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Coronary Artery Calcification and Cardiovascular Disease in Children with Chronic Kidney Disease

Sara Paoli, M.D.[1] and **Mark M Mitsnefes, M.D., M.S.**[2]

[1] University “SAPIENZA”, Department of Pediatrics, Pediatric Nephrology Policlinico Umberto I, Rome, Italy

[2]Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

Abstract

Purpose of the review—Cardiovascular diseasae is the leading cause of death in children and young adults with end-stage renal disease (ESRDS). As adults, children with advanced chronic kidney disease (CKD) have extremily high prevalence of traditional and uremia-related cardiovascular risk factors. Coronary artery calcification is one of the earliest cardiovascular markers detected in children with ESRD. The purpose of this review is to examine new development in pathogenesis of coronary artery calcification and to describe recently published studies on this topic in children with CKD.

Recent findings—There is growing evidence that fibroblast growth factor 23 (FGF23) and Klotho factor play a key role in the development of coronary artery calcification in ESRD. Recent studies have shown that induction of vascular calcification begins in early normophosphatemic CKD by reduction of vascular Klotho and increased FGF23 secretion. Pediatric studies confirmed the presence of abnormal FGF23 and Klotho metabolism and the association of increased circulating FGF23 with coronary artery calcification in children with CKD.

Summary—New development in our understanding of the mechanisms of vascular calcification in patients with early CKD require a further investigation whether control of FGF23/Klotho metabolism will prevent or delay the development of CC and other cardiovascular outcomes.

Keywords

cardiac calcification; FGF23; Klotho; chronic kidney disease

INTRODUCTION

Recent study of more than 20,000 children initiating dialysis therapy demonstrated that mortality rates in pediatric ESRD have improved dramatically between 1990 and 2010 [1**]. Despite this improvement, the overall mortality rates are still 30 times higher than in

Corresponding Author: Mark Mitsnefes, M.D. M.S. Nephrology and Hypertension Cincinnati Children's Hospital Medical Center 3333 Burnet Ave, MLC 7022 Cincinnati, Ohio 45229 (513) 636-4531 (phone) (513) 636-7407 (fax) mark.mitsnefes@cchmc.org.

Conflict of Interest None

the general pediatric population and cardiovascular disease remains the most common cause of death [1]. The likely reason for such a high cardiovascular mortality is that children with CKD have extremely high prevalence of traditional and CKD-related cardiovascular risk factors [2]. Early markers of cardiomyopathy, such as left ventricular hypertrophy and dysfunction and early markers of atherosclerosis, such as increased carotid artery intima-media thickness and carotid arterial wall stiffness are frequently found in this patient population, especially in those on maintenance dialysis. Coronary artery calcification, known early marker of increased cardiovascular mortality in adults with CKD, can also be seen in children with advanced CKD. In general population, elevated LDL-C and systolic BP levels during adolescence are independent predictors of adulthood coronary artery calcification [3]. In contrast, in children with CKD, disturbunces in mineral metabolism are the likely cause vascular calcification [4].

In a recent review, Shroff et al [5] summarized a model of intact human vessels from CKD and dialysis patients to study the earliest changes involved in initiation of calcification *in vivo*. Experiments using this model showed that ectopic vascular calcification is a very similar process to physiological bone formation. Under conditions similar to CKD (increased calcium-phosphorus product) , vascular smooth muscle cells (VSMC) undergo phenotypic transformation and upregulate expression of miniralization-regulating proteins that are normally restricted to bone and cartilage. Dialysis vessels also showed VSMC loss due to apoptotic cell death further increasing local concentration of calcium thus promoting vicious cycle of VSMC death, vesicle release and accelerating calcification. The most interesting and current development in our understandoing of the mechanisms of vascular calcification in CKD involves two relatively new factors: fibroblast growth factor 23 (FGF23) and Klotho. In the current review, we provide an update on the role of these factors in pathogensis of CC in patients with CKD.

Klotho, FGF23, and vascular calcification

FGF23 and Klotho serve as major regulators of phosphorus homeostasis in both health and CKD. The FGF23, a bone-derived hormone, is a potent phosphaturic hormone that increases the rate of urinary excretion of phosphate and inhibits renal production of 1,25 (OH)2D, thus helping to mitigate hyperphosphatemia in CKD patients [6]. There are two forms of Klotho. One is a multi-functional single-pass transmembrane protein that belongs to the family 1 glycosidases (MW 130-kDa), and is expressed primarily in the renal tubules of the kidney and the choroid plexus in the brain, and another is free in circulation (soluble Klotho) [7]. Klotho exerts multiple actions on the kidney including regulation of 1,25 Vitamin D3 production and modulation of urinary phosphate, calcium and potassium excretion [7]. Klotho's gene product functions as a coreceptor for FGF23. Currently, it is well-accepted from multiple animal models and human studies that elevated levels of FGF23 and decreased levels of Klotho are the earliest hormonal abnormalities involved in minearl metabolism in CKD [6, 7]. There is evidence emerging that disturbunces in Kloto –FGF23 metabolism are important contributor to vascular calcification in patients with CKD.

Lim et al for the first time described endogenous Klotho expression in human arteries and human aortic smooth muscle cells [8**]. Samples were collected from healthy people

donating a kidney and CKD patients undergoing a renal transplant. Immunohistochemistry analysis demonstrated Klotho protein expression in the medial layer of arteries from healthy individuals, but marked reduction in arteries from patients with CKD. Importantly, reduced vascular Klotho expression in CKD patients was associated with extensive medial calcification. Subsequent series of mechanistic experiments of Klotho knockdown animals demonstrated that 1) development of arterial calcification is through a Runx2 and myocardinserum response factor – dependent pathway; 2) vascular cells are Klotho-dependent target tissue for FGF23; 3) vitamin D receptor activators can restore procalcific stress, Klotho expression and unmask FGF-23 anticalcific effects. Authors concluded that local vascular Klotho is an endogenous inhibitor of vascular calcification and also serves as a co-factor required for vascular FGF23 signaling.

The results of this study were confirmed by Fang et al [9] who examined FGF23 and Klotho expression in an animal model of early CKD before evident hyperphosphatemia. The authors used the atherosclerosis-bearing low-density lipoprotein–deficient mouse (ldlr -/-) fed with high-fat, Western-type diets (40% of calories from fat). The CKD was induced using unilateral renal injury and contralateral nephrectomy. Vascular calcification and decreased expression of Klotho in the aorta was evident in mild CKD. As in the study by Lim et al [8], Runx2 was identified as a critial transcription factor in osteoblast transition in the aorta. The study also found elevated levels of another osteocytic biomarker protein, scerostin, implicated in bone turnover of CKD [10].

In another study, Hu et al [11] found increased vascular tissue calcium content in CKD animals and in Klotho deficient ($Kl^{-/-}$) animals. As expected, Klotho was undetectable in $Kl^{-/-}$mice (Klotho knock out mice). Importantly, Klotho was significantly decreased in kidney and barely detectable in the blood and urine of CKD mice indicating that CKD is a state of “pan deficiency” of Klotho. In another set of experiments, Klotho levels and their effects on vascular calcification were compared in wild (*WT*), $Kl^{-/+}$, and in transgenic (*Tg-Kl)* mice before and after CKD induction. Baseline Klotho was lower in $Kl^{-/+}$ mice comparing to *WT* and the highest in *Tg-Kl* mice. Despite induction of CKD, transgenic mice that overexpressed Klotho had preserved levels of Klotho, enhanced phosphaturia, better renal function, and much less calcification compared with wild-type mice with CKD. Conversely, Klotho-haploinsufficient mice with CKD had undetectable levels of Klotho, worse renal function, and severe vascular calcification. This study determined that Klotho ameliorated vascular calcification by enhancing phosphaturia, preserving glomerular filtration, and directly inhibiting phosphate uptake by VSMCs. The authors speculated that Klotho replacement therapy may be important in slowing progression of CKD as well as preventing and reversing vascular calcification and other CKD complications.

The role of FGF23 in inducing coronary artery calcification was questioned in study by Scialla et al [12]. Among 1501 patients enrolled in Chronic Renal Insufficiency Cohort (CRIC) study, 983 participants (65%) had prevalent coronary artery calcification and 693 (46%) had thoracic aortic calcification, each defined as an Agatston score of >0. Overall, there was no significant association between FGF23 and the prevalence or severity of coronary calcium. However, FGF23 was associated with the severity of thoracic aortic calcification in patients with nonzerro Agaston score. Interestingly, experimental data

demonstrated no expression of FGF23 in human or mouse VSCMs or in normal or calcified mouse aortas. In contrast, higher levels of serum phosphate were strongly associated with coronary artery calcification independent of FGF23 and elevated phosphate concentrations induced calcification in vitro. Authors suggested that while phosphate induces calcification, FGF23 may promote the progression, but not the genesis, of calcification in large vessels like aorta. Similarly, Lindberg et al showed no impact of FGF23 on vascular calcification and endothelial response in bovine VSMC and in a murine ex vivo model of endothelial function [13]. Finally, Jimbo et al showed that FGF23 enhances phosphate-induced vascular calcification by promoting osteoblastic differentiation involving the ERK1/2 pathway [14].

Pediatric studies: FGF23, Klotho, and coronary artery calcification in CKD

As in adults, similar changes in FGF23 and Klotho are observed in children with CKD. Recent pediatric study of 110 children with various stages of CKD showed that changes in FGF23 and Klotho levels are the earliest mineral abnormalities: even in the presence of well-controlled serum phosphorus, high FGF23 and low soluble Klotho levels are already seen [15*]. In this study, there was a strong inverse correlation between plasma FGF23 and estimated GFR in the total cohort including patients with pre-dialysis CKD, dialysis patients and patients with functioning graft; Klotho levels decreased with decreasing GFR in the CKD 1–5 and dialysis patients. Decreased Klotho levels were also associated with increased FGF23 levels in the CKD and dialysis patients. Transplanted patients also showed an inverse correlation between FGF23 and GFR but no association was found between Klotho and GFR. There are very few studies evaluating coronary calcification in children with CKD [2]. Most current studies have been performed by Srivaths et al [16-18]. In their initial study, coronary artery calcification was assessed by ultrafast computerized tomogram (CT) in 16 patients receiving maintenance hemodialysis for more than 2 months [16]. Five (31%) of 16 patients had coronary artery calcification. Patients with coronary artery calcification were older, had longer dialysis vintage, lower cholesterol and higher phosphorus and calcium-phosphorus product levels. Authors concluded that renal osteodystrophy control (high phosphorus and calcium-phosphorus product) and malnutrition (low cholesrterol) may contribute to coronary artery calcification development. Using stored samples of these patients, FGF23 level was measured and compared in children with and without CC [17]. Serum FGF23 was elevated in all 16 children on maintenance hemodialysis and was positively associated with serum phosphorus level. Children with coronary artery calcification had significantly higher serum FGF23 levels than children without coronary artery calcification. In a follow up study, the CT was repeated one year after the initial evaluation [18]. Three patients with initial coronary artery calcification progressed based on increased Agaston score; one patient developed new and none of the patients resolved coronary artery calcification. Not surprisingly, children with progressive coronary artery calcification had higher serum phosphorus and FGF23 levels. Interestingly, all but one patient with no calcification at baseline remained calcification-free. This suggests the presence of protective factors such as higher use of non-calcium based phosphate binders (sevelamer), genetic factors, and higher levels of calcification inhibitors. Since most of the patients without coronary artery calcification remained calcification-free at follow-up, the authors did not recommend routine screening by CT in all pediatric CKD patients, due to relatively high radiation exposure.

FGF23 and Klotho control as potential therapeutic target in CKD patients

Since a decrease in serum FGF23 is one of the earliest abnormalities of mineral metabolism observed in CKD, monitoring of this factor may be important in our understanding of the disease progression and potential therapeuticc target in clinical practice, even in early stages of CKD.

Lau et al [19] tested two vitamin D receptor agonists in a mouse CKD model where dietary phosphate loading induced aortic medial calcification. Mice were given intraperitoneal calcitriol or paricalcitol in physiological doses three times per week for three weeks. Treatment with these vitamin D receptor agonists was associated with half of the aortic calcification compared to no therapy. No difference between the two agents was found. Vitamin D receptor agonist therapy was associated with increased serum and urine Klotho levels, increased phosphaturia, correction of hyperphosphatemia, and lowering of serum FGF23. Interestingly, in the setting of a high phosphate diet, serum parathyroid hormone and calcium levels were not significantly altered by treatment. Study concluded that Klotho was upregulated by vitamin D receptor agonist therapy independent of changes in serum parathyroid and calcium.

Block et al [20] determined FGF23 and coronary artery calcification as secondary end-points in the randomized placebo-controlled pilot clinical trial of safety and efficacy of different phosphate-binders in patients with moderate CKD and normal or near-normal serum phosphorus concentration. Treatment with phosphate binders significantly lowered serum and urinary phosphorus and attenuated progression of secondary hyperparathyroidism, but it resulted in progression of coronary artery and abdominal aortic calcification, particularly in those randomized to calcium acetate arm. The effects on intact FGF23 were different by binder type: patients treated with sevelamer carbonate, a non-calcium based phosphate binder, experienced a significant decrease in intact FGF23 ($P=0.002$ versus placebo), whereas those patients treated with calcium acetate had a significant increase ($P=0.03$ versus placebo) and those patients treated with lanthanum carbonate were no different compared with placebo. The authors concluded that the safety and efficacy of phosphate binders in patients with CKD and normal serum phosphorus remain uncertain. To date, there have been no pediatric clinical trials in children with CKD assessing the effect of phosphate binders on FGF23 or Klotho levels.

Another study of adults on maintenance dialysis showed that values of FGF23 were lower in patients receiving short daily hemodialysis when compared with conventional hemodialysis (three times a week), despite greater use of vitamin D sterols and equivalent serum phosphorus and PTH concentrations [21]. The authors suggested that FGF23 levels may be a more sensitive biomarker of cumulative phosphate burden than single or multiple phosphorus determinations and better marker to assess dialysis adquacy than currently utilized Kt/V.

CONCLUSION

Cardiac calcification is present and progresses in children with advanced CKD. Animal and human studies determined that CKD is a state of Klotho deficiency and elevated FGF23, the

ealiest markers of abnormal bone metabolism and factors associated with inducing (Klotho) or progression of (FGF23) cardiac calcification. Future studies should determine if correction of Klotho and FGF23 is associated with regression of cardiac calcification and improving of cardiovascular outcomes in patients with CKD.

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Key Points

- Cardiovascular disease is the most common cause of death in children with advanced CKD
- Coronary artery calcification is frequent in children on maintenance dialysis
- Abnormal mineral metabolism is the likely cause of cardiac calcification in CKD
- Increased FGF23 and Klotho deficiency are early abnormalities involved in the development of cardiac calcification in CKD