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Pre-Existing Venous Calcification Prior to Dialysis Vascular Access Surgery

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Abstract

Vascular calcification is present in arterial vessels used for dialysis vascular access creation prior to surgical creation. Calcification in the veins used to create a new vascular access has not previously been documented. The objective of this study was to describe the prevalence of venous calcification in samples collected at the time of vascular access creation.

67 vein samples were studied. A von Kossa stain was performed to quantify calcification. A semi-quantitative scoring system from 0–4+ was used to quantify the percentage positive area for calcification as a fraction of total area (0=0; 1+ = 1–10%; 2+ =11–25%; 3+ = 26–50%; 4+ >50% positive).

22/67(33%) samples showed evidence of venous calcification. Histologic examination showed varying degrees of calcification within each cell layer. Among the subset of patients with 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. The mean semi-quantitative scores of the 22 samples with calcification were 0.18±0.08, 1.2±0.14, 1.6±0.13, and 0.36±0.12 for the endothelium, intima, media, and adventitia, respectively.

Our results demonstrate that vascular calcification is present within veins used to create new dialysis vascular access, and located predominately within the neointimal and medial layers.

Keywords

Vascular Calcification; Hemodialysis Vascular Access; Vascular Access Stenosis

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Disclosure

Dr. Lee is a consultant for Proteon Therapeutics. Dr. Roy-Chaudhury is on the advisory board/consultant for Pervasis Therapeutics, Inc., Proteon Therapeutics, WL Gore, Bioconnect Systems, Philometron and NanoVasc and receives research support from BioConnect Systems, Shire, Proteon Therapeutics, and WL Gore. These funding sources had no involvement in the design or execution of this study.

Introduction

Aggressive venous neointimal hyperplasia is the most common histologic lesion seen in arteriovenous fistula (AVF) and graft (AVG) failure [1–6]. While the majority of the research in vascular access dysfunction has focused on the mechanisms of neointimal hyperplasia development after AV access creation, recently, our group and others have reported that the health of the vessel (artery and vein) may play an important role in the short and long-term outcomes of AVFs [7–9]. Progressive arterial calcification due to uremia plays an important role in accelerated cardiovascular mortality in end stage renal disease patients compared to the general population[10–12]. Emerging evidence has shown that vascular calcification in arteries used to create new vascular accesses may play an important role in vascular access failure[13, 14]. However, venous stenosis is the most common lesion in vascular access dysfunction and there have been no previous publications describing presence of venous calcification in the vessels used to create a new vascular access. Thus, the main objective of this study was to describe the prevalence of venous calcification and its distribution within the venous wall, in samples collected at the time of dialysis vascular access creation.

Methods

Study Population

67 patients requiring new vascular access placement, from 2008–2010, were recruited in our vascular access clinic for evaluation into this study. Prior to each evaluation a pre-operative ultrasound mapping of both extremities, or angiography, was performed to evaluate vessel diameters and stenosis. Patients were consented in our vascular access clinic, during access placement evaluation, to obtain venous tissue specimens at the time of vascular access surgery. Demographic data was collected at the time of recruitment. Data pertaining to the site of access placement and specific vessel obtained was collected at the time of surgery. Institutional Review Board approval was obtained to conduct this study.

Specimen Collection and Processing

Venous tissue specimens were collected at the time of surgical creation of vascular access. During the surgery, an approximately 8–10mm circumferential segment of vein was removed near the planned anastomosis site in each patient and immediately fixed in formalin.

Each venous tissue sample, fixed in formalin, was embedded and cut into 2–3 tissue blocks of 3–4 mm thickness using previously described techniques [2, 7]. Each piece was paraffin-embedded and then sliced into 4μm sections for histological and histochemistry studies.

Histochemistry Studies

Sections from each tissue block were evaluated for the presence of calcification with von Kossa staining using standard techniques. In brief, deparaffinized slides were placed in 5% silver nitrate for 10 to 60 minutes with exposure to an ultraviolet light or 100 watt incandescent desk lamp, then rinsed and placed in 5% sodium thiosulfate for 2 to 3 minutes. Finally, the slides were rinsed and stained with a nuclear fast red stain. A brown or black color on the specimen indicated a positive stain.

The degree of calcification, based on the intensity of the Von Kossa stain, was scored by an independent investigator blinded to the identity of the tissues. A semi-quantitative scoring system from 0–4+ was used to quantify the percentage positive area for calcification as a fraction of total area (0=0; 1+ = 1–10% positive; 2+ =11–25% positive; 3+ = 26–50%

positive; 4+ >50% positive) for each cell layer, endothelium, intima, media, and adventitia. Mean values for calcification for all samples were calculated.

Statistics

The distribution of study variables was characterized according to means ± S.E. and proportions. All statistical analyses were performed using JMP® 8.0 (Cary, NC) statistical software package.

Results

In total, 67 vein specimens were collected for this study. 22/67 (33%) samples showed evidence of venous calcification (Figure 1). Histologic examination showed varying degrees of calcification within each cell layer. Among the subset of vein samples with calcification (n=22), 4/22 (18%), 19/22 (86%), 22/22 (100%), and 7/22 (32%) had calcification present within the endothelium, intima, media, and adventitia, respectively (Table 1). The mean semiquantitative scores of the 22 samples with calcification were 0.18±0.08, 1.2±0.14, 1.6±0.13, and 0.36±0.12 for the endothelium, intima, media, and adventitia, respectively (Table 1). The average calcification scores were significantly higher in the media compared to the intima (p=0.0064).

Discussion

Aggressive venous neointimal hyperplasia is the most common histologic lesion seen in AVF and AVG dysfunction[2, 3, 5, 6, 15]. However, our group has recently reported that severe preexisting vascular changes is present in the veins used to create new vascular access prior to surgery[7]. Specifically, in a small group of patients, we found that venous neointimal hyperplasia is present in the majority of veins used to create new vascular accesses and may be associated with lower AVF and AVG maturation[7]. Furthermore, Wasse et. al have also recently reported that pre-existing venous neointimal hyperplasia is present in the majority of end stage renal disease and chronic kidney disease patients receiving a new vascular access[16]. Given the potential importance pre-existing vascular changes may play in access maturation, specifically in AVFs, understanding and elucidating new mechanisms that are associated with vascular vasodilation will play a key role in development of novel therapies for vascular access stenosis. In this descriptive study, we focused on reporting the prevalence of venous calcification and the location of the calcification within the vein.

Cardiovascular mortality, secondary to accelerated intimal and medial calcification, in ESRD patients is dramatically increased when compared to the general population[13]. Arterial vascular calcification likely not only contributes to the high prevalence of cardiovascular disease in ESRD patients, but also may play a major role in vascular access dysfunction. A recent study by Wang et. al has shown that one-third of uremic radial arteries collected at the time of AVF creation had arterial calcification predominately in the media of the vessel[14]. While the artery plays an important role in AVF maturation, the most common lesion seen in AVF nonmaturation is venous stenosis in the juxta-anastomotic region[17]. In our study we report that one-third of veins used to create new vascular accesses already have calcification present at the time of surgery. Furthermore, similar to Wang et al in arteries[14], we have found that the predominant area of calcification in the vein is also within the media.

What is the clinical significance of calcification in veins in the context of vascular access stenosis and maturation prior to access creation? In arteries, medial calcification leads to increased stiffness and decreased vascular reactivity[18, 19]. However, veins respond to vascular injury differently from arteries. At a molecular and physiologic level veins produce less nitric oxide and prostacyclin compared to arteries[1, 20]. Thus, pre-existing venous

calcification may further reduce the vein's capacity to vasodilate after AV access creation, an essential process in AVF maturation. Furthermore, the mechanisms that lead to development of venous calcification are currently unknown. In arteries it has been hypothesized that vascular smooth muscle cells derive from a common mesenchymal precursor cell, and vascular smooth muscle cells, under different pathologic conditions, transform into osteoblast-like cells, which then play a critical role in recruitment and production of mediators that lead to calcium deposition[14, 21, 22]. In veins, it is likely a similar process occurs, and determining the origins of these osteoblast-like cells and whether they migrate from the adventitia, perhaps in a similar fashion as neointimal cells[1], will play a critical role in understanding AVF maturation and developing future therapies to treat AVF non-maturation.

Conclusions

We have shown that vascular calcification is present within veins used to create new dialysis vascular access, and predominately located within the intima and media layers. Future studies are needed to evaluate the role of pre-existing venous calcification and AVF maturation and mechanisms of venous calcification in patients with advanced CKD.

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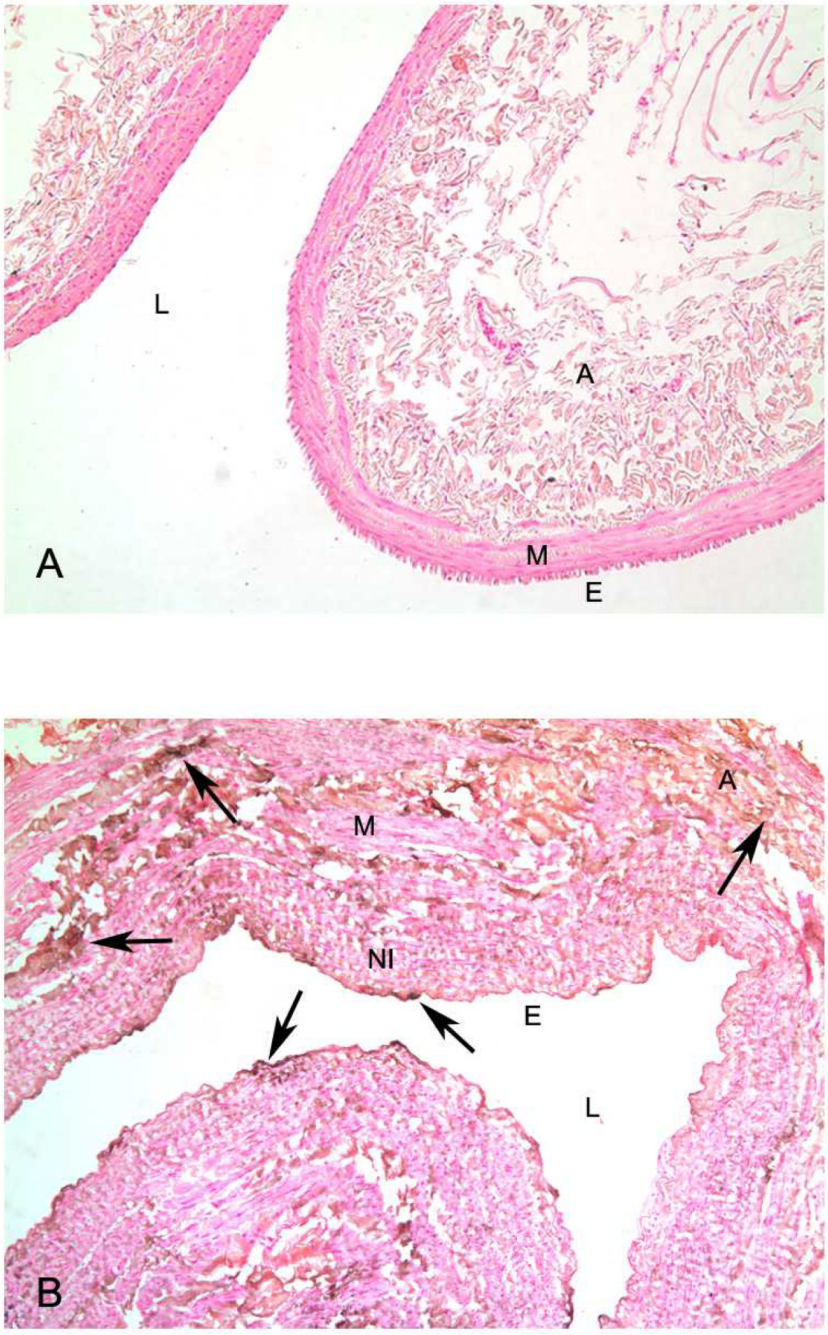

Figure 1.
Panel A. Representative histological section of normal vein. Note thin media (M) and no neointima present. There is also no calcification present after Von Kossa stain. Panel B. Representative histological section of vein obtained at the time of fistula creation with Von Kossa stain. Note presence of calcification (brown and black stain) in endothelium (E), neointima (NI), media (M), and adventitia (A).

Table 1

Proportion of Vascular Calcification and Mean Semiquantitative Score by Cell Layer Among Patient Samples with Calcification

	Endothelium	Intima	Media	Adventitia
Proportion of Vascular Calcification	4/22(18%)	19/22 (86%)	22/22 (100%)	7/22 (32%)
Semiquantitative Score	0.18±0.08	1.2±0.14	1.6±0.13	0.36±0.12