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Review Article

Association of Serum Phosphate and Related Factors in ESRD-Related Vascular Calcification

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Vascular calcification is common in ESRD patients and is important in increasing mortality from cardiovascular complications in these patients. Hyperphosphatemia related to chronic kidney disease is increasingly known as major stimulus for vascular calcification. Hyperphosphatemia and vascular calcification become popular discussion among nephrologist environment more than five decades, and many researches have been evolved. Risk factors for calcification are nowadays focused for the therapeutic prevention of vascular calcification with the hope of reducing cardiovascular complications.

1. Introduction

Vascular calcification is a kind of extraosseous calcification and is associated with aging physiologically, and a number of disorders including ESRD, diabetes mellitus, and cardiovascular disease pathologically. Multifactorial processes contribute to VC in which derangements in calcium and phosphorus homeostasis plays an important role and becomes popular therapeutic target nowadays. In ESRD patients with vascular calcification, a mixture of intimal and medial calcification has been observed in the effected vessels with dominant medial involvement. The risk of CVD mortality in ESRD patients with vascular calcification is 20 to 30 times higher than that of the general population [1–5].

Although phosphate is important for diverse cellular and physiological functions, impaired renal function with resultant phosphate accumulation with consequent bone and mineral disorders and vascular calcification are major problems among nephrologists. The increased risk of CVD mortality by hyperphosphatemia was partially explained by the predisposition of this population to vascular calcification [6–8]. (Figure 1) Even in early stage CKD, serum phosphorus level disturbances are proved to promote vascular calcification, hypertension, myocardial hypertrophy, and heart failure [9–11]. Current understanding of relationship between phosphorus and those disorders becomes popular in medical field, with the hope of halting or retarding the vascular calcification from the very early status in those patients.

2. Traditional Concepts in ESRD Patients with Vascular Calcification

2.1. Vascular Calcification in ESRD Patients. Vascular calcification (VC), an extra osseous calcification of arteries, is strongly associated with CKD patients with or without hemodialysis. Two types of VC include neointimal calcification, which occurs in large and medium-sized arteries, and medial calcification, which occurs in arteries of any size, including arterioles. VC is an important indicator of atherosclerosis, and its occurance directly predict prognosis of atherosclerotic disease [12]. Uremic atherosclerotic plaques are more calcified and fibroatheromatous than those in aging, with similar cellular infiltrates [13] and more of tunica media involvement [14, 15]. VC in ESRD patients, those especially found in the tunica media of large arteries, may lead to increased stiffening and decreased compliance of these vessels. Consequent increased arterial pulse wave velocity, pulse pressure, and impair arterial dispensability result

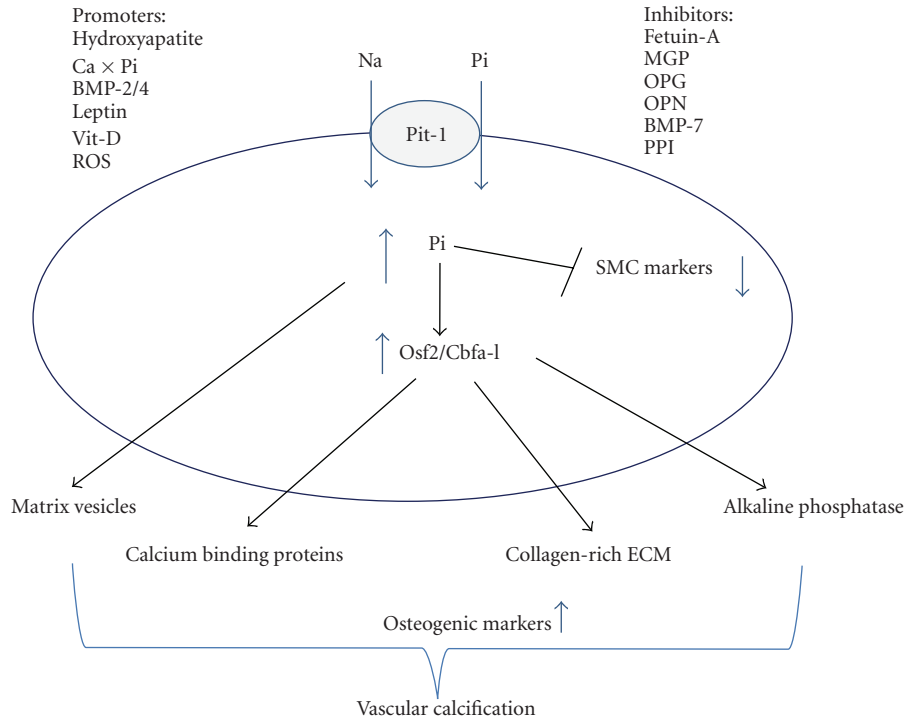

Figure 1: Mechanisms of VSMC osteogenesis during vascular calcification in chronic kidney disease. VSMC upregulate expression of transcription factors Osf2/Cbfa1 which were enhanced by ROS, leptin, vitamin D, increased CaxP product, or high PO_4 (Pi) levels induced by Pit-1. VSMC activation occurs in part as a result of the phenotypic switch of VSMCs into osteoblast-like cells. VSMCs that have acquired an osteogenic phenotype express ALP and produce hydroxyapatite crystals. Calcification inhibitors such as PPi inhibit hydroxyapatite precipitation, whereas fetuin-A, MGP, OPG, OPN, and BMP-7 antagonize calcification. VSMC: Vascular smooth muscle cells, Osf2/Cbfa1: Osteoblast-specific transcription factor, ROS: Reactive oxygen species, CaxP product: Calciumx phosphate produce, PO4(Pi): Phosphate, Pit-1: Sodium-phosphate cotransporter-1, ALP: Alkaline phosphatase, PPi: Pyrophosphate, MGP: Matrix Gla protein, OPG: Osteoprotegerin, OPN: Osteopontin, BMP: Bone morphogenic protein.

in increase afterload and left ventricular hypertrophy, which finally compromise coronary perfusion with development of congestive heart failure [7, 10, 16–19].

2.2. Risk Factors of Vascular Calcification in ESRD Patients. Risk factors for premature VC in ESRD patients are different from the traditional atherogenic risk factors. Hyperparathyroidism and alteration in Ca-P mineral metabolism, especially hyperphosphatemia, modulate renal osteodystrophy and vascular medial calcification [20, 21]. Microinflammation with chronically elevated acute phase protein CRP relates with intimal calcification and predicts the CV mortality. The presence of C. pneumoniae in arterial walls and atherosclerotic lesions also related this persistant infection with atherosclerotic vascular lesions in CKD with or without dialysis patients [13, 19]. Longer hemodialysis duration is also found to be significantly relate with severe vascular calcification [4, 5, 21, 22]. Furthermore, hyperglycemia and hyperphosphatemia are two most significant factors to be considered in ESRD patients with and without diabetes mellitus, respectively [23].

2.3. Role of Phosphate in ESRD with Vascular Calcification. Serum phosphate concentration is usually maintained within 2.5 to 4.5 mg/dL by a variety of mechanisms until renal disease has progressed to approximately CKD stage 5 or ESRD [24, 25]. Adaptation of nephrons in attempt to preserve phosphate homeostasis in ESRD patients plays an important role for VC. Hyperphosphatemia result in secondary hyperparathyroidism, calcium and vitamin D derangements, vascular calcification, and mineral bone disorders. Additionally, hyperparathyroid state and altered vitamin D status in ESRD patients also play a major role in extraosseous calcifications [1, 26–28]. Higher serum phosphorus levels may increase serum PTH levels even in healthy individuals [29]. Our previous study revealed serum PTH levels may stimulate inflammatory marker IL-6 production in HD patients [30, 31]. Higher levels of serum IL-6 and hsCRP also associated with increased VC and CVD risk. Higher serum phosphorus levels also inhibit 1,25-dihydroxyvitamin D synthesis [32, 33]. Lower 1,25-dihydroxyvitamin D status was associated with myocardial dysfunction [27, 34] and increased coronary vascular calcification [26, 27, 35]. The effects of vitamin D on vascular calcification are biphasic pattern with both excess, and deficiency may promote its development [17].

2.4. Mechanism of Vascular Calcification. Vascular calcification involves two distinct events, smooth muscle cell

transformation and mineralization. Normally, blood vessels express inhibitors of mineralization, like pyrophosphate, matrix Gla protein, fetuin A, and loss of these inhibitors may result in spontaneous vascular calcification. Fetuin A also plays a role in inhibition of CaxP precipitation [36].

The presence of bone-like tissue including osteoblast-like cells and hematopoietic elements within atherosclerotic plaque suggest VC as osteogenic differentiation of vessel wall cells [37–40]. A signal from AS plaque induces expression of potent osteogenic differentiation factor BMP-2a, with bone matrix and calcium hydroxyapatite crystals deposition in the arterial wall cells [41, 42]. Osteoprotegerin (OPG) [43, 44] inactivates the osteoclasts by blocking RANK activation. In dialysis patients, serum OPG levels are associated and predict progressive vascular calcification especially when CRP level is increased. Unregulated, degenerative calcification process within advanced AS plaque is progressive and severe in ESRD patients.

Hyperphosphatemia plays a very important role in ESRD related arteropathy [6, 7, 45]. In hyperphosphatemic environment (P > 2.4 mM), the vascular smooth muscle cell culture systems revealed both osteochondrogenic phenotypic change and mineralization through sodium-dependent phosphate cotransporter, Pit-1 [39, 46, 47]. There was loss of smooth muscle-specific gene expression and upregulartion of bone differentiation genes that translate into differential factors (Osteocalcin, osteopontin and Runx2, etc.), with the resultant osteogenic differentiation. Calcium deposition also occurs in prolonged hypercalcemic (>2.6 mM) environment. Calcium increases Pit-1 mRNA levels, increases smooth muscle cell sensitization to phosphorus, and results in osteoblastic differentiation. Elevated serum calcium was also associated with elevated CaxP product, altered alkaline phosphatase, and decreased marix Gla protein, which together play a role in VC [48].

Recent study revealed that calcium phosphate deposition may trigger the osteogenic changes. Calcium phosphate deposition (CPD) occurs as a cellular independent phenomenon which depends on calcium, phosphate, and hydroxyl ions concentration, but not on CaxPi concentration products [49]. A mouse lacking the VC inhibitor matrix-Gla protein had spontaneous extensive extraosseous calcifications despite normal Ca and P concentrations [50]. Mineralization is actively inhibited and prevented in the arteries. The loss of calcification inhibitors and CPD may lead to specific osteogene expression and VSMC differentiation and finally result in vascular calcification [49].

A genetic mechanism of hyperphosphatemia on vascular calcification was cited by Wu-Wong et al. in 2007. Elevated P modulates through VDR mRNA stability and PPARγ-mediated gene expression may lead to its detrimental effects including vascular calcification [20].

The calcium-sensing receptor (CaSR), which is expressed in vascular wall, plays key role in inhibition of vascular calcification in patients with chronic kidney disease (CKD). After CaSR stimulation, many intracellular signaling events occur via MEK1/ERK1,2 and PLC pathways and lead to proliferation of vascular smooth muscle cells. CaSR-mediated PLC activation is important for SMC survival and protection against apoptosis [51, 52]. Calcimimetic agents act on CaSR increasingly known nowadays for treatment of SHPT and CKD-MBD. Calcimimetics activate vascular calcium-sensing receptor and modulate the expression of VC inhibitor proteins like matrix Gla [53]. Calcimimetics were shown to have better plasma PTH controlling than calcitriol in uremic rats [54]. It also revealed that combination of Calcimimetics with calcitriol prevents VC and reduced mortality. Apart from controlling serum PTH levels, these agents also reduce calcium (Ca), phosphate (P), and CaxP product levels, which is more beneficial than other calcium containing phosphate binders and vitamin D analogues [53, 54]. These studies revealed that those agents may even reverse some vascular abnormalities in CKD.

3. Contemporary Concepts in ESRD Patients with Vascular Calcification

3.1. Role of Inflammation and Oxidative Stress. HD patients with secondary hyperparathyroidism may be under an environment of increased serum inflammatory cytokines and oxidative stress [30, 55]. High PTH levels, inflammation, and oxidative stress may involve together in CKD-MBD and cardiovascular calcification in SHP patients. Our recent study showed calcitriol can effectively suppress PTH secretion and reduce inflammatory markers including CRP and IL-6 [31]. While using vitamin D analogues in CKD patients, we have to note that, on one side, with increasing survival benefit, and on the other side, with some risks of vascular calcification. Thus, vitamin D in its form of less hypercalcemic agent paricalcitol, combination therapy with calcimimetic agents is becoming popular nowadays. Zhao et al. [56] demonstrate recently that increased mitochondrial membrane potential may lead to intracellular and mitochondrial reactive oxygen species (ROS) stimulation, with resultant superoxide production, and increased oxidative stress. These ROS mediate p65 nuclear translocation which is found to associate with phosphate-induced VC [56]. Osteogenic differentiation factor bone morphogenic protein-2a (BMP-2a), a well-known mediator of VC, is nowadays found to be a molecular link between oxidative stress and arterial stiffness due to vascular calcification [41, 57].

3.2. FGF23-Klotho Axis. The fibroblast growth factor–23 (FGF-23) and klotho genes are growingly known to regulate phosphate homeostasis recently [58]. Fibroblast growth factor-23 inhibits proximal renal and intestinal phosphorus absorption and klotho gene express in renal distal convoluted tubules and parathyroid gland. In mice, deletions of klotho and FGF-23 genes found to result in hyperphosphaemia, vascular calcification, arteriosclerosis, elevated $1,25(OH)_2D$, and osteopenia.

Patients with CKD have elevated FGF-23 levels early in the course of disease before clinically significant hyperphosphatemia occurs. Increased circulating FGF-23 target the remnant nephrons, enhance phosphate excretion, and inhibit $1,25(OH)_2D_3$ production. Thus, frank hyperphosphatemia does not develop until GFR < 30 mL/min. FGF-23 concentrations directly correlate with renal function. Higher

FGF-23 levels indicate rapidly progressive CKD, and also predict mortality in HD patient, thus, FGF-23 level may become important as creatinine level in kidney function assessment in future [59]. FGF-23 in earlier CKD increase phosphate excretion and prevent the development of vascular calcification. Higher FGF-23 also lead to reduced serum levels of $1,25(OH)_2D$ in early CKD despite high PTH. Its phosphaturic and hypovitaminosis D effects are more important than PTH in earlier stages of CKD. The resultant low serum $1,25(OH)_2D$ and hypocalcemia enhance PTH secretion and progress to secondary hyperparathyroidism. FGF-23, together with cofactor klotho, act on FGF receptor in parathyroid gland and reduce its secretion. But, in later stages of CKD, FGF23 resistance and hyperphosphatemia lead to secondary HPTH despite high FGF23 levels. Due to progressive nephron loss, the number of nephrons responds to FGF-23 reduced and phosphate retension occurs in late stage CKD. Abnormally high FGF23 levels in later stages of CKD lead to vascular dysfunction, left ventricular hypertrophy, and early mortality. Aortic calcification can be predicted by measuring FGF-23 level in hemodialysis patients [60]. Phosphate binders or use of long-acting PTH analogs to reduce intestional phosphate reabsorption in earlier stages CKD may reduce FGF-23 production and prevent those complications. In future, FGF-23 may be an important therapeutic target in management of CKD and hyperphosphatemia.

3.3. Role of Metabolic Acidosis. Metabolic acidosis which is very common in CKD patients may lead to bone dissolution by osteoclast activation and osteoblast inhibition [61, 62]. Our studies prove that acute correction of metabolic acidosis improves osteoblast function, increases $1,25(OH)_2D_3$ levels, and attenuates circulating PTH activity in chronic renal failure patients, and it underlines the importance of maintaining normal acid-base homeostasis in chronic renal failure [63–65]. Mendoza et al. [66] hypothesize an assumption that the response of extraosseous calcification to metabolic acidosis may be similar to that of bone, and, given that metabolic acidosis impairs bone mineralization, it is likely to attenuate the extraosseous calcification processes. They investigate the in vivo effect of metabolic acidosis on the development of vascular and other soft-tissue calcifications in a rodent model of uremia. Their results show that metabolic acidosis eventually prevents the development of calcitriol-induced extraskeletal calcifications in uremic rats, even with elevated plasma Ca and P levels. Metabolic acidosis plays a complex mechanism in VC, including stimulating of the solubility of Ca-P deposits, suppressing parathyroid secretion, inhibiting some osteogenic enzymes, blocking bone matrix formation, modulating the upregulation of Pit-1, and finally blocking phosphate uptake by the arterial smooth muscle cells [61].

3.4. Role of Adipocytokine Leptin. Increased BMI and abdominal obesity are well known to be related with metabolic syndrome and cardiovascular complications. Mediators released from adipose tissue are recently shown to be playing a role in promoting CVD. It has been shown in our previous study that adipocytokine leptin level is a marker for body adiposity in hemodialysis patients [67]. Studies conducted by Zeadin et al. [68] and Martin et al. [69] showed that a positive correlation between plasma leptin levels and CAC. It has been demonstrated that leptin promote osteoblastic differentiation of vascular smooth muscle cells and, thus, possibly lead to vascular calcification.

3.5. Role of Salivary Phosphate. In uremic patients, hyperphosphatemia is found to be poorly controlled even with the use of phosphate binders and dietary phosphate limitation in addition to dialysis. Savica et al. [70] found the role of salivary phosphate in worsening hyperphosphatemia in CKD patients. The salivary phosphorus ratio in dialysis patients was more than two times compared with healthy controls, and salivary phosphorus was five times higher than serum phosphorus in those patients. Thus, salivary phosphate binders could be an efficient approach in treating hyperphosphatemia in those patients.

3.6. Role of Pyrophosphate. Recent data suggest that deficient in pyrophosphate (PPi), a potent inhibitor of vascular calcification, may increase the medial vascular calcification in advanced kidney disease [71]. Levels of PPi are reduced in hemodialysis patients [72]. Hydrolysis of PPi is increased in aortas from uremic rats because of upregulation of alkaline phosphatase, providing a mechanism for vascular deficiency of PPi. Therefore, the data suggest that exogenous PPi may be useful in treating or preventing uremic vascular calcification. O'Neill and colleagues [73] show in uremic rats that systemic administration of pyrophosphate prevents or reduces uremia-related vascular calcification, without overt negative consequences for bone and without calcium pyrophosphate deposition disease. These findings prompt further research into the potential of pyrophosphate as treatment for vascular calcification in chronic kidney disease patients.

3.7. New Concept on Measures to Halt Phosphorus-Related Vascular Calcification. Factors that relate with vascular calcification process in CKD patients include phosphorus activation of the Pit-1 receptor, bone morphogenic proteins 2 and 4, leptin, endogenous 1,25 dihydroxy vitamin D, vascular calcification activating factor, and measures of oxidative stress. These entities may become future targets for diagnosis and treatment since standard hydroxymethylglutaryl-CoA reductase inhibitors have been shown to be failing to attenuate the progressive VC in those patients.

Dietary and therapeutic phosphorus control stands out as an important and basic step of preventing progressive VC in CKD patients. Dietary phosphorus control plays an important regulatory role in mineral homeostasis. Phosphorus is absorbed throughout the intestines and via 2 separate processes, a sodium-independent paracellular pathway and sodium-dependent carrier-mediated transcellular pathway (NaP-IIb) [74]. Low dietary phosphorus level may increase that type II b Na-P cotransporters protein expression on enterocytes with increasing P absorption. The percentage of dietary absorption varies with intake. At high levels of

intake (>10 mg/kg/day), approximately 70% of the ingested P is absorbed, whereas at lower levels of intake, as much as 80%–90% may be absorbed. Net intestional P absorption may be somewhat lowered in patients with ESRD. Inorganic phosphate is an important P which is found mostly in food additives like enhanced meat products, cereal and snack bars, flavored waters, and frozen meals, with 90% to 100% absorption. Thus, the principle of Pi management in ESRD patients is to restrict dietary Pi intake to 1~1.4 g/day, usage of phosphate binders as needed, and if still noncompliant can increase the dialysis time (Nocturnal HD) and frequency (Short Daily HD) with target levels of Pi < 4.6 mg/dL in CKD stage 3,4 and 3.5–5.5 mg/dL in CKD stage 5.

With concept of insufficient phosphorus removal by conventional hemodialysis, phosphate binders are taking to reduce gastrointestional phosphate absorption by virtually all hemodialysis patients. Due to potential risk of VC with calcium overload, Calcium-based phosphate binders are nowadays replaced by non-Ca-based ones. Sevelamer HCl [75–77] and Lanthanum carbonate [78–80] are the non-Ca-based phosphate binders well known nowadays with beneficial effects on vascular calcification. Sevelamer found to exert many benefits on VC by increasing fetuin-A levels, reducing systemic inflammation and modulating lipid profiles [81–83]. Efficacy and safety of new non-calcium, iron-based phosphate binder SBR759 was studied by Block et al. in a phase I clinical trial. Risks of iron accumulation and hypocalcemia were addressed but appear to be well tolerated [84].

Pyrophosphate, serve as an endogenous inhibitor of calcification and prevent hydroxyapatite formation in the vessel walls [85–87]. Pyrophosphate stands important defense against calcification in vessels without adverse effects on bone [73]. Thus, pyrophosphate becomes recent popular medication research in progress. Nitrogen-containing bisphosphonates (NCBPs) (e.g., ibandronate, alendronate, risedronate, and zoledronate) may inhibit the cardiovascular calcification [88, 89]. Etidronate may also reduce chronic inflammatory response and decrease OPG concentrations with the resultant decrease in vascular calcification in dialysis patients [90].

Niacin lowers serum phosphate and increases HDL cholesterol in dialysis patients. Niaspan (prolonged-release nicotinic acid) lowers serum phosphate and increases HDL cholesterol in dialysis patients [91, 92]. Patients with ESRD often have markedly elevated salivary phosphate concentration, independent of food content. A recent study on thirteen HD patients with serum phosphate levels > 6.0 mg/dL chewed 20 mg of chitosan-loaded chewing gum twice daily for 2 wk at fast in addition to oral compound found to be a useful approach for improving treatment of hyperphosphatemia in HD patients [93]. An experiment on ammonium chloride on uremic rats having a high phosphorus diet and calcitriol treatment may prevent vascular calcification [66]. This explains that the alkaline pH environment which occurs during regular HD even for a short time may lead to vascular calcification [94]. Thiosulfate, previously taken for urolithiasis events, nowadays seem to reduce vascular calcification [95]. Additional clinical trials on Thiosulfate are still underway [96]. Vitamin K analogues serve as cofactors for gamma-carboxylation of proteins including coagulation factors and matrix gla protein (MGP). MGP regulate vascular calcification, and its deficiency may lead to severe VC and CVD [50].

4. Conclusion

Vascular calcification started with extracellular phosphate regulation of vascular smooth muscle cell osteogenic differentiation and calcium phosphate mineralization. Generalized inflammatory condition in ESRD patients also suggested playing as an important factor in VC. FGF23-Klotho axis in regulating serum P levels and their roles in CVD become uncovered recently, while newer concepts of PTH and vitamin D are still evolving. It is increasingly accepted that factors promoting VC and inhibiting VC are in balance with excess of any one may relate with adverse effects in CKD-MBD and VC. Management trends are shifting from treating VC in older days to preventing VC in the new era. For the last, but not the least, the main target for all those efforts is to improve survival and life quality of these ESRD patients.

Authors' Contributions

C.-M. Z. and K.-C. L. contributed equally to this work.

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