Prevalence and severity of vitamin D deficiency in patients with diabetic foot infection

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Abstract

The aim of the present research was to study the prevalence and severity of vitamin D deficiency in patients with diabetic foot infection. Patients were enrolled in two groups: diabetic patients with foot infection (n 125) as cases and diabetic patients without the infection as controls (n 164). Serum 25-hydroxyvitamin D (25(OH)D) was measured by RIA. Data were presented as means and standard deviations unless otherwise indicated and were analysed by SPSS. Results revealed that 25(OH)D (nmol/l) was significantly lower (40·25 (SD 38·35) *v.* 50·75 (SD 33·00); $P<0.001$) in cases than in controls. Vitamin D inadequacy (25(OH)D < 75 nmol/l) was equally common in cases and controls (OR 1·45, 95 % CI 0·8, 3·0; $P=0.32$), but cases had a greater risk of vitamin D deficiency (25(OH)D < 50 nmol/l) than controls (OR 1·8, 95 % CI 1·1, 3·0; $P=0.02$). Risk of severe vitamin D deficiency (25(OH)D < 25 nmol/l) was significantly higher in cases than in controls (OR 4·0, 95 % CI 2·4, 6·9; $P<0.0001$). Age, duration of diabetes and HbA1c were significantly higher in cases than in controls and therefore adjusted to nullify the effect of these variables, if any, on study outcome. The study concluded that vitamin D deficiency was more prevalent and severe in patients with diabetic foot infection. This study opens up the issue of recognising severe vitamin D deficiency (<25 nmol/l) as a possible risk factor for diabetic foot infections and the need for vitamin D supplementation in such patients for a better clinical outcome. This could be substantiated by similar data from future studies.

Key words: Diabetic foot infection: Vitamin D deficiency: Infection

Vitamin D is a pleiotropic hormone known to play an immunomodulatory role[1,2], in addition to Ca and bone metabolism. Receptors for its activated form have been identified on pancreatic β cells and immune cells[3,4]. Evidence is available linking vitamin D deficiency with bacterial and viral infections[5,6]. Foot infection accounts for 20 % of hospitalisation of diabetic patients annually[7]. Immunological defects[8] in addition to neuropathy and vascular abnormality are the prime contributors in the pathogenesis of diabetic foot and subsequent infections. Different studies have shown that deficiency of vitamin D leads to immune cell dysfunction, β cell damage and impaired insulin production[3,9,10]. In addition to hyperglycaemia, vitamin D deficiency could also be linked to an altered immune system of patients with diabetes, rendering them susceptible to foot infection and unfavourable prognosis. Thus, we were tempted to hypothesise vitamin D deficiency to be more common and severe in diabetic foot infected patients. We hence designed the present study to find out the prevalence and severity of vitamin D deficiency in patients with diabetic foot infection.

Patients and methods

Patients

In the present study, patients attending the diabetic foot and endocrine outpatient clinics of the University Hospital were enrolled. A total of 289 patients with diabetes were enrolled and divided into two groups depending on their presentation on clinical examination as cases and controls. Cases consisted of diabetic patients with clinical evidence of foot infection (n 125); diabetic patients without evidence of any systemic infection (n 164) served as controls. A detailed clinical history, including age, sex, duration of diabetes, and concomitant and anti-diabetic medications, was recorded on a preset proforma. Infection was clinically diagnosed by culture positivity and/or

Table 1. Vitamin D status and other characteristics of study participants
(Number of participants, mean values and standard deviations; mean values with their standard errors)

	Cases		Controls		
	Mean	SD	Mean	SD	P
n	125		164		
Male	85		103		
Female	40		61		
Age (years)	53·6	10·7	51·0	10·8	0·039
DODM (years)					0·049
Mean	6·9		5·4		
SEM	0·53		0·52		
HbA1c (mmol/mol)	86	6·0	77	8·0	0·024
BMI (kg/m²)	24·0	3·7	24·7	4·2	0·246
25(OH)D (nmol/l)	40·25	38·35	50·75	33·00	0·012

DODM, duration of diabetes mellitus; 25(OH)D, 25-hydroxyvitamin D.

leucocytosis in the presence of fever. Patients having clinical evidence of vascular insufficiency or taking immuno-suppressants, multivitamins, Ca supplements and drugs that interfere with vitamin D metabolism were excluded from the study. Cases and controls were simultaneously enrolled throughout the study period of 1 year. Informed written consent was obtained from all participants. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by Institute Ethics Committee, Banaras Hindu University.

Sample collection

Blood samples with and without anti-coagulant were collected for estimating glycosylated HbA1c and serum 25-hydroxyvitamin D (25(OH)D), respectively.

Serum 25-hydroxyvitamin D assay

Serum 25(OH)D was estimated by RIA using a commercial kit (Diasorin) following the manufacturer's protocol. All the tests were run in triplicates and tubes were counted for 60 s. The mean of the three readings was taken to plot the graph and calculate the concentration of samples. Intra-assay and inter-assay CV were 11·7 and 12·5 %, respectively.

Adequacy of vitamin D

Different cut-offs of vitamin D were chosen, i.e. <25, <50 and <75 nmol/l for evaluating the most appropriate risk

factor in patients with diabetic foot infection. The three cut-offs of vitamin D deficiency chosen were in accordance with the recommendation of Endocrine Society practice guidelines[11] and Institute of Medicine definitions[12], but in the context with its role in immune modulation and not with Ca homeostasis.

Statistical analysis

Data are presented as means and standard deviations unless otherwise indicated. Statistical analysis was conducted by using SPSS 16.0 (SPSS Inc.). Group means were compared by independent *t* test and OR were calculated for the three study cut-offs of vitamin D deficiency to estimate the risk point for diabetic foot infection. Those variables that were significantly different in cases and controls and known to influence immune status were also adjusted independently before analysis.

Results

25(OH)D was significantly lower in patients with foot infection than in those without infection (40·25 (SD 38·35) *v.* 50·75 (SD 33·00); $P<0·001$) (Table 1). Prevalence of vitamin D inadequacy at the three study cut-offs chosen, i.e. <75, <50 and <25 nmol/l was 87·4, 70 and 45·6 %, respectively, in cases, whereas the corresponding values in controls were 82·6, 56·2 and 17·3 %.

To further specify the risk point of vitamin D deficiency for diabetic foot infection, OR were calculated (Table 2). Vitamin D inadequacy (25(OH)D < 75 nmol/l) was equally common in cases and controls, but cases had a greater risk of having vitamin D level <50 nmol/l than controls. Also, the risk of having severe vitamin D deficiency (25(OH)D < 25 nmol/l) was much higher in cases than in controls. Factors such as age ($P=0·039$), duration of diabetes ($P=0·049$) and HbA1c ($P=0·024$) were significantly higher in cases than in controls, and were adjusted to nullify their effect, if any, on the test variable (Table 2). It was found that the outcome was similar to the observation without adjustment.

Discussion

In the present study, vitamin D status was evaluated in view of its immune-regulatory role in diabetic foot infected patients who are known to have immune dysfunction[13–15] and susceptibility to microbial infection. The data analysis revealed

Table 2. Risk assessment of vitamin D deficiency at three cut-off values for diabetic foot infection
(Odds ratios and 95 % confidence intervals)

Vitamin D cut-off	Unadjusted OR	95 % CI	Age		HbA1c		Duration of diabetes	
			Adjusted OR	95 % CI	Adjusted OR	95 % CI	Adjusted OR	95 % CI
<25 nmol/l	4·0**	2·4, 6·9	4·3**	2·5, 7·5	3·7**	2·1, 6·4	3·8**	2·0, 7·0
<50 nmol/l	1·8*	1·1, 3·0	1·9*	1·2, 3·2	1·8*	1·0, 3·0	1·5	0·9, 2·6
<75 nmol/l	1·5	0·8, 3·0	1·7	0·8, 3·5	1·6	0·8, 3·4	1·9	0·9, 4·0

*$P<0·05$, **$P<0·0001$.

that the patients with foot infection had comparatively higher prevalence of vitamin D deficiency (<50 nmol/l) than those who had no evidence of infection ($71 \cdot 2$ $v.$ $57 \cdot 3$ %). Prevalence of severe vitamin D deficiency (<25 nmol/l) was remarkably higher in cases ($46 \cdot 4$ %) than in controls ($17 \cdot 6$ %). Foot infection in patients with diabetes reflects their poor immune status compared to patients with diabetes. Our assumption of the vitamin D deficiency in diabetic foot infection to be more common and severe was substantiated by the findings of the present study. Subjects who were enrolled as cases mostly belong to the rural part of northern India and due to the common practice they had comparatively more hours of daily sun exposure than the control subjects who were fit and indoor workers. Mobility was not a concern when sun exposure time was longer in cases than in controls. There was no difference in the sampling season of cases and controls. We were not intended to elaborate the cause of vitamin D deficiency in this study. We observed that irrespective of aetiology, vitamin D deficiency was common and severe in diabetic foot infection.

Researchers have linked vitamin D with several other immunological alterations that are associated with increased susceptibility towards infection. It has also been shown that active vitamin D_3 stimulates the phagocytosis and killing of bacteria by macrophages[16] and is a potent suppressor of interferon-γ-mediated macrophage activation[17]. It suppresses T cell proliferation and decreases the production of the T helper type 1 cytokines while promoting the production of T helper type 2 cytokines[18]. T helper type 2 cells primarily play a role in response to extracellular pathogens (most bacteria and parasites). In addition to hyperglycaemia, deficiency of vitamin D might also increase the risk of infection in diabetic foot patients by further depleting the immune cells' response against infection. The role of vitamin D in defence against tuberculosis has been studied. It was shown that vitamin D modulates the immunological response to intracellular pathogen *Mycobacterium tuberculosis* particularly by inducing cathelicidin antimicrobial peptide gene expression[19].

Studies have also shown the effect of vitamin D on glucose homeostasis, insulin resistance and β cell dysfunction in subjects at risk of type 2 diabetes[20] and on markers of inflammation in non-diabetic adults[21]. Studies are lacking on vitamin D status of patients with diabetic foot infection and its effect on host defence. This study has 99 % power to define 25(OH)D <25 nmol/l as the risk point for diabetic foot infection. Age, duration of diabetes and HbA1c were significantly higher in patients with diabetic foot infection than in patients with diabetes, and were therefore adjusted to nullify their effect, if any, on vitamin D concentration. It is a preliminary study with a lacuna that the effect of vitamin D deficiency on immunological parameters was not studied directly, as the aim was to determine the prevalence of vitamin D deficiency in diabetic foot infection. The effect of vitamin D deficiency and replacement on various immunological parameters could be incorporated in the future plan of similar studies. The strength of our present study is that the cut-off value of <25 nmol/l had a significant relationship with the poor host defence against bacterial infection in diabetic foot and that this also indicates a new cut-off of vitamin D deficiency for immune dysfunction.

In conclusion, the study supports the hypothesis that vitamin D deficiency is more prevalent and severe in diabetic foot infection. The level of vitamin D observed to influence Ca homeostasis is possibly lower for enhancing susceptibility to infection in patients with diabetes. This study opens up an issue of recognising vitamin D deficiency as a possible risk factor for diabetic foot infections and suggests the need for vitamin D supplementation in such patients to prevent or to adjuvant the antibiotic therapy for control of infection.

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