Vascular Access Calcification and Arteriovenous Fistula Maturation

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Abstract: The vascular access serves as the "lifeline" for the hemodialysis patient, but in recent years has become the "Achilles Heel" of the hemodialysis procedure. The vascular health of the vessels used to create arteriovenous accesses is paramount to vascular access maturation and successful outcomes. Vascular calcification is widely present in the vessels of chronic kidney disease (CKD) and end stage renal disease (ESRD) patients, and may also potentially impact the vascular access remodeling process. Few studies have been published to date evaluating vascular calcification as it relates to vascular access outcomes. However, an improved understanding of how vascular calcification plays a pathophysiological role in hemodialysis vascular access dysfunction may improve therapies to treat arteriovenous fistula (AVF) non-maturation and the health of the vessels prior to vascular access creation.

INTRODUCTION

Vascular access dysfunction remains a major cause of morbidity and mortality in hemodialysis patients [1-4]. Arteriovenous fistulas (AVF) remain the preferred form of vascular access due to the lower rates of infection and maintenance procedures to treat stenosis compared to arteriovenous grafts (AVG), if the AVF successfully matures for hemodialysis. However, recent publications have reported a high proportion of non-maturing AVFs among our hemodialysis patients [5-7]. Furthermore, several recent publications have also demonstrated the presence of neointimal hyperplasia from patient veins and arteries collected at the time of new surgical access creation [8-13]. This suggests that the baseline vascular health of both the arteries and veins used to create new vascular accesses may play an important role in AVF maturation [1]. Vascular calcification has been demonstrated to play an important role in the cardiovascular mortality in CKD and ESRD patients [14], but with fewer published studies looking at its role in hemodialysis vascular access. In the context of AVF maturation, this review will; (1) discuss the pathophysiology of vascular calcification in CKD and ESRD, (2) highlight the major research studies evaluating vascular calcification in hemodialysis vascular access, and (3) identify future areas of research to better elucidate the role of vascular access calcification in hemodialysis vascular access dysfunction.

PATHOPHYSIOLOGY OF VASCULAR CALCIFICATION IN CKD

Cardiovascular events are the major etiology of mortality in ESRD patients [15]. Arterial vascular histologic changes

and remodeling occur in response to increasing hemodynamic alterations and increasing uremia from progressive CKD. The arterial vascular pathology associated with CKD and ESRD is commonly characterized by the presence of significant intimal and medial calcification [16]. In addition to CKD and ESRD, calcification has also been associated with increasing age, hypertension, diabetes, dyslipidemia, and smoking [17]. Thus, there has been a tremendous interest in developing therapies to reduce, minimize, and treat vascular development and complications, which may also have implications to vascular access creation and functional use [9].

Atherosclerosis and Arteriosclerosis in CKD and ESRD

Atherosclerosis is a process, whereby, the artery wall begins to thicken as a result of deposition of calcium, cholesterol, low-density lipoproproteins, and inflammatory cells, such as macrophages. When these products are not sufficiently removed and deposit within the vessel wall, the process of plaque formation and development (atheroma) initiates [16,18,19].

Calcification is an important part of the atherosclerosis process and generally develops within the intimal layer of the vessel wall in patients with kidney disease [16]. Atherosclerotic lesions are present in an intermittent pattern along the length of the artery leading to vascular stenosis and occlusions, and subsequently ischemia [16].

Arteriosclerosis refers to stiffening and loss of elasticity of the arteries. The process typically involves arterial intima and media thickening with or without significant atherosclerotic disease [16,20,21] and is frequently characterized by increased vascular wall thickness and lumen vasodilation (vascular remodeling). The consequences of adverse vascular remodeling, referred to as poor compliance or distensibility, lead to decreased ability of the artery to hemodynamically adapt to the large pulsatile flow that occurs during intermittent ventricular ejection [16,22].

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The clinical and functional significance of vascular calcification in CKD and ESRD patients is very significant and important. Vascular calcification can lead to a number, a histologic and structural changes to the artery which impact vessel hemodynamics as well as elasticity [16]. Guerin *et al.* have reported vascular remodeling, as measured by common carotid artery distensibility and aortic pulse wave velocity, to be proportional to the presence of vascular calcifications [16,17].

In a study by Ibels *et al.* [23], evaluating arterial histology from uremic patients, several key findings were reported: (1) uremic arteries demonstrated the presence of fibrous neointimal hyperplasia, calcification within the internal elastic lamina, medial elastic fibers, and damage and duplication of the internal elastic lamina [23], suggesting the predominance of vascular calcification within uremic vessels and providing further evidence for the loss of elasticity in CKD and ESRD and (2) the degree of neointimal thickness and vascular arterial calcium concentration correlated with the duration of uremia, suggesting acceleration of vascular arterial changes as a consequence of uremia [23]. These changes to the vasculature from uremia and complications of advanced CKD may potentially impact vascular access maturation and development of future stenosis.

Biology of Vascular Calcification in CKD and ESRD

The mechanisms of vascular calcification in CKD and ESRD remain an active area of research. In this section, the mechanisms of vascular calcification will be briefly described. Vascular calcification is the end result from an imbalance between cellular mediators which promote and inhibit mineralization. In addition to proper homeostasis of vascular mineralization, the current research to date has demonstrated that one major mechanism involved in regulation of vascular calcification is vascular smooth muscle cell (VSMC) damage [24]. Normally, VSMCs play an important role in inhibiting calcification, but in pathologic environments such as kidney disease their normal function is compromised and they develop osteogenic phenotypic changes that favor deposition of minerals that leads to calcification [24]. It is also hypothesized that when VSMCs become apoptotic, they release apoptotic bodies which accumulate calcium and initiate the calcification process [24-26]. Furthermore, VSMCs have been reported to bud matrix vesicles from their plasma membranes [24,26,27]. These small membrane bound particles form a microenvironment capable of concentrating calcium and phosphate and allowing for crystal nucleation [24,26,27].

Expression of osteogenic markers such as matrix γ-carboxyglutamic acid protein (MGP) [28,29], osteopontin (OPN) [30], and bone morphogenetic protein-2 (BMP-2) [31], by VSMCs has been described to be associated with vascular calcification *in vivo* [24]. Temporal gene expression studies from normal and calcified arteries, which include arteries from uremic patients, have reported that VSMCs express core binding factor α-1 (Cbfa1)/Runx2 and Sox9 [24,32,33] and transcription factors for osteoblastic and chondrocytic differentiation at sites of calcification from *in vivo* studies [24]. When studies were performed to study the temporal expression of these proteins to evaluate the relationship with onset of calcification, the VSMCs in the normal vessel wall expressed constitutive inhibitors of calcification such as MGP [24,33]. However, when examining calcified arteries these inhibitors were found to be down-regulated and expression of VSMC mineralization mediators was up-regulated [24,29].

Studies from transgenic animal models have demonstrated the importance of a number of proteins that play an important role in vascular calcification [24]. These include murine knockout models for MGP [34], BMP-2 [29,31], SMAD-6 [24,35], ecto-nucleotide pyrophosphate/phosphodiesterase 1 (ENPP1) [36], Klotho [37], and Fetuin-A [38].

There remain many unanswered questions about the mechanisms underlying vascular calcification and the role of CKD and uremia in accelerating the vascular calcification process. Improving our understanding of the pathophysiological regulation of vascular calcification may have therapeutic potential in regards to treating cardiovascular disease in kidney disease and in hemodialysis vascular access.

CLINICAL STUDIES EVALUATING VASCULAR CALCIFICATION AND HEMODIALYSIS VASCULAR ACCESS DYSFUNCTION

Histologic Studies of Arterial Coronary Calcification

The leading cause of mortality in patients with CKD and ESRD is still cardiovascular disease [39]. The high risk of elevated cardiovascular events is even present in early stages of CKD [40]. Recent studies from autopsies of coronary artery samples have shed light on this theory. Schwarz *et al.*, in a comparative study of coronary atherosclerotic lesions in autopsy samples from 27 patients with CKD (21 with ESRD) and 27 age- and sex-matched controls, demonstrated that atherosclerotic plaque was more calcified in patients with CKD compared to their control group [41]. A recent study from Nakano *et al.*, from 126 patients reported that the proportion of advanced atherosclerotic lesions is higher at a lower estimated GFR with a higher prevalence of calcified coronary lesions and thickened arterial intima [42]. However, no medial calcification was noted in this study [42]. Nakamura *et al.,* in their study of autopsied coronary arteries from subjects with different levels of CKD (stage I/II, stage III, stage IV/V, stage VD) showed that intimal calcification was present in all groups, but most prominent and severe in the CKD Stage VD group compared to CKD Stage IV/V group [43]. However, medial calcification was seen in a minority of CKD Stage IV/V group [43]. The collective studies above demonstrate that vascular calcification progressively worsens with decline of kidney function. All studies have demonstrated the presence of intimal calcification, with calcification less prominent within the media.

Vascular Calcification in Upper Extremity Vessels and Vascular Access

There are several studies that have documented the presence of vascular calcification in upper extremity vessels used to create new vascular access. Wang *et al.*, collected 30 radial artery specimens from ESRD patients at the time of new arteriovenous fistula creation and found that 37% of

arteries had calcification (20% mild/moderate and 17% severe calcification) [44] with calcium deposition primarily located in the medial layer of the artery. In a study of 65 patients, Kim *et al*., reported that micro-calcification from radial and brachial arteries, using von Kossa staining, was present in 55.4% of patients [45]. Calcification was primarily observed in the medial layer (unlike coronary arteries) and not seen in the intimal layer [45]. Venous calcification and vascular access outcomes were not reported in this study. Lee *et al*., using Von Kossa staining evaluated 67 veins collected at the time of new vascular access creation [46]. Twenty-two of 67 (33%) samples showed the presence of venous calcification [46]. Among the subset of patients with documented venous calcification, 4/22 (18%), 19/22 (86%), 22/22 (100%), and 7/22(32%) had calcification present within the endothelium, intima, media, and adventitia, respectively [46]. This study did not report the association of pre-existing venous calcification with vascular access outcomes. Finally, Allon *et al*., in 50 CKD patients undergoing AVF placement, reported arterial micro-calcification in 2/3 of arterial samples [8]. Among samples with the presence of arterial micro-calcification, there was a trend towards an association with AVF non-maturation [8].

There are also several studies that have evaluated vascular calcification after vascular access creation. Schlieper *et al.,* found in a study of 212 patients that 49 patients (23%) had presence of vascular access calcification (in AVF and AVG) using plain X-ray [47]. Furthermore, they reported that the presence of vascular access calcification was significantly associated with patient mortality [47]. Finally, Touissant *et al.,* in 28 hemodialysis patients, using computed tomography imaging fistulograms, showed that vascular calcification was predominately within the aorta (75%) and subclavian arteries (89.3%), but minimal vascular calcification was present at the level of the AVF [48].

The studies above, collectively, demonstrate the presence of both calcification in both veins and arteries used to create new vascular access with possible association with mortality when detected after vascular access creation. There is an urgent need for more investigative studies to understand the pathophysiological mechanisms of calcification development in arteries and veins prior to and after vascular access creation.

FUTURE PERSPECTIVES

Cardiovascular disease remains one of the leading causes of death in advanced CKD patients and a major contributing factor in cardiovascular mortality in ESRD patients [39]. In addition, vascular calcification, not only contributes to the high prevalence of cardiovascular disease, but also likely influences successful vascular access creation, maturation, and long-term patency of AVF and AVG. Future research needs to be focused on how the uremic milieu accelerates vascular calcification and disrupts endothelial function. Recognizing that vascular calcification appears to be a regulated process, identifying key pro-calcification and inhibitory factors will be crucial to understand the role of vascular calcification as it relates to vascular access dysfunction. One approach is to develop AVFs in animal models targeting the vascular calcification process, where

specific genes related to vascular calcification, such as Klotho and osteoprotegerin are knocked out. Another key question is whether vascular access calcification worsens after creation of the vascular access and use of dialysis. There are no clinical studies to date that have evaluated the role of vascular calcification of the brachial or radial arteries in the same individual prior to vascular and after access creation, or the natural progression of vascular calcification in AVFs in experimental models. The mechanisms leading to calcification in AVFs may be different from those leading to calcification seen in coronary arteries due to local hemodynamic effects and injury at the arteriovenous anastomosis, thus, development of experimental models is crucial to determine the role of calcification in vascular access, particularly AVF maturation.

CONCLUSION

Vascular calcification is present in both arteries and veins, used to create new vascular access. Future studies in this area need to focus on the mechanisms that lead to vascular calcification in the uremic milieu and the impact of calcification on vascular access outcomes. The complex pathogenesis of vascular calcification is still poorly understood, thus the prevention and progression of vascular calcification will likely require a multi-faceted therapeutic approach.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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