VASCULAR AND VALVULAR CALCIFICATIONS IN CHRONIC KIDNEY DISEASE: AN UPDATE

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ABSTRACT

In chronic kidney disease (CKD) and end-stage renal disease patients cardiovascular disease is the main cause of morbidity and mortality, with incidence of cardiac related mortality increasing as renal function declines. Even after controlling for traditional cardiovascular risk factors such as smoking, age, gender, dyslipidaemia, and arterial hypertension, patients with CKD have a higher incidence of major cardiovascular events. CKD is characterised by the presence of many other non-traditional cardiovascular risk factors, such as chronic inflammation and accelerated atherosclerosis, oxidative stress, and especially, secondary hyperparathyroidism. This review will summarise the current evidence on vascular calcifications and valvular heart disease in CKD patients, from pathophysiology to therapeutic strategies.

Keywords: Chronic kidney disease (CKD), vascular calcifications, valvular calcifications, secondary hyperparathyroidism.

BACKGROUND

In chronic kidney disease (CKD) and end-stage renal disease patients cardiovascular disease is the main cause of morbidity and mortality, with incidence of cardiac related mortality increasing as renal function declines. Even after controlling for traditional cardiovascular risk factors such as smoking, age, gender, dyslipidaemia, and arterial hypertension, patients with CKD have a higher incidence of major cardiovascular events. CKD is characterised by the presence of many other non-traditional cardiovascular risk factors, such as chronic inflammation and accelerated atherosclerosis, oxidative stress, and especially, secondary hyperparathyroidism.1

It has been postulated that the high incidence of valvular and vascular calcifications could be mainly due to systemic chronic inflammation;2 however, new findings have indicated that chronic inflammation alone cannot explain the extent of degenerative calcifications (involving the arterial media layer) that are observed in CKD patients.3,4 This article will review the latest insights into cardiovascular calcifications, which are potent predictors of cardiovascular morbidity and mortality in CKD patients.

CLINICAL FEATURES AND DIAGNOSIS

Two types of vascular calcification affect the majority of patients with long-standing CKD and ESRD: arterial media calcification (calcific arteriosclerosis or Mönckeberg’s sclerosis), and accelerated calcification of the intimal plaque (calcific atherosclerosis).3,4 The latter probably represents the last step in classical atherosclerosis,
whereas medial calcification is non-inflammation based and associated with the duration of haemodialysis, calcium phosphate disorders, diabetes, and ageing.\textsuperscript{3,4} Cardiac calcifications (i.e. myocardial or valvular calcifications) mainly involve valve leaflets, discussed forthwith, although pathophysiological pathways seem to be similar to those of vascular calcifications. Another rare form of vascular calcification is calcific uraemic arteriolopathy (also known as calciphylaxis),\textsuperscript{5,6} but discussion of this topic lies beyond the scope of this review.

The presence of vascular and valvular calcifications can be associated with other cardiovascular findings related to arterial stiffness and myocardial fibrosis. Vascular calcifications are frequently related to reduced arterial elasticity, higher pulse pressures, and pulse-wave velocities resulting in cardiomyocyte hypertrophy and left ventricular hypertrophy (LVH).\textsuperscript{7-9} Uraemia-associated valvular disease (i.e. stenotic mitral and aortic valvular disease) is therefore accountable for increased afterload and contributes to worsening of LVH. CKD-associated LVH is related to cardiomyocyte and interstitial fibrosis, which can induce systolic and/or diastolic heart failure as well as arrhythmias, and is responsible for higher rates of sudden cardiac death compared with the general population.\textsuperscript{10} Diagnosis of vascular and valvular calcifications can be provided by ultrasound or plain X-ray radiograms. Echocardiography is crucial in detecting and staging both mitral and aortic calcifications and it is fundamental to the evaluation of younger CKD patients and those awaiting a kidney transplant.

**PATHOPHYSIOLOGY AND CLINICAL CORRELATIONS**

Vascular and valvular calcifications usually result from an imbalance between promoting and inhibiting factors (Table 1). The main constituents of calcifications are calcium and phosphate, especially as hydroxyapatite aggregates.\textsuperscript{3} It has been well established that higher mortality rates in CKD patients can be explained by high calcium and phosphate levels, which lead to osteoblastic modifications of vascular smooth muscle cells (VSMCs) and fibrosis development.\textsuperscript{3} CKD patients also show higher concentrations of bone morphogenic protein (BMP) 2, which is involved in the calcification process.\textsuperscript{5} Unbalanced calcium and phosphate levels are also associated with high parathyroid hormone and fibroblast growth factor 23 (FGF23) levels.\textsuperscript{11,12} Osteocytes produce FGF23 in response to dietary phosphate overload.\textsuperscript{11} FGF23 increases renal phosphate excretion and reduces the levels of 1,25-dihydroxyvitamin D and parathyroid hormone. In CKD patients, increased levels of FGF23 therefore act as a compensatory mechanism to normalise phosphate concentrations as renal function worsens. The FGF23-mediated compensation of phosphate balance may have deleterious trade-offs. High levels of FGF23 are associated with mortality, CKD progression, and calcification in CKD patients.\textsuperscript{11} Indeed, high FGF23 leads to an increased risk of secondary hyperparathyroidism due to the inhibition of 1-alpha-hydroxylase.

Other extracellular matrix molecules may play a role in vascular mineralisation and arterial remodelling, such as Type 1 collagen, bone sialoprotein, fibronecin, and decorin.\textsuperscript{13,14} Arterial remodelling results in VSMCs switching to osteoblastic cells, leading to vascular calcification. Matrix metalloproteinases and cathepsin can also modulate and promote vascular calcifications and their levels are elevated in the blood even in patients with early CKD.\textsuperscript{15,16} Among calcification-promoting agents, palmitic acid can promote mineralisation through activation of acyl-coenzyme A synthetases;\textsuperscript{17} therefore oxidised low-density lipoprotein can also induce osteoblastic switching in VSMCs.\textsuperscript{18} Finally, chronic systemic diseases (diabetes and hypertensive diseases) have been associated with cardiovascular calcifications. Drugs such as warfarin could promote calcifications by inhibiting the vitamin K cycle and reducing fetuin-A levels.\textsuperscript{19-22} Calcification inhibitors that provide tight control of calcium metabolism and precipitation will now be discussed.

Fetuin-A is a systemic inhibitor of ectopic calcification as several experimental studies have shown.\textsuperscript{20-24} In haemodialysis patients, low blood fetuin-A levels are associated with chronic inflammation and vascular/valvular calcifications. It has also been demonstrated that small vesicular structures derived from VMSCs accumulate fetuin-A to prevent calcification;\textsuperscript{23} fetuin-A acts by binding BMP2, BMP4, and BMP6, blocking osteochondrogenic activity.\textsuperscript{24} Matrix Gla protein is another calcification inhibitor as demonstrated in studies where matrix Gla protein-deficient mice have died as a result of massive bleeding caused...
by calcified vessel rupture.\textsuperscript{25,26} Osteoprotegerin is mainly expressed on VMSCs and endothelial cells and it inhibits osteoclastic function and bone reabsorption; its deficiency can lead to accelerated osteoporosis and vascular calcification, while high blood levels are associated with atherosclerotic disease.\textsuperscript{27,28} Osteopontin (OPN) is an extracellular phosphoprotein with a high affinity for hydroxyapatite and it can be found in mineralised tissues; its deficiency is associated with accelerated vascular calcification.\textsuperscript{29} OPN usually inhibits calcium crystal growth and promotes osteoclastic function; it is not present in normal arteries.\textsuperscript{30}

Calcification inhibitor/promoter imbalances lead to deep modifications in VSMC phenotype and function. Before the deposition of calcium in the vessel wall, VMSCs undergo differentiation into osteoblastic-like cells and downregulate production of smooth muscle specific genes such as \textit{smooth muscle actin} and SM22. Simultaneously, as previously discussed, these cells upregulate markers of osteochondrogenesis such as OPN, osteocalcin, and alkaline phosphatase. Osteoblast/chondrocyte-like VSMCs are able to produce a collagen matrix and form calcium and phosphorus-enriched matrix vesicles (MV) promoting vascular wall mineralisation and beginning the calcification pathway.\textsuperscript{30-32} Endothelial and vascular calcification also seems to be increased by α-elastin, an elastin-derived peptide; however elastin degradation secondary to ESRD in mice has demonstrated a lack of medial calcification development.\textsuperscript{30-32} As previously described, calcium and phosphorus-enriched MV seem to promote vascular calcification. MV have been found in atherosclerotic plaques associated with intimal calcification and in non-atherosclerotic patients with arterial medial calcification, such as those with CKD.\textsuperscript{33} In CKD patients, elevated calcium and phosphate levels have been shown to induce release of 30–300 nm large MV from cultured VSMCs, and elevated calcium levels also enhance mineral formation from MV.\textsuperscript{34} New evidence demonstrates that macrophages also play a role in the histogenesis of procalcific vesicles similar to MV at atherosclerotic plaque sites.\textsuperscript{35}

Other extracellular vesicles are represented by apoptotic bodies that can contribute to CKD vascular calcifications; it has been shown that elevated calcium and phosphate levels can be accountable for VMSC apoptosis.\textsuperscript{36} Released apoptotic bodies can accumulate calcium and lead to widespread calcifications along the vessel walls.\textsuperscript{36} Elevated levels of MV are also associated with cardiovascular mortality in ESRD patients and they are often correlated with aortic pulse wave velocity and carotid intimal media thickness.\textsuperscript{37} Circulating MV are detectable in menopausal women with coronary calcifications and coronary heart disease and they have been directly correlated with coronary artery calcium score.\textsuperscript{38}

Heart valvular calcifications mainly occur on mitral and aortic valves. Prevalence of mitral annulus calcifications (MAC) is between 8% and 15% in the general population and there is a median prevalence of 14–18% in CKD patients dependent on CKD stage, with a higher incidence in those presenting other cardiovascular risk factors.\textsuperscript{39-43}

### Table 1: Promoters and inhibitors of cardiovascular calcifications.

<table>
<thead>
<tr>
<th>Promoters</th>
<th>Inhibitors</th>
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</thead>
<tbody>
<tr>
<td>BMP2, BMP4</td>
<td>Matrix Gla protein</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>Osteopontin</td>
</tr>
<tr>
<td>Bone sialoprotein</td>
<td>Osteoprotegerin</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>Fetuin-A</td>
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<tr>
<td>Calcium and phosphate ions</td>
<td>Klotho</td>
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<tr>
<td>Oxidative stress</td>
<td>Pyrophosphate</td>
</tr>
<tr>
<td>Inflammatory cytokines (IL-6, IL-1, TNF)</td>
<td>Carbonic anhydrase</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Vitamin K</td>
</tr>
<tr>
<td>Coumadin derivatives</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Matrix vesicles and apoptotic bodies</td>
<td>Sodium thiosulfate</td>
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</tbody>
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BMP: bone morphogenic protein; IL: interleukin; TNF: tumour necrosis factor.
MAC are thought to be an age-related process but, especially in CKD patients, they represent the culmination of an active process quite similar to medial and atherosclerotic calcification.\textsuperscript{44} Presence of MAC is also related to incidence of arrhythmias and sudden cardiac death.\textsuperscript{45,46} MAC can be defined as a chronic degenerative process of the fibrous component of the mitral valve\textsuperscript{45,47} and can be easily detected by standard two dimensional echocardiography, both in M and B-mode. Echocardiography usually detects MAC as a hyperechoic band beneath the posterior mitral leaflet with the M-mode; B-mode (Figure 1) ultrasound allows physicians to localise MAC in the angle between the left ventricular posterior wall and the posterior mitral leaflet.\textsuperscript{45,48} Unfortunately, echocardiography does not distinguish between calcification and collagen; electron beam computed tomography (CT) and multislice (spiral) CT are more effective, non-invasive techniques for assessing cardiac and coronary calcifications.\textsuperscript{40,49} New three dimensional ultrasound devices provide a cheaper alternative to CT and they provide complete information regarding localisation and extension of calcifications, allowing evaluation of the mitral valve area.\textsuperscript{50}

MAC was initially considered a degenerative, age-related process only;\textsuperscript{51} however, accumulating evidence points toward a regulated process with features similar to both medial and atherosclerotic cardiovascular calcification on the basis of pathological findings and strong correlations with other pre-existing cardiovascular risk factors.\textsuperscript{41,52} A strong correlation has also been demonstrated between MAC and carotid atherosclerotic disease, peripheral artery disease, and coronary artery disease.\textsuperscript{53} Degenerative calcification of the mitral annular area is accelerated by conditions that increase mitral valve stress such as hypertension, aortic stenosis, and hypertrophic cardiomyopathy,\textsuperscript{45,54} with a strong relationship between LVH and severity of MAC.\textsuperscript{55} MAC is common in patients with CKD because of an increased prevalence and severity of cardiovascular risk factors and atherosclerotic disease.\textsuperscript{56}

Figure 1: Large mitral annulus calcification in a Stage 4 chronic kidney disease patient.
Furthermore, there is growing evidence that the abnormal calcium-phosphorus metabolism observed in patients with chronic renal failure has a direct role in the pathogenesis of MAC.\textsuperscript{45,57}

MAC is usually an incidental finding in patients evaluated by ultrasound for cardiovascular disease (CVD). Calcified mitral annulus is not always associated with symptoms, precluding true evaluation of MAC prevalence in the general population and in CKD patients. In large observational studies, the prevalence of MAC was 42\% in elderly patients with known CVD and 28\% in CKD patients at any stage of disease.\textsuperscript{31} MAC generally has little or no effect on left ventricular inflow or mitral valve function because leaflets are usually spared;\textsuperscript{45} severe mitral annulus involvement may occasionally lead to mitral regurgitation or stenosis.\textsuperscript{45,58,59} Patients with MAC also have a higher incidence of arrhythmias such as atrioventricular block, bundle branch block, intraventricular conduction delay, and especially in CKD patients, sudden cardiac death probably due to direct extension of calcific deposits in the atrioventricular node and bundle.\textsuperscript{46,60} Several community-based studies have evidenced a strong association between the presence of mitral valve calcifications and atrial fibrillation (AF) that seems to be independent of other risk factors for AF and development of acute coronary syndrome.\textsuperscript{61}

In patients with MAC, AF is probably a result of involvement of the inter and intra-atrial conduction systems and only partially mediated by left atrial dilation.\textsuperscript{61,62}

As previously mentioned, >50\% of deaths among ESRD patients are caused by CVD. Valvular heart disease is common in ESRD patients, with an incidence rate 5-times greater than that reported in the general population; its prevalence ranges from 9.5–36\%.\textsuperscript{63-66} Aortic valve calcification (AVC; Figure 2) is the most common valvular abnormality in the general population as well as in patients on haemodialysis.\textsuperscript{52} In the general population, incidence of AVC increases with age, occurring mainly in those >65 years old,\textsuperscript{67,68} while in CKD patients AVC is seen at a younger age and with associated secondary hyperparathyroidism. FGF23 levels could be considered as strong predictors of aortic valve disease in CKD patients.\textsuperscript{69}

Figure 2: Aortic valvular calcification in a haemodialysis patient.
TREATMENT STRATEGIES

 Moderate-to-severe CKD (with estimated glomerular filtration rate [eGFR] <30 mL/min) is characterised by an impairment of calcium-phosphate metabolism with low calcium and high phosphate levels due to secondary hyperparathyroidism. Haemodialysis patients worldwide are treated with bicarbonate dialysis using sodium bicarbonate as the base. Post-dialysis alkalosis may result in precipitation of calcium phosphate in soft tissues, including vessel walls, especially in the presence of high serum calcium levels (e.g. due to high dialysate calcium or treatment with 1,25-dihydroxyvitamin D) and may contribute to the pathogenesis of CVD.70 The appropriate dialysate calcium concentration should be chosen on the basis of the clinical characteristics of each patient for personalised dialysis therapy.71 For instance, in order to avoid positive calcium balance and increased risk of vascular calcification, 1.25 mmol/L dialysate calcium concentration should be chosen for patients taking supplements of calcium salts, active vitamin D metabolites, and calcium-containing phosphate binders. In contrast, for patients prone to cardiac arrhythmias, special caution is warranted when reducing from a 1.5 to 1.25 mmol/L dialysate calcium concentration. Current opinion encourages calcium levels of 1.25 mmol/L or lower; however some authors suggest an individualised approach.72,73 On the other hand, phosphate blood levels increase in patients with moderate-to-severe renal failure. Advanced CKD stages (with eGFR <30 mL/min) are characterised by hyperphosphataemia, also due to excessive dietary intake. Together with diet, phosphate binder therapy should be started with calcium-based or calcium-free phosphate binders; the majority of clinical studies show better outcomes with calcium-free phosphate binders.74,75 Some studies have shown the relevance of vascular calcification progression on mortality rate and the critical role of diet and phosphate intake on survival.76-79 Indeed, slow progression of cardiovascular calcifications, and even delayed progression of kidney disease may be attained in patients who follow a phosphate-restricted diet plus sevelamer in comparison with either unrestricted diet alone or with calcium acetate. Concerning vitamin D levels and the effects of its supplementation on the onset and progression of vascular and valvular calcifications, recently reported data are conflicting.80 The ADVANCE study has investigated the role of calcimimetics (e.g. cinacalcet) regarding the calcification progress in haemodialysis patients; the trial was conducted in 360 patients randomised to cinacalcet plus low-dose calcitriol or vitamin D analogue. The primary endpoint was to evaluate the percentage change in coronary artery calcification from baseline to Week 52; results did not differ between the two arms, although excessive use of vitamin D analogue in the combination arm could be a confounding factor.81,82 A small pilot study has investigated the potential role of a magnesium-containing phosphate binder83 but no data are available to substantiate the hypothesis that magnesium interferes with calcification in CKD. Finally, in 50 patients with CKD Stages 3-4, alendronate was tested but results showed that progression of vascular calcification compared with placebo over 18 months was not affected.84

CONCLUSIONS

 Different factors and several pathophysiological pathways can contribute to vascular and heart valvular calcification development in CKD patients. These various patterns (atherosclerotic calcification, media sclerosis, and valvular disease) could overlap but VSMCs are the most directly involved in the calcification process using the mechanisms described in this article. Therapy has to be aimed at both correcting the imbalance between calcification promoters and delaying cardiovascular calcification progression, reducing phosphate levels, and monitoring the clinical and biochemical features of secondary hyperparathyroidism.

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