LĖTINE INKSTŲ LIGA SERGANČIŲ VAIKŲ KRAUJAGYSLIŲ KALCIFIKACIJA IR KALCIFIKACIJOS PROFILAKTIKA

VASCULAR CALCIFICATION AND CALCIOPROPHYLAXIS WITH CKD IN CHILDREN

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ABSTRACT

Cardiovascular disease (CVD) is a major contributing factor of morbidity and mortality in chronic kidney disease (CKD) patients. Increased burden of CVD is partly due to damage to the arterial vessels by the uremic environment. Purpose of this article is to outline main calcification mechanism taking place and address each of the occurring biochemical and mineral imbalances in CKD population. In end stage renal disease (ESRD) calcification promoters and inhibitors intertwine with each other resulting in vascular calcification, a subject of great complexity. Vascular calcification share many similarities to bone ossification and vascular smooth muscle cells (VSMCs) are crucial to the process. Steps to undergo osteochondrocytic conversion are explained as well as biomarkers possibly postponing or reversing the process. Data from clinical trials, animal model in \textit{in vivo} and \textit{ex vivo} human vessel models are used to represent vascular calcification and it’s contributing factors.

INTRUDUCTION

Arterial remodeling is described as changes in the vessel’s wall in response to stimuli. Arterial wall structure alterations can be induced by (I) direct injury, (II) atherogenic factors and/or (III) changes in hemodynamics\(^1\). There are two main types of arterial wall calcification – intimal and medial. Intimal calcification is known as atherosclerotic vascular disease, in which atherosclerotic plaques are formed by macrophage and vascular smooth muscle cells (VSMC’s) in sites of lipid accumulation induced by endothelial damage\(^2,3\). Medial calcification or Monckeberg’s sclerosis is a form of tunica media calcification with concentrating vessel wall thickening without changes in intima. Arterial remodeling in ESRD encompasses broader spectrum of underlying mechanisms such as not obstructive arterial remodeling, altered hemodynamics and metabolic abnormalities associated with chronic uremia\(^4\). While atherosclerotic calcification increases vascular stiffness which leads to systolic hypertension and left ventricular hypertrophy, arteriosclerotic lesions escalate risk of ischemic heart disease and its complications. “Classical” and CKD related risk factors often mix together making it difficult to determine type of calcification and therefore possible clinical outcomes in adults. Pediatric patients “lack” traditional atherosclerosis risks and as a result are suitable for vascular calcification study population.

Hemodynamic alterations in CKD

Main structural arterial wall alterations induced by hemodynamics are (I) changes in arterial lumen and/or arterial wall thickness and (II) rearrangements of cellular elements and extracellular matrix of the vessel wall\(^1,5\). Acute changes induce vasomotor adjustment, while chronic changes cause changes in vessel geometry and structural composition. Characteristic of remodeling is determined by (I) nature of...
hemodynamic stimuli and (II) presence of intact endothelium. Blood pressure is main culprit determining arterial wall stretch and is one of the contributors that drives arterial remodeling. Data have shown that constant increase of arterial blood flow proportionally increases vessel lumen, while decrease of flow reduces inner diameter. Mechanical forces cause remodeling of the sensor cells which transmit mechanical force into effector cells. Endothelial cells are the main candidates for such a role. Some factors such as age, sex, mean BP are non-specific while others such as high blood flow velocity and systemic blood flow rate are more specific for ESRD. Chronic volume overload (anaemia, sodium and water retention etc.) is common in this population, and although not being the driving force of arterial calcification, it still “helps” to create perfect starting conditions for arterial remodeling and letting biochemical and mineral substances (calcium, phosphate PTH, FGF23 etc.) imbalance promote and drive calcification further.

Predicting calcification

Intima media thickness (IMT) and pulse wave velocity (PWV) measurement are methods of detection of arterial wall remodeling. Both of them respectively can be used to assess arterial changes in vivo, although data for using these methods in CKD patients are scarce. Even though not invasive, in routine clinical practice they are still rarely used. Carotid IMT (cIMT) detectable changes already are seen in predialysis patients. Systolic blood pressure (SBP) and serum phosphate are strong predictors of increased IMT, other factors are serum calcium and 25-hydroxyvitamin D concentrations. PWV predictors include SBP, 25-hydroxyvitamin D, and Parathyroid hormone (PTH). Age matched controls are not used in the trials. Height matched controls are considered more reliable, especially in pediatric patients. IMT and PWV stabilization or even reversal can be achieved with renal transplantation, this is contributed to calcium and phosphate excess removal from the blood, as well as normalization of BP. Removal of uremic environment may contribute to the increase of calcification inhibitors such as Fetuin-A, which plays a role in arterial remodeling, although further investigations are required. As studies show increased cIMT is associated with dislipidaemia, hypertension and being overweight or obese compared with healthy controls (Figure 1). Previous studies pointed out that arterial modeling is as well related to PWV. As arterial wall stiffness increases, blood pressure and pulse pressure tends to rise. IMT and PWV are important tools providing evaluation to ongoing vascular calcification as well as cardiovascular risk.

**Calcium and phosphate induced calcification**

Epidemiological studies showed relation between mineral imbalance in CKD patients and calcification. In the past it was believed that high serum calcium and phosphorus levels lead to passive accumulation on arteries. However, recent studies have shown that vascular calcification is cell mediated process which resembles bone formation and vascular smooth muscle cells (VSMC’s) play considerable role in the process. VSMC’s, fibroblasts, mesenchymal cells have the potential to differentiate into osteo/chondrocytic cells. Conversion to osteocytes and osteochondrytic cells is regulated by the same factors that mediate bone formation. In uremic environment calcification inhibitors are kept to the minimum, whereas calcification promoters are in abundance. Calcium and phosphorus have direct effects on VSMC’s, although together they accelerate calcification by forming hydroxyapatite nanocrystals. The mechanism consist of: (I) increased phosphate concentration in VSMCs; (II) high serum calcium downregulation on calcium sensing receptor (CaSR) expression; (III) VSMC apoptosis and (IV) Calcium-phosphate nanocrystals undergoing phagocytosis. Phosphatase concentration in VSMCs increases as it travels through concentration dependant sodium cotransporters in time and concentration-dependant manner. Calcium is required for VSMC contractility and its concentration is regulated by CaSRs as well as voltage gated calcium channels. CaSR function is to regulate myogenic tone in vessels. Data have shown that decrease of CaSR function results in increased calcification while elevated receptor sensitivity leads to opposite. Phosphate helps to activate osteochondrytic genes within VSMCs, after which production of alkaline phosphatase (ALK) is initiated that consecutively inactivates osteochondrytic mineralization inhibitors such as pyrophosphates and osteopontin resulting in free phosphate release. High calcium environment causes VSMCs to apoptosis, and as a result releases even more calcium and subsequently cell death.

![Figure 1. Box-percentile plots illustrating the distribution of cIMT measurements in 101 Children of the CKiD and in 97 healthy controls. The numerical values of the 50th percentile are shown. cIMT, carotid artery intima-medial thickness; CKiD, Chronic Kidney Disease in Children study. Adapted from Brady et al., 2012.](image-url)
follows\textsuperscript{10}. Formed calcium-phosphate nanocrystals undergo lysosomal phagocytosis and degradation inside VSMCs resulting in high intracellular Ca levels which leads to cell death. In this manner, vicious cycle is formed\textsuperscript{10}.

**PTH impact**

PTH directly regulates calcium and phosphorus concentrations and is one of main agents determining calcification and metabolic bone disorders (MBD’s) as well as independently predicting mortality\textsuperscript{13}. One out of 6 children undergoing peritoneal dialysis have radiological and/or clinical MBD signs. In pediatrics it’s essential to maintain positive calcium balance because of undergoing bone development and growth. PTH concentrations are strongly determined by residual renal function, dialysis intervals, gender, pubertal status and serum bicarbonate. Acidosis due to low serum bicarbonate levels causes PTH levels to rise. It showed that severe hyperthyroidism occurred 10% higher with each year on peritoneal dialysis\textsuperscript{16}. Serum PTH levels in CKD patients are subject of ongoing debate. Kidney Disease Outcomes Quality Initiative (KDOQI), European Pediatric Dialysis Working Group (EPDWG) and Kidney Disease: Improvement of Global Outcome (KDIGO) all suggest specific ranges of serum PTH levels, although these ranges differ significantly\textsuperscript{17–19}. Such differences arise from trying to balance calcium concentration between two extremes; high PTH leads to extraosseous calcifications and low PTH causes impaire longitudinal growth as well as osteopenia. Current pediatric guidelines focus is avoiding extraosseous calcifications thus promoting mild to moderate hyperparathyroidism in order to maintain low normal serum calcium as well as inorganic phosphate. However all current consensuses operate on limited evidence. KDOQI recommend target values of 200–300 pg/ml while KDIGO go as high as nine fold the upper normal limit. Borzych et al. analysis revealed significant numbers of patients with MBD complications while their PTH concentration was within recommended target values. This study also suggested PTH interval in which lowest complication rate (extraosseous calcifications as well as osteopenia) was observed (figure 2). While debate about recommended values continues, PTH remains as one of the contributing factors in process of MBD and vascular calcification.

**Effects of vitamin D supplementation**

Vitamin D and its analogs are commonly used as a treatment of secondary hyperparathyroidism in ESRD. All the supplements work through activating Vitamin D receptor activators (VDRA’s). VSMC’s have 1 and 25 $\alpha$-hydroxylase enzyme systems and also express VDRA’s, enabling vitamin D supplementation usage inside cells. Doxerciferol, pericalcitol, alfalcacidiol and calcitriol are usual pharmaceutical choices\textsuperscript{17}. In healthy persons with normal kidney function inverse correlation between vitamin D and vascular calcification degree was shown\textsuperscript{20}. Although activation of VDRA’s should promote calcification there is conflicting data supporting the claim that bimodal effect exists as shown in study by Shroff R. et al.\textsuperscript{21} (figure 3). However one must consider the fact that in patients with ESRD supraphysiological doses of vitamin D are administered. It has been demonstrated that independently of P and Ca different level of VDRA’s can cause different effects\textsuperscript{22}. Abnormal effects on vasculature are caused by either too high or too low levels of vitamin D metabolites\textsuperscript{21}. It is discussed – which
VDRA should be used? No studies so far evaluated effects on calcification. KDIGO recommends to start analogs only when PTH is persistently rising and remains above the normal limits, while KDOQI recommendations being more specific – therapy should be started when serum 1,25(OH)D are >30 ng/ml and PTH levels are above recommended depending on CKD stage (table 1). The effect of vitamin D metabolites on VSMC’s has not been analyzed yet. The primary goal for starting these medications are to keep serum calcium, phosphate and PTH in their respective target ranges while preserving bone growth, vascular calcification being second concern.

**Asociation with FGF23 and s-klotho.**

Fibroblast growth factor 23 (FGF23) is a bone derived protein hormone which is responsible for phosphate metabolism via renal phosphate excretion and inhibiting 1α-reductase and thus reducing 1,25(OH) vitamin D production. Two forms of klotho exist: one is multifunctional transmembrane protein of 1 glycosidases family (MW 130 kDa) which is expressed mainly in renal tubules and choroid plexus of the brain; the other is unbound, free (soluble klotho; s-klotho). S-klotho regulates 1,25(OH) vitamin D production as well as phosphate, calcium and potassium excretion through kidneys. In CKD and dialysis patients decreased klotho levels are associated with increased FGF23 levels. FGF23 association was shown with left ventricular hypertrophy, impaired left ventricular function, vascular calcification, heart failure, CKD progression and mortality in adult patients. Animal study has shown that transgenic mice who had overexpression of klotho with induced CKD had preserved their levels of klotho compared to control groups and furthermore had better kidney function due to enhanced phosphaturia, and direct inhibition of phosphate intake by VSMC’s. It has been demonstrated that even relatively small renal function decline causes 100 fold FGF23 increase. It has shown that at the sites of calcification VSMCs undergo osteochondrocytic change due to increased expression of mineralization regulating proteins which usually are restricted to bone and cartilage. Duer et al have put speculations to an end revealing that bone and ectopic vascular calcification is essentially the same and indistinguishable and that mineralized plaque in vessel wall is composed of hydroxyapatite crystals (Figure 4).

From clinical point FGF23 and klotho have the potential to show early renal function decline, or even stop/regress artery calcification and lower cardiovascular morbidity.

**Vascular and bone calcification**

In the areas where calcification is mandatory resident cells have specific mechanisms enabling calcification in the extracellular matrix. VSMCs are type of mesenchymal cell that can differentiate to different mesenchymal cell types (osteoblasts or chondrocytes) under specific conditions which arise in CKD related uremic environment. Under normal circumstances VSMC calcification is prevented by mineralization inhibitors such as MGP or Fetoin-A. Nonetheless, when inhibitors are low and Ca levels are high VSMCs produce mineralization competent vesicles which contain hydroxyapatite. Vesicles also contain alkaline phosphatase which degrades pyrophosphatase, thus enabling hydroxyapatite crystal growth by creating phosphate source. Raised ALK levels are found in dialysis patients as well as in calcific uremic arteriopathy. It has shown that at the sites of calcification VSMCs undergo osteochondrocytic change due to increased expression of mineralization regulating proteins which usually are restricted to bone and cartilage.

### Table 1: Target Range of Intact Plasma PTH by Stage of CKD

<table>
<thead>
<tr>
<th>CKD Stage</th>
<th>GFR Range (mL/min/1.73 m²)</th>
<th>Target “intact” PTH (pg/mL [pmol/L])</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30-59</td>
<td>35-70 [3.85-7.7 pmol/L] (OPTONION)</td>
</tr>
<tr>
<td>4</td>
<td>15-29</td>
<td>70-110 [7.7-12.1 pmol/L] (OPTONION)</td>
</tr>
<tr>
<td>5</td>
<td>&lt;15 or dialysis</td>
<td>150-300 [16.5-33.0 pmol/L] (EVIDENCE)</td>
</tr>
</tbody>
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Figure 4. Comparison of 13C spectra of mineralized plaque and bone. Adapted from Duer et al., 2008.
Changes in vessels *ex vivo* and *in vivo*

Studies *in vivo* have been performed on medium sized muscular arteries removed from dialysis patients as well as healthy individuals to show changes which take place under stressful conditions which arise in CKD\(^6\). Several clinical studies have suggested that intact levels of mineralization inhibitors protect predialysis patients from vascular calcification\(^{10,11}\). Calcium load in vessels extracted from dialysis patients was almost twice as high as in predialysis vessels and calcium load strongly correlated with the time of dialysis. It appears that high Ca x P environment is not sufficient to create conditions of accelerated calcification and exposure to dialysis provides medium for changes in vessel wall that enhances calcification. Performed histology and immunohistochemistry demonstrated that VSMCs loss is apoptosis attributed. Apoptotic cell death was not detected in predialysis patients with similar calcium load suggesting that dialysis related factors cause VSMC apoptosis. Hydroxyapatite laden vesicles containing MGP and Fetoin-A in deposited areas was observed as well. As VSMC death occurs it reduces the calcification inhibitors production\(^{15}\) as well as increase local concentrations of calcium\(^{13}\). In *ex vivo* study\(^{13}\) cultured vessel rings from predialysis, dialysis patients and healthy controls were exposed to high calcium or high calcium and high phosphorus media up to 21 days to mimic uremic conditions which arise in CKD. It was shown that healthy individual vessels were resistant to calcification even in highly calcifying environment. Contrary, predialysis and dialysis vessels revealed time-dependent calcification which was greater in dialysis group under identical conditions. Evidence suggests that damaged VSMCs and compromised inhibitory mechanisms prepare vessels for accelerated calcification. In media with high calcium and high phosphorus calcification was greater than in media with high phosphorus, emphasizing potency of calcium to induce VSMC death and calcification. Findings of vessels with histologically overt calcification *ex vivo* are prone to greater calcium accumulation *ex vivo* and that after nidus of calcification forms it acts as further accelerator of calcification. Models display the importance of intact inhibitory systems which provide defense against detrimental uremic conditions while highlighting potency of accelerating calcification once it occurs. Mechanisms of calcification in VSMCs are shown in figure 5\(^{10}\).

CONCLUSIONS

As management of CKD becomes better, patients no longer die of renal failure and its direct consequences, but from cardiovascular disease and related complications. Vascular calcification begins in predialysis stage and on dialysis becomes more rapid. Early detection methods such as IMT and PWV provide some insight although are unable to differentiate between intimal and medial change. Vessel biopsy models provide important mechanistic insights demonstrating main processes taking place in calcification. Although multiple factors causing calcification are known, mineral imbalance, specifically calcium and phosphate, plays key role in initiation and progression. Treatment options for imbalances are available, although some of the guidelines require further research and clarity. Vasculature remains difficult treatment target and while vitamin D analogs remain a therapeutic option, renal transplantation is still the solution and most effective option in stopping or even reversing calcification. Calcification inhibitors in time may emerge as treatment choice in preserving normal vasculature function, meanwhile mineral imbalance management remains main focus point in CVD prevention.

LITTERATURE

5. Zarins CK, Zatina MA, Giddens DP, Ku DN, Gladov S. Shear stress regulation of artery lumen diameter in experimental ath-

![Figure 5. Mechanisms of calcification in VSMCs in culture and in intact vessel rings in predialysis and dialysis vessels. Shroff, Long, & Shanahan, 2013.](image-url)

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