; \( P = .002 \)) increased risk of valvular calcification.

**COMMENT**

Valvular or vascular calcification is prevalent in patients with ESRD and is considered to be largely the result of derangements in calcium-phosphorus metabolism. In the present study, using carotid artery IMT as a marker of atherosclerosis, we demonstrate that even in patients with no clinical atherosclerotic vascular complications, calcification of the mitral valve, aortic valve, or both is associated with statistically significantly greater carotid artery IMT, indicating more atherosclerosis, than in patients with no valvular calcification. Carotid artery IMT is a strong predictor of cardiovascular events in the general population.\(^{20}\) The extent of atherosclerosis of the carotid arteries also reflects the severity of arterial damage in other vascular territories.\(^{21,22}\) Recent studies\(^{23,24}\) in the ESRD population demonstrated that carotid artery IMT is predictive of cardiovascular death. Taken together, our results indicate that valvular calcification not only reflects an excessive calcium-phosphorus load but is also a marker of atherosclerosis in the ESRD population. Our findings are in keeping with those of studies\(^{25-29}\) in the general population that report associations between valvular calcification and atherosclerosis of the entire vascular system, including the coronary arteries, aorta, peripheral vascular system, and carotid arteries. Furthermore, calcifications of the mitral and aortic valves were associated with increased risk of cardiovascular mortality in the general population.\(^{6,7}\) A recent study\(^ {32}\) by our group demonstrated that valvular calcification is an important predictor of cardiovascular mortality in patients with ESRD. Moreover, the mortality rate was not significantly different between patients with either valvular calcification or atherosclerotic vascular disease,\(^ {12}\) indicating that valvular calcification has similar prognostic implications to other atherosclerotic vascular complications. Taken together, the present data provide evidence that valvular calcification is a marker of atherosclerosis in patients with ESRD despite differences in the pathogenic mechanisms of valvular calcification compared with the general population.

Calcification in patients with ESRD is considered to be different from that in patients without ESRD in that it occurs not only in the intima in association with atherosclerotic plaque but also typically in the media.\(^ {30}\) A recent study\(^ {31}\) reported increased plaque burden and calcification in patients with chronic renal failure compared with controls. Moreover, the results of a pathological study\(^ {32}\) indicated that the calcium deposits in atherosclerotic plaques were more extensive in patients with ESRD than in those without ESRD who had coronary artery disease, which was attributed to deranged mineral metabolism associated with renal failure. In this study, patients with valvular calcification showed a higher prevalence of calcification and plaque in the carotid arteries and more segments with calcium deposits and plaque in the carotid arteries. These findings suggest that valvular calcification is also a marker of vascular calcification and plaque burden in patients with ESRD, although it is not known whether the vascular calcification is present in the intima or the media. Previous studies reported close correlations between valvular and vascular calcification in patients with ESRD\(^ {33}\) and without ESRD.\(^ {34,35}\) Other than showing infiltration of inflammatory cells, lipoproteins, and calcium deposits, bone matrix proteins were observed in areas of valvular and vascular calcification,\(^ {40}\) suggesting similarities in the pathogenesis of valvular and vascular calcifications. Our study provides evidence that calcifications of the carotid arteries and cardiac valves are indeed associated syndromes and that valvular calcification represents the presence of a systemic atherosclerotic and arterial calcification process in patients with ESRD.

Although valvular calcification is considered a marker of atherosclerosis, we found no statistically significant differences in other traditional atherogenic risk factors, including age, sex, hypertension, diabetes mellitus, smoking, and cholesterol, between patients with and without valvular calcification. This finding may be related to the relatively small sample size and sampling bias because patients with clinical atherosclerotic vascular disease were deliberately excluded from the study to assess the clinical significance of valvular calcification in asymptomatic individuals. On the other hand, C-reactive protein remained independently associated with valvular calcification in patients with no atherosclerotic vascular disease, suggesting an association between calcification and inflammation. Because more men than women have atherosclerotic vascular disease, the exclusion of patients with clinical atherosclerotic vascular disease may also explain why there was a female preponderance in the group with valvular calcification, in contrast to a previous study\(^ {4}\) by our group, which showed a greater prevalence of men with valvular calcification.

Contrary to findings from previous studies,\(^ {6,7}\) we found no statistically significant association between serum calcium \( \times \) phosphorus product and valvular calcification.
in patients without atherosclerotic vascular disease. This may be partly related to the study power. On the other hand, the exclusion of patients with clinical atherosclerotic vascular disease may have weakened the association between calcium × phosphorus product and valvular calcification. Furthermore, a single serum calcium × phosphorus product measured at the time of cardiac and carotid examination may have missed this association. In this study, high-resolution ultrasonography was used to assess carotid artery calcification and plaque and valvular calcification. Although echocardiography is simple, noninvasive, and radiation free and we demonstrated high intraobserver reproducibility with ultrasonographic detection of calcification, it does not allow accurate quantification of calcification. Electron beam computed tomography sensitively detects and quantifies coronary calcium. Several recent studies demonstrated that electron beam computed tomography permits the quantification of valvular calcification with high inter- and intrascan reproducibility and thus provides a more accurate, noninvasive means of assessing coronary arterial and valvular calcification. Using electron beam computed tomography, the progression of aortic valvular calcification was shown to correlate with the progression of coronary artery calcification, a surrogate marker of atherosclerotic plaque burden. Taken together with our findings, this correlation suggests that valvular calcification also represents a marker of increased plaque burden in the ESRD population.

There is increasing evidence from studies in the general population that statin treatment is associated with reduced progression of calcific aortic stenosis. Valvular calcification is associated with inflammation and is a form of atherosclerosis in patients with ESRD. Considering the pleiotropic effects of statins, including anti-inflammation and reduction of extraosseous calcifications (as in the coronary artery), other than cholesterol lowering, prospective randomized studies are warranted to evaluate whether statin treatment may slow the progression of valvular and vascular calcification in the ESRD population.

This study has several limitations. First, the sample size was relatively small. Second, the different biochemical variables were measured at a single time and did not detect changes across time. Third, the study was performed in patients undergoing peritoneal dialysis. Whether our findings can be generalized to patients undergoing hemodialysis requires further evaluation. Nevertheless, our findings have several important implications. First, given that valvular calcification is a noninvasive marker of atherosclerosis and predicts cardiovascular death in patients with ESRD, more active screening is required to identify asymptomatic patients undergoing dialysis with valvular calcification for earlier active intervention. Second, because valvular calcification represents part of the atherosclerotic and vascular calcification process and has prognostic importance, further study is needed to examine whether therapeutic strategies that reduce the atherosclerotic burden are also effective in retarding the progression of vascular and valvular calcification in the ESRD population. In conclusion, we show that valvular calcification is associated with inflammation and represents a marker of atherosclerosis and vascular calcification in patients with ESRD.

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