

Phosphate overload accelerates vascular aging in uremic patients

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ABSTRACT: Vascular calcification is a very common event in patients affected by diabetes and chronic kidney disease (CKD). Recently, it has been well documented that abnormalities in mineral and bone metabolism in CKD patients are associated with increased morbidity and mortality. Elevated serum phosphate and calcium-phosphate product levels play an important role in the pathogenesis of vascular mineralization in uremic patients and also appear to be associated with increased cardiovascular mortality. Together with classical passive precipitation of calcium-phosphate in soft tissues, during the last decade it has been demonstrated that inorganic phosphate may cause extraskeletal calcification directly through a real "ossification" of the tunica media in the vasculature of CKD patients. Therefore, control of phosphate retention is now an even more crucial target of treatment in patients affected by chronic kidney disease. (Heart International 2006; 2: 6-11)

KEY WORDS: Vascular calcification, Phosphate, Chronic kidney disease

INTRODUCTION

Vascular calcification is a common event in patients affected by diabetes and chronic kidney disease (CKD) (1, 2). In fact, these patients develop extensive medial calcification, which causes increased arterial stiffness and high morbidity and mortality due to cardiovascular events (3). Calcification of soft tissues and blood vessel walls occurs frequently in dialyzed patients compared to the non-uremic population (4-6). A growing number of risk factors are associated with vascular mineralization in dialysis patients (inflammation, age, time on dialysis, etc.), but abnormalities in bone mineral metabolism represent a major determinant (Tab. I). Clearly, hyperphosphatemia, elevated serum calcium-phosphate product levels, and secondary hyperparathyroidism are associated to vascular calcification, although their roles have been incompletely investigated (7, 8).

In addition to these classic alterations in bone and mineral metabolism, an active process has been documented (9). Enhanced serum phosphate levels have been recently investigated as inducing factors on extraskeletal calcification in uremic population. In particular, elevated blood levels of phosphate associate with ectopic calcifications and increased risk of calciphylaxis (10-12). *In vitro* studies demonstrated that human aortic smooth muscle cells calcify when incubated in a high

TABLE I - RISK FACTORS FOR CARDIOVASCULAR DISEASE IN CHRONIC RENAL FAILURE PATIENTS

Classic	Typical
Obesity	Secondary hyperparathyroidism
Family history	Hyperphosphatemia
Smoking	Inflammation
Hypertension	Hyperhomocysteinemia
Diabetes	
Dyslipidemia	
Gender	

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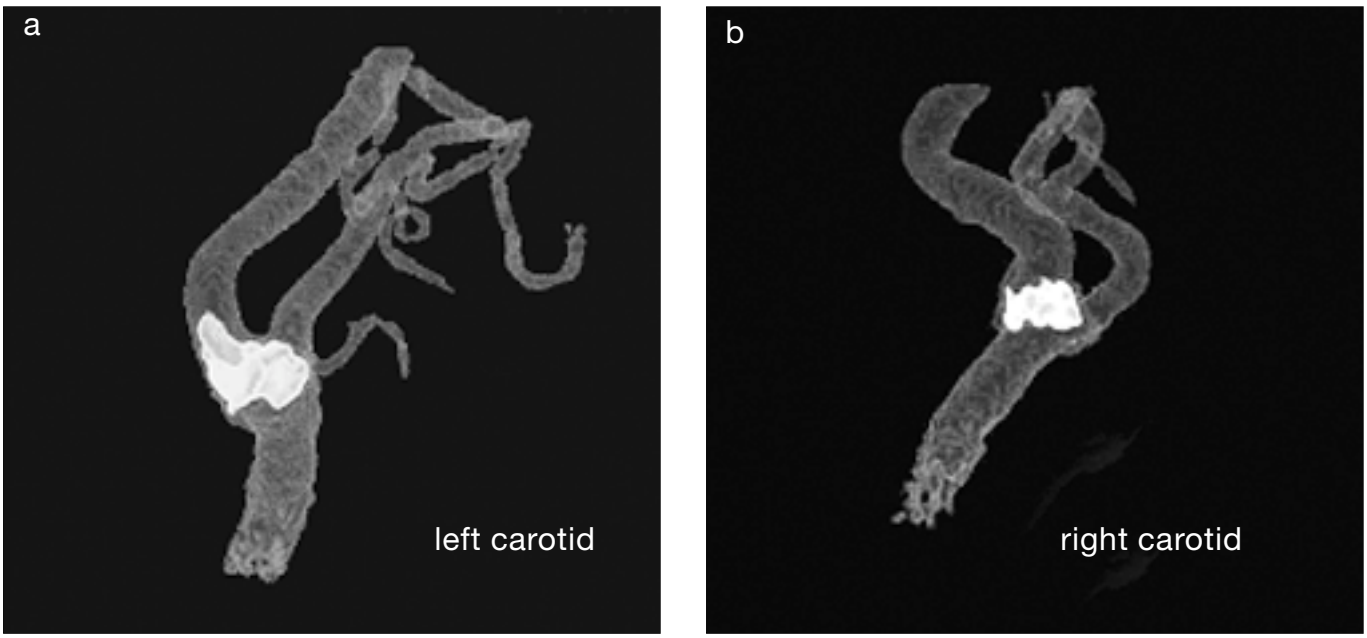

Fig. 1 - *Vascular calcification in CKD. A representative hemodialysis patient with remarkable bilateral carotid artery calcifications. Doppler analysis revealed a 60% stenosis in the left carotid artery (a) and a 70% stenosis in the contralateral artery (b). The patient had a sudden death 5 months after this CT scan was performed.*

phosphate medium, where calcium and calcitriol are not changed (13).

In the last decade, several studies defined calcification of atherosclerotic lesions as an active process similar to bone formation. Furthermore, vascular calcification was shown to involve not only a passive calcium-phosphate deposition on atherosclerotic vessels but also an active "ossification" of vascular structures.

Since vascular calcifications are predictive of higher morbidity and mortality, the control of serum phosphorus in CKD patients becomes crucial in preventing increases in calcium-phosphate product, secondary hyperparathyroidism, and so far vascular mineralization (14). Recent studies have shown that new pharmacological tools may be useful to prevent vascular calcifications in animals and humans (15-17).

In this review, we analyzed the role of phosphate on inducing vascular mineralization in CKD patients.

Cardiovascular risk in uremic patients

Abnormalities in mineral and bone metabolism are very common in end-stage renal disease patients. In this population, parathyroid hyperplasia and enhanced secretion of PTH characterize secondary hyperparathyroidism (18). Parathyroid gland enlargement and high circulating levels of PTH (19) are major contributors to increased bone resorption, a feature of renal osteodystrophy. Hypocalcemia, hyperphosphatemia, and vitamin D deficiency are the main regulators of hyperparathyroidism secondary to renal failure (20).

In the last decade, large evidence has been accumulated indicating that disturbances in mineral and bone metabolism in chronic renal disease patients associate with increased morbidity and mortality. In fact, cardiovascular events are the most frequent cause of death in patients with chronic renal failure (3). Calcification of soft tissues and blood vessel walls (Fig. 1) occurs frequently in dialyzed patients compared to the non-uremic population (21). Hyperphosphatemia and increased calcium-phosphate product are important contributors to vascular calcifications in uremic patients and also appear to be associated with increased mortality (7). In particular, elevated blood levels of phosphate associate with ectopic calcifications and increased risk of calciphylaxis (12). Unfortunately, the pathogenic effects of hyperphosphatemia, high calcium-phosphate product, and secondary hyperparathyroidism on enhancing vascular calcification in chronic renal failure are still poorly

understood.

Since vascular calcifications are predictive of higher morbidity and mortality, the control of serum phosphorus in patients with chronic renal failure is crucial in preventing increases in calcium-phosphate product, secondary hyperparathyroidism, and so far ectopic calcifications (12). In the past, the standard treatment for the hyperphosphatemia of chronic renal failure consisted of dietary phosphate restriction, dialysis treatment efficiency, and administration of phosphate-binders (aluminum salts, calcium carbonate or acetate). Recent studies described the limitations of calcium salts as phosphate-binders and the elevated calcium load in patients with advanced renal failure (14). Moreover, with such therapeutic approach more than 50% of patients did not achieve a good control of serum phosphate levels.

Recently, new phosphate binders, that do not contain aluminum or calcium, and therefore lack the side effects associated with classical phosphate-binders, opened new perspectives in preventing ectopic calcification in end-stage renal disease. Recent studies have shown that new therapeutical tools may be useful to prevent vascular calcifications in animals and humans (17).

Active regulation of vascular mineralization by phosphate

During the last decade, several investigators have been deeply interested in studying the mechanisms of vascular calcification (22). In fact, extraskeletal mineralization seems to be characterized not only by a passive calcium-phosphate deposition in the vasculature, but also by an active transformation of arterial walls in bone structures regulated by genes associated with osteoblastic functions (7). Moreover, recent studies demonstrated phosphate regulation of vascular calcification and provide some insights into the mechanisms for phosphate–induction of metastatic calcifications. Jono et al (13, 23) showed that high phosphorus levels in the incubation media (2 mmol/L) increased human aortic smooth muscle cells (HSMCs) calcification. Phosphate-containing mineral deposition was predominant in the extracellular matrix (13). Furthermore, these *in vitro* studies indicated that high phosphate directly increases HSMCs calcification by inducing the expression of the sodium-phosphate cotransporter Pit-1 and the osteoblast-specific genes Osf2/Cbfa-1 (13). Cbfa-1 is a transcription factor that regulates the expression of osteocalcin (24), one of the key osteoblast-specific genes *in vitro* and *in vivo.*

Other studies identified a potential role of phosphate on "active" regulation of vascular calcification. *In vivo* studies by Kuro-o et al (25) illustrate a 2-fold increase in serum phosphate levels in the KLOTHO-gene mutant mice, resulted in increased calcium-phosphate product with the development of vascular calcifications and osteoporosis, in the presence of normal renal function.

Further evidence has been reported on the function and action of a bone-associated protein: osteopontin. In two recent studies, Wada et al (26) proposed that osteopontin acts as an inhibitor of calcification of vascular smooth muscle cell (VSMC) cultures, while Jono et al (27) demonstrated that the phosphorylation of osteopontin was a mandatory step to inhibit VSMCs calcification. Thus, osteopontin is both an important bone mineralization modulator and a potent inhibitor of extraskeletal mineralization. In very recent review article, Giachelli et al (28) analyzed the opposite roles of phosphate and osteopontin on regulating vascular calcification. In fact, while phosphate induces directly vascular smooth muscle cell mineralization, osteopontin may stimulate cellular mineral resorption and inhibit calcium-phosphate deposition. Furthermore, smooth muscle cells isolated from osteopontin knock-out mice (OPN $^{-/-}$) have a significantly higher calcification grade when they are incubated in a medium containing elevated concentrations of inorganic phosphate (29).

In summary, several studies support an active role of phosphate on regulating arterial mineralization, "similar" to bone formation. However, new evidence is necessary to better understand pathogenic mechanisms of vascular calcification in chronic kidney disease, and the potential role of phosphate on controlling directly this disease.

Prevention of uremia-induced extraskeletal calcification by reducing serum phosphate levels

Over the past 40 years, different studies have shown that phosphate retention not only induces secondary hyperparathyroidism but also accelerates kidney failure

by inducing renal calcifications (30). Clearly, Gimenez et al (31) showed that patients with higher serum calcium-phosphate product, renal calcium content, and histologic calcium deposition were those with serum creatinine levels above 1.5 mg/dL. In experimental renal failure, high dietary phosphate-induced acceleration of kidney function deterioration is associated with renal calcium-phosphate deposition and tubular-interstitial damage (32).

The control of serum phosphorus levels is critical in preventing ectopic calcification in CKD. Recently, using an experimental model of chronic renal failure, we compared the effects of sevelamer hydrochloride, a non-calcaemic phosphate-binder, and calcium carbonate in the control of serum phosphate, secondary hyperparathyroidism, and ectopic calcifications (33). Sevelamer-treated rats showed a significant reduction of renal calcium deposition compared to both uremic untreated animals and rats taking calcium carbonate. In addition, in long-term experimental chronic renal failure, the phosphate-binder sevelamer controls serum phosphorus and secondary hyperparathyroidism, and greatly attenuates vascular calcification (34).

In humans, cardiovascular calcification often occurs with advanced age, atherosclerosis, and diabetes mellitus. Several studies showed a higher prevalence of coronary plaques in dialyzed patients compared to the non-uremic population (10, 21). In addition, vascular alterations in CKD are associated with accelerated atherosclerosis and vascular stiffening, and left ventricular hypertrophy which are major causes of cardiovascular mortality in hemodialysis patients (3). Both accelerated atherosclerosis and mineralization of arterial intima and media caused arterial stiffness and hypertension (35).

Increases in serum phosphate and calcium-phosphate product levels are associated with arterial calcification in renal failure. Ongoing evidence demonstrates that calcium-containing phosphate-binders can increase calcium load. A new non-invasive imaging technology, electron beam computed tomography (EBCT), demonstrated that both hyperphosphatemia and increased calcium-phosphate product cause a progressive increase in calcium deposition in the coronary arteries, mitral and aortic valves in patients with advanced renal failure (10). A study of adolescents and young adult hemodialysis patients by Goodman et al (11) noted a correlation between coronary artery calcification detected by EBCT and duration of dialysis, serum phosphorus and calcium-phosphate product levels, and daily intake of calcium. Another study in 200 hemodialysis patients by Chertow et al (14) showed that sevelamer attenuated the progression of coronary and aortic calcification better than calcium-based phosphate binders after 12 months. Subjects treated with sevelamer had lower serum calcium, total cholesterol, and LDL levels compared to subjects treated with calcium-based phosphate binders.

It should also be kept in mind that a more efficient dialysis can be of great help in reducing or eliminating phosphate retention in dialysis patients. Minutolo et al (36) proved that hemodiafiltration is more effective than hemodialysis in removing phosphate, without differences in phosphate levels at the end of dialysis. Pierratos et al (37) showed that with long , nocturnal hemodialysis (performed six to seven nights per week for 8 to 10 h during sleep at home), weekly removal of phosphate was twice as high as conventional hemodialysis. Moreover, all patients discontinued their phosphate binders and increased dietary phosphate and protein intake. However, daily hemodialysis is very time-demanding for patients and is more expensive, so that at the moment it can be applied to a very limited number of subjects.

CONCLUSIONS

Hyperphosphatemia is an independent risk factor for higher incidence of cardiovascular events in patients with chronic kidney disease. In addition, increased phosphorus and calcium-phosphate product levels are important contributors to vascular calcifications in these patients. Also, it is well known how hyperphosphatemia accelerates the progression of secondary hyperparathyroidism, the concomitant bone loss and consequent calcium-phosphate precipitation. The control of serum phosphorus levels may reduce vascular calcification by decreasing calcium-phosphate product and reducing the active process through regulation of specific genes. Consequently, more efficient phosphate removal by dialysis, along with new calcium-free and aluminum-free phosphate binders have been proposed for the control of hyperphosphatemia in chronic renal failure. Recent studies have shown that new therapeuti-

cal tools may be useful to prevent vascular calcifications in animals and humans. Additional investigations are necessary to examine the relative effect of different phosphate-binders on mortality for cardiovascular events.

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