Brief Communication

Vascular calcification in diabetic foot and its association with calcium homeostasis

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ABSTRACT

Introduction: Vascular calcification (VC), long thought to result from passive degeneration, involves a complex process of biomineralization resembling osteogenesis, frequently observed in diabetes and is an indicator of diabetic peripheral vascular disease with variable implications. **Aim and Objective**: To study the association between vascular calcification and calcium homeostasis in diabetic patients with foot ulcers without stage 4, 5 chronic kidney disease. **Materials and Methods**: A total of 74 patients with diabetic foot ulcer were enrolled, and VC was detected by X-ray and Doppler methods. Serum calcium, phosphate, alkaline phosphatase (ALKP), fasting and post-prandial glucose levels, and glycosylated hemoglobin (HbA1C) were recorded. Serum iPTH and 25 (OH) vitamin D were estimated by immune radiometric assay and radioimmunoassay, respectively. Data was analyzed by SPSS 16.0. **Results:** Vascular calcification was present in 42% of patients. Significant difference in the mean (±SD) of vitamin D, HbA1C, and eGFR was observed in VC +ve compared to VC –ve. There was no significant association of age, duration, BMI, PTH, Ca, PO4, ALKP with that of VC incidence. Severe vitamin D deficiency was more common in VC +ve (51.6%) compared to in VC –ve (18.6%). Sub-group analysis showed that the risk of VC was significantly higher (RR = 2.4, $P < 0.05$, 95% C.I. = 0.058-2.88) in patients with vitamin D < 10 ng/ml compared to others. **Conclusion:** Vitamin D deficiency could be a risk for vascular calcification, which possibly act through receptors on vascular smooth muscle cells or modulates osteoprotegerin/RANKL system like other factors responsible for VC in diabetic foot patients.

Key words: Diabetic foot, vascular calcification, calcium homeostasis

Introduction

Vascular calcification (VC), long thought to be result of passive degeneration, involve a complex, regulated process of biomineralization resembling osteogenesis. VC is an important development in progression of vasculopathy in diabetes mellitus (DM) and contributes to significantly high prevalence of peripheral vascular disease and lower extremity amputation in these subjects. VC is also associated with other manifestation of cardiovascular diseases like hypertension, coronary insufficiency, and increased mortality in patients with DM.

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Traditionally, medial arterial calcification (MAC) has been associated with ageing, advanced chronic kidney disease (CKD), and long-standing DM with diabetic neuropathy. Factors that potentiate MAC in DM may include metabolic and hormonal along with activation of receptor activator of nuclear factor Kappa B lignad/osteoprotegerin (RANK-L/OPG) signaling pathway.[1] RANK-L/OPG pathway modulated directly or indirectly by large number of factors including interleukins, sex steroids, thyroid, parathyroid hormone (PTH), 25 (OH) vitamin D, lipoprotein etc.[2]

The literature on the role of 25 (OH) vitamin D in VC is ambiguous. Experimentally higher 25 (OH) vitamin D level have been associated with increased VC while *in vivo,* lower level of 25 (OH) vitamin D seems to have this effect. This suggests that 25 (OH) vitamin D may have a biphasic relation with risk promoting VC in both excess and deficiency.[3] We designed the present study to find out the prevalence of vascular calcification in diabetic foot

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patients without stage 4, 5 chronic kidney disease (CKD) and its association with calcium homeostasis.

Materials and Methods

A total of 74 diabetic patients with all grades of foot ulcer were enrolled in this study. After clinical evaluations, data regarding age, sex, weight, height, duration of diabetes, and smoking was recorded. VC was detected by X-ray, antero-posterior and lateral view of both feet and by Doppler methods. Serum calcium, phosphate, alkaline phosphatase (ALKP), fasting and post-prandial plasma glucose levels, and glycosylated hemoglobin (HbA1C) were recorded. Overnight fasting blood samples was collected at 8-10 am and stored at –80°C. Serum iPTH and 25 (OH) vitamin D were estimated by immune radiometric assay (IRMA) and radioimmunoassay (RIA), respectively. The co-efficient of inter-and intra-assay variation for iPTH assay were 10.3% and 7.7% and for 25 (OH) vitamin D were 12.5% and 11.7%, respectively. Data was represented as mean (±SD) unless otherwise indicated and analyzed by SPSS 16.0. Independent '*t*' test, Chi-square test, correlation, and risk analysis was performed wherever applicable.

Results

Vascular calcification was present in 42% of the diabetic foot patients. Significant difference in the mean (±SD) of 25 (OH) vitamin D, HbA1C, and estimated glomerular filtration rate (eGFR) was observed in VC +ve patients compared to VC –ve. [Table 1]. There was no significant association of age, duration of diabetes, BMI, PTH, Ca^{2+}, PO_4^{2-}, ALkP with that of VC incidence in study subjects. Severe 25 (OH) vitamin D deficiency was more common in VC +ve patients (51.6%) compared to VC –ve patients (18.6%) as shown in Figure 1. Sub-group analysis based on different status of 25 (OH) vitamin D (<10, 10-20 and >20 ng/ml) showed that the risk of VC was significantly higher (RR = 2.4, $P < 0.05$, 95% C.I. = 0.058-2.88) in diabetic foot patients with 25 (OH) vitamin D < 10 ng/ml compared to others.

25 (OH) vitamin D was significantly correlated with calcium level ($r = 0.488$; $P < 0.001$) and negatively with alkaline phosphatase ($r = –0.213$; $P = 0.06$). Calcium showed significant negative correlation with alkaline phosphatase ($r = –0.391$; $P = 0.001$).

Discussion

Principal observations in this study are high prevalence of peripheral VC in diabetic foot patients with relatively preserved renal function. Higher incidence

Table 1: Comparison of clinical and biochemical characteristics of diabetic foot patients with and without vascular calcification

Parameters	Vascular calcification Present	Vascular calcification Absent	*P* value
N	31	43	
Sex (M/F) [No]	(22/9)	(33/10)	
Neuropathy (%)	58 (18/31)	44 (19/43)	
Age (years)	54.5 (±5.3)	54.8 (±10.1)	0.9
Duration of Diabetes (years)	9.5 (±5.3)	7.3 (±6.0)	0.9
BMI (Kg/m^2)	22.6 (±4.5)	22.6 (±3.1)	0.1
HbA1C (%)	11.6 (±2.6)	9.0 (±1.4)	<0.001
25 (OH) Vitamin D (ng/ml)	12.6 (±8.4)	16.4 (±7.4)	0.04
iPTH (pg/ml)	48.5 (±12.7)	38.7 (±5.7)	0.4
Calcium (mg/dl)	8.3 (±1.5)	8.7 (±1.2)	0.2
Phosphorus (mg/dl)	3.5 (±.84)	3.8 (±0.7)	0.1
Alkaline phosphatase (IU/L)	582.1 (±103.4)	491.4 (±48.6)	0.3
eGFR (ml/min/1.7 m^2)	44.1 (±9.6)	58.6 (±11.6)	<0.001

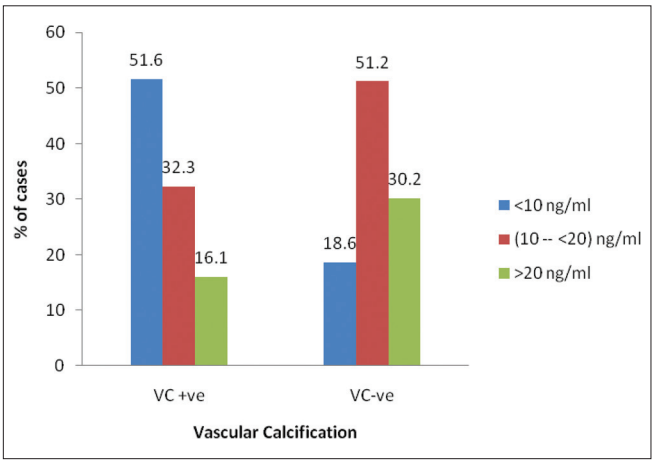

Figure 1: Distribution of diabetic foot patients with respect to their vitamin D status and presence of vascular calcification

of severe vitamin D deficiency in VC +ve diabetic foot patients suggested the possibility of its contribution in development of VC in diabetic foot. Earlier studies have also suggested that vitamin D deficiency could lead to calcification, consequently to increased stiffness and impaired compliance of arterial walls.[4] The endothelial and vascular smooth muscle cells have local 1-alpha hydroxylase activity and Vit-D receptors. Absence of these receptor activations would promote calcification through decreased expression of inhibitory factors of calcifications.[5]

We also observed that VC was associated with uncontrolled glycemia that was evidenced by HbA1C value and reduced glomerular filtration rate as reported previously, but no significant relation of VC with Ca^{2+} and PO_4-soluble product, PTH or ALKP was found in our study. It might be possible that some other factors or mechanisms are

involved in the formation and the progression of VC apart from the high calcium and phosphate microenvironment. It is evident from present study that vitamin D is significantly associated with VC in diabetic foot, the mechanism of which needs to be elucidated.

Various studies have shown both stimulatory and inhibitory action of vitamin D on OPG and stimulatory actions of vitamin D on RANKL.[5] Vitamin D alone and either along with RANKL/OPG, possibly many other factors like HbA1c, peripheral neuropathy, eGFR, may play a complex role in the pathogenesis of the development of VC. OPG levels reflect the response of the innermost endothelial cell layer to inflammation, and vitamin D is a known modulator of inflammatory markers. Thus, in view of the previous reports[6] and observations of our study, we propose that the deficiency of vitamin D leads to altered inflammatory response, which may through modulation of RANKL/OPG leads to the formation/progression of VC in diabetic foot.

Conclusion

Our study signifies that vitamin D deficiency could be a risk for vascular calcification possibly acting through receptors on vascular smooth muscle cells or modulating RANKL/OPG system like other factors responsible for VC in diabetic foot ulcer patients.

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