IJMS
Vol 44, No 6, November 2019

Original Article

Evaluation of Serum Vitamin D Levels in Diabetic Foot Infections: A Cross-Sectional Study in a Tertiary Care Center in South India

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Received: 12 February 2018
Revised: 25 April 2018
Accepted: 13 May 2018

Abstract

Background: The impact of diabetic foot infections is enormous in India. Studies on vitamin D levels in diabetes mellitus foot infections are scarce. The primary objective of the present study was to compare the serum vitamin D level between diabetics with foot infections and those without foot infections and the secondary objective was to assess the association between the vitamin D level and the severity of foot infections and outcomes.

Methods: The study included 176 type 2 diabetics who attended Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India, between September 2012 and June 2014. The serum vitamin D level was measured for 88 diabetics with foot infections (Group 1) and 88 without foot infections (Group 2) using the ELISA 25OH vitamin D DIAsource kit (DIAsource ImmunoAssays S.A., Belgium) and compared. Both groups were followed up for 6 months for outcomes. The qualitative variables were analyzed using the χ^2 test and the quantitative variables using the Student *t* test. The statistical analyses were performed using SPSS, version 17.0. A P value of less than 0.05 was considered significant.

Results: The mean serum vitamin D level was not significantly different between the two groups (P=0.306). Among the patients in Group 1 who either required amputations or died, 97.44% had subnormal vitamin D levels in contrast to 59.18% in those who were grafted or achieved wound healing (P=0.001). Among those who achieved wound healing within 6 months, 78.9% had normal vitamin D levels (P=0.0006).

Conclusion: The study found no significant difference in the serum level of vitamin D between diabetics with and without foot infections. However, vitamin D deficiency was associated with a poor outcome in diabetics with foot infections.

Please cite this article as: Danny Darlington CJ, Suresh Kumar S, Jagdish S, Sridhar MG. Evaluation of Serum Vitamin D Levels in Diabetic Foot Infections: A Cross-Sectional Study in a Tertiary Care Center in South India. Iran J Med Sci. 2019;44(6):474-482. doi: 10.30476/ijms.2018.44951.

Keywords ● Amputations ● Diabetic foot ● Vitamin D deficiency

What's Known

- Vitamin D has several non-hormonal functions and its deficiency has been implicated in the pathogenesis of diabetes mellitus.
- Role of vitamin D in the pathogenesis of diabetic foot infections has not been studied in the Indian population and hence the present study.

What's New

- Deficiency of vitamin D was associated with a poor outcome in diabetic foot infections. Vitamin D deficiency was more common in the diabetic foot infections with delayed wound healing and in those requiring major amputations.

Introduction

Foot infections account for limb deformity, limb loss, and significant long-term morbidity and mortality in patients with diabetes mellitus. Diabetic foot infections have a significant impact on the quality of life. Given the rising global burden of diabetes mellitus, the impact of foot infections is enormous.

This calls for the effective prevention and early treatment of diabetic foot infections.[1] Vitamin D has several non-endocrine actions. Several studies have reported the immunomodulatory, endocrine, and metabolic effects of vitamin D. Around one billion of the world population have either vitamin D deficiency or insufficiency.[2] Vitamin D deficiency is very common in India, with a prevalence rate of 70% to 100% in many parts of the country.[3] Vitamin D deficiency has been linked to the complications of diabetes, both microvascular and macrovascular. Considering the immunomodulatory action of vitamin D and its association with diabetic vascular complications, vitamin D may have a role in the development of foot infections and their prognosis.[4] However, studies assessing the association between serum vitamin D level and diabetic foot infections are sparse in the literature. The primary objective of the present study was to compare the serum vitamin D level between diabetics with foot infections and those without foot infections. The secondary objective was to assess the association between vitamin D level and the severity of foot infections and outcomes in type 2 diabetes mellitus.

Patients and Methods

Study Design

The present study is a cross-sectional study involving 176 type 2 diabetic patients. Overall, 310 diabetic patients were enrolled; however, only 176 who met the inclusion criteria and consented were included in the study. They were equally divided into two groups of 88 each. Diabetic patients with clinical evidence of foot infections were included in Group 1, and diabetic patients without evidence of any infection were included in Group 2. Vitamin D level in both groups was measured. All the patients were followed up for a period of 6 months for outcome and prognostic assessment (figure 1).

Sample Size Calculation

The sample size was calculated using OPENEPI® software. It was estimated with an estimated difference in the level of vitamin D as 15 between the groups at 5% level of significance and 80% power. With an estimated dropout rate of 10% and using the statistical formula for hypothesis testing for two means, the sample size was calculated to be 88 in each group.

Inclusion and Exclusion Criteria

Type 2 diabetic patients attending the diabetic clinic, surgery outpatient department, and emergency medical services of Jawaharlal Institute of Post Graduate Education and Research (JIPMER), Pondicherry, India, between September 2012 and June 2014 were included in the study. The presence of ulcer with pus or the presence of cellulitis was taken as clinical evidence of infection in this study.

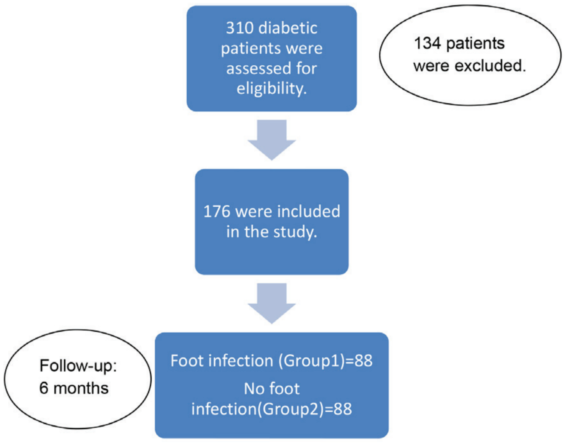

Figure 1: Flowchart shows the study design.

Exclusion Criteria

1. Type 2 diabetic patients with the Hansen disease or varicose veins or peripheral arterial diseases
2. Patients on vitamin D or calcium supplementation or on drugs that interfere with vitamin D metabolism
3. Patients with infections in sites other than the foot

Study Procedure

A detailed clinical history, including age, gender, duration of diabetes and concomitant anti-diabetic medications, body mass index (BMI), and history of smoking, was recorded on a preset proforma. Clinical assessment, including peripheral pulses examination, and laboratory investigations, including HbA1c levels, wound swab, tissue culture, and sensitivity were carried out. The diabetic foot infections in the study population were graded using Wagner grading: grade 0, intact skin; grade 1, superficial ulcers of the skin or subcutaneous fascia; grade 2, ulcers extending into the tendon, bone, or capsule; grade 3, deep ulcers with osteomyelitis or abscess; grade 4, gangrene of the toes or the forefoot; and grade 5, gangrene of the mid-foot or the hind-foot. Wagner grading was used in the present study as it is a well-established and bedside clinical grading which can be done without the need for any special investigations in the limited resource setting common in Indian hospitals. The complications of diabetes mellitus like peripheral neuropathy and nephropathy were assessed. Peripheral neuropathy was assessed using the Semmes–Weinstein 5.07/10g monofilament testing, and nephropathy was assessed using urinary albumin levels and 24-hour urine protein estimation.[5]

Vitamin D Estimation

The estimation of serum 25-hydroxyvitamin D (25[OH] vitamin D) in both groups was done using the DIAsource 25-Hydroxyvitamin D Total Enzyme-Linked Immunosorbent Assay Kit (DIAsource ImmunoAssays S.A., Belgium). Venous blood samples of 5 mL were collected without anticoagulants for estimating serum 25 (OH) vitamin D from all the 176 patients at the time of presentation. The blood samples were centrifuged and the serum samples were stored in a deep freezer at -40 °C, protected from light, and thawed just before the assay. The estimation of vitamin D level was performed only after the 6-month follow-up period was completed to eliminate observer bias. All the observations and analyses were made by a single observer to avoid inter-observer variability.

In the first step, total vitamin D present in the serum was dissociated from binding proteins in the serum and allowed to bind to a specific monoclonal antibody. After washing, a fixed amount of vitamin D with biotin and horseradish peroxidase was added to the microplate and allowed to compete with the unlabeled 25 (OH) vitamin D molecules and the 25 (OH) vitamin D bound to the monoclonal antibody. After this step, a chromogenic solution containing Tetramethylbenzidine was added. Tetramethylbenzidine acts as a substrate for the peroxidase enzyme, thereby producing color change. The amount of the substrate turnover was measured colorimetrically by absorbance at 450 nm, which was inversely related to the serum 25 (OH) vitamin D concentration. A vitamin D level of equal to or greater than 30 ng/mL was considered normal, 10 to less than 30 ng/mL was taken as vitamin D insufficiency, and 0 to less than 10 ng/mL was considered to be deficient.[3, 6]

Treatment of Diabetic Foot

All the diabetic foot patients with active infections were admitted and started on intravenous antibiotics empirically after sending the pus or wound swab for culture. The blood glucose level was controlled using insulin (short-acting and long-acting as and when needed). Thorough debridement until viable bleeding tissues was done. Pus pockets were drained, and amputation was done when the limb was non-viable. Daily dressing and minor debridement were performed. The patients were discharged when either wound cover was achieved (grafting/amputation with flap) or the active infection was treated.

Follow-up and Outcome Measures

The serum vitamin D level was assessed and compared between the two groups. The severity of the diabetic foot infections was graded using Wagner grading. All the patients were followed up every 2 weeks for 6 months to assess the outcome and prognosis. The severity and duration of the foot infections, number of the sessions of debridement, requirement of major/minor amputations, time duration for skin cover, and mortality were recorded. Subgroup analysis was performed among Group 1 patients to compare the serum vitamin D level with various outcomes.

Statistical Analysis

The statistical analyses were performed using the SPSS software version 17.0. (Chicago, SPSS Inc.). The distributions of the data on the categorical variables such as gender, smoking, diabetic medications, and the duration of diabetes were presented as frequencies or percentages and were compared between the two groups using the χ^2 test. The continuous variables like age, BMI, blood glucose, HbA1c, and vitamin D were compared between the groups by using the independent Student *t* test. Various outcomes on vitamin D level were analyzed using the χ^2 test. The mean values were compared using Student *t* test. The statistical analyses were carried out at 5% level of significance, and a P value of less than 0.05 was considered statistically significant.

Ethical approval: The institutional ethics committee's approval was obtained (No. IEC/SC/2012/5/221). The nature, methodology, and risks involved in the study were explained to the patients and informed written consent was obtained. All the information collected was kept confidential, and the patients were given full freedom to withdraw at any point during the study. All the provisions of the declaration of Helsinki were followed in this study.

Results

Totally, 310 diabetic patients were assessed for eligibility for the study. Nevertheless, only 176 met the inclusion criteria and consented and were included in the study. They were equally divided into two groups of 88 each, and follow-up was done for 6 months. There were five deaths among the diabetic foot patients. No patient in either of the groups was lost to follow-up.

The demographic profile of both groups was similar, eliminating any confounding factors between the two groups (table 1). The groups, however, differed in terms of the BMI and the mean HbA1c level (P=0.0001) (table 1). In the present study, 62.5% of Group 1 patients, who underwent major amputations, had a BMI less than 25 kg/m^2. Gender was not found to have an influence on wound outcomes in the present study. It, however, affected the mortality rate among diabetics with foot infections. All the five patients who expired were men, that was statistically significant (P=0.035). This can be attributed to chronic smoking and co-morbidities like hypertension and coronary artery disease, which are more prevalent in men. All the five men who expired were smokers.

Foot Infections

The Wagner grading of the foot wounds was associated with the outcome in the present study. With respect to grading, 29.5% of the wounds were grade 1, 21.60% were grade 2, 26.30% were grade 3, and 22.60% were grade 4. Wagner grades 3 and 4 were associated with a poor outcome in the present study. Amputations were required in 90% of the grade 4 and 52% of the grade 3 foot infections (P=0.0001). None of the grade 1 patients required amputations. Pseudomonas species was the most common organism isolated in the present study (26%) (figure 2). Leukocytosis indicating the presence of active infection was present in 55.7% of the cases and only in 18.2% of the controls on presentation, which was statistically significant (P=0.0001).

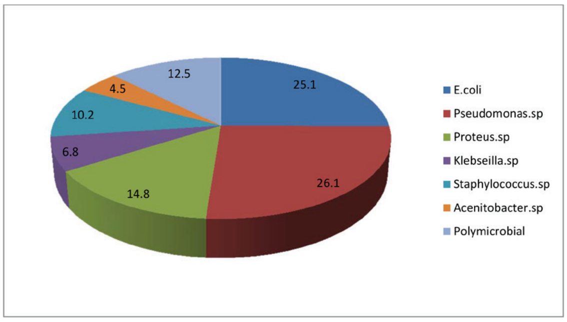

Figure 2: Figure shows the microbiological profile of the diabetic foot infections in the study (numbers indicate percentages).

Outcome of the Diabetic Foot Infections

Among the diabetic foot patients, 18.2% underwent major amputations, 20.5% underwent minor amputations, 8% had their wounds grafted, 5.7% expired in the hospital, and 47.7% were discharged with a raw area after the control of the infection and followed up on an outpatient basis until wound healing (figure 3).

Neuropathy and Vitamin D Level

Major amputations were required in 28.57% of Group 1 patients with neuropathy, which was statistically significant (P=0.005), compared with only 5.12% of Group 1 patients without neuropathy who underwent major amputation. However, the vitamin D level was not significantly related to diabetic neuropathy in the diabetic foot patients in our study (P=0.06) (table 2).

Nephropathy and Vitamin D Level

Nephropathy was present in 23% of the

Table 1: Demographic profile of the diabetic patients with and without foot infections

Demographic Parameter	Diabetics with Foot Infections	Diabetics without Foot Infections	P value
Age	56.58±8.99	58.08±10.37	0.307 *
Body mass index	23.21±4.07	25.51±4.03	0.0001*
Presentation blood glucose	297.81±109.58	226.34±73.96	0.0001*
Gender			
Male	48 (47.52)	40 (53.33)	0.4460#
Female	53 (52.48)	35 (46.67)	
Smoking			
Smoker	29 (32.95)	38 (43.18)	0.1625#
Non-smoker	59 (67.05)	50 (56.82)	
Anti-diabetic treatment			
oral hypoglycemic agents	66 (75)	33 (37.5)	0.0736#
Insulin	22 (25)	55 (62.5)	
Duration of diabetes mellitus			
Recently diagnosed (%)	18 (20.45)	13 (14.77)	0.5694#
1–10 y	63 (71.60)	66 (75)	
>10 y	7 (7.95)	9 (10.23)	
HbA1c levels			
Mean±SD	10.52±2.59%	8.49±1.84%	0.0001*

*Student *t* test; #χ^2 test; N=Number of patients; Numbers indicated in the parentheses are percentage values

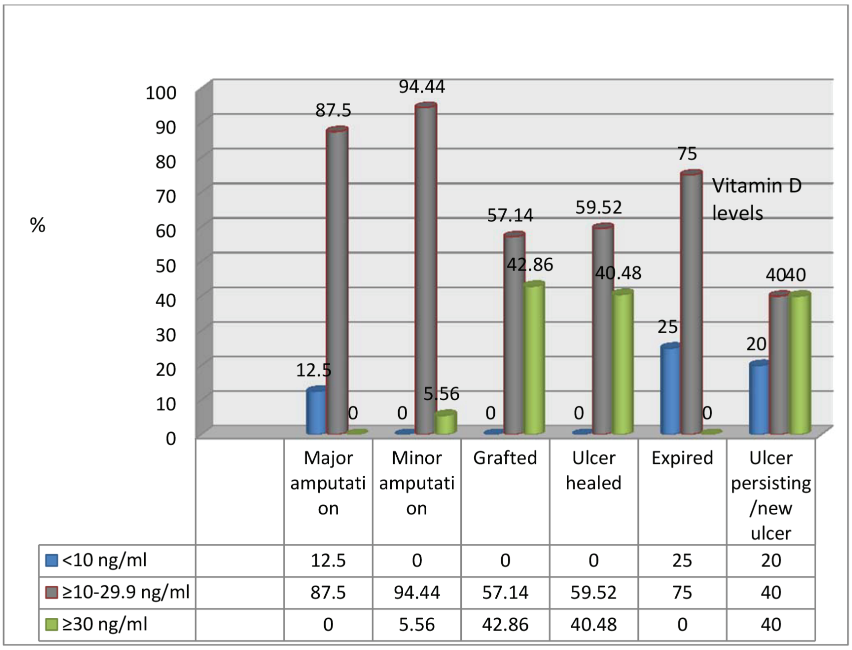

Figure 3: Bar chart depicts the serum vitamin D levels and the outcomes of the diabetic foot patients included in the study (numbers indicate percentages).

Table 2: Subgroup analysis of the diabetics with and without foot infections and their outcomes in the present study with reference to the serum vitamin D level

Group	Vitamin D levels		P value
	Subnormal (<30 ng/mL)	Normal (≥30 ng/mL)	
Diabetics with foot infections N=88 (%)	67 (76.14)	21 (23.86)	0.306#
Diabetics without foot infections N=88 (%)	66 (75)	22 (25)	
Diabetics with foot infections mean±SD (ng/mL)	19.38±5.32	42.32±19.06	0.00001*
Diabetics without foot infections mean±SD (ng/mL)	21.91±5.16	47.67±14.59	0.0001*
Diabetics with foot infections HbA1c <7 N=8 (%)	6 (75)	2 (25)	0.937*
Diabetics with foot infections HbA1c ≥7 N=80 (%)	61 (76.25)	19 (23.75)	
Neuropathy in diabetics with foot infections N=49 (%)	41 (83.67)	8 (16.33)	0.0629#
Amputation/death in diabetics with foot infections N=39 (%)	38 (97.44)	1 (2.56)	0.001#
SSG/ skin cover in diabetics with foot infections N=49 (%)	29 (59.18)	20 (40.82)	
Wound healing duration>6 months in diabetics with foot infections N=27 (%)	17 (62.96)	10 (37.04)	0.0006#
Amputation/death in diabetics with foot infections mean±SD (ng/mL)	17.03±5.10	30.22	0.014*
SSG/ skin cover in diabetics with foot infections mean±SD (ng/mL)	22.45±3.73	42.92±19.30	0.0001*

*Student *t* test; #χ^2 test; N=Number of patients; Numbers indicated in the parentheses are percentage values; SSG: Split skin graft

cases, and it was shown to be related neither to vitamin D level nor to the outcome of the diabetic foot infections in this study. However, only 23% of Group 1 patients had nephropathy, which was too small proportion to derive any statistically significant observation.

Vitamin D Levels and the Outcome of the Diabetic Foot Infections

There was no statistically significant difference in vitamin D level between the diabetics with and without foot infections in the present study. However, among the diabetic

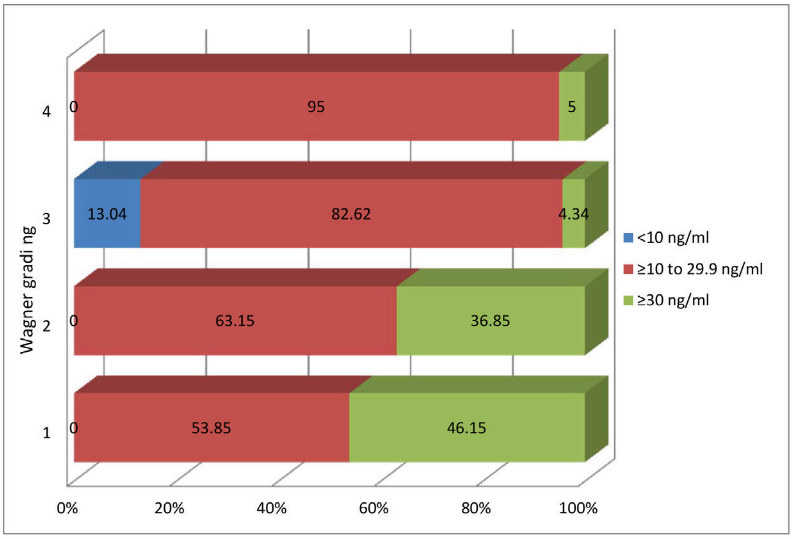

Figure 4: Bar chart depicts the vitamin D status in the different Wagner grades of the diabetic foot infections included in the study (numbers indicate percentages).

foot patients with a poor outcome (amputation or death), 97% had subnormal vitamin D levels with a mean vitamin D level of 17.03±5.1 ng/mL, which was statistically significant (P=0.001) (figure 4 and table 2). Most of the diabetics with foot infections (67%) required more than 6 months for achieving wound cover on follow-up when vitamin D levels were subnormal. Among the foot infections requiring fewer than 6 months for wound cover, 78.9% had normal vitamin D levels, which was statistically significant (P=0.0006) (table 2).

Discussion

The present study is a hospital-based cross-sectional study analyzing the effects of the serum vitamin D level on the outcome of diabetic foot infections. It analyzed the serum vitamin D level in 176 diabetic patients, who were divided into two groups: diabetics with foot infections and without foot infections. The serum vitamin D level was found to be similar in both groups. However, among the diabetic foot patients, vitamin D level was significantly lower than the patients with a poor outcome (death or amputation) in comparison with the patients who achieved wound healing.

A BMI of less than 25 kg/m^2 and more than 29 kg/m^2 has been associated with a higher risk of amputations in previous studies.[7] Several studies have shown a J-shaped association between BMI and diabetic foot ulcers.[8] The poor outcome in the group of patients with a BMI of less than 25 kg/m^2 in the present study can be due to the effects of malnutrition on immunity and wound healing, which is more common in Indian patients. Our study included very few patients with a BMI of above 29 kg/m^2 and hence the outcome in such patients cannot be commented based on the present study.

Among the diabetic foot patients with persisting ulcers or recurrent infections requiring amputations, 80% were Wagner grade 3 or 4. This is in accordance with a study by Yekta and others,[8] who reported that 93% of the diabetic foot infections of Wagner grade 3 or above among their patients required amputations.

The duration of diabetes mellitus and the type of treatment received were not found to have a significant influence over the outcome of the diabetic foot infections in the present study. This is contrary to the results of the studies by Wang and colleagues[9] and Ndosi and others,[10] who reported a higher risk of amputation and death with longer durations of diabetes and insulin treatment. The above authors concluded that patients having diabetes mellitus for longer durations ran the risk of having more microvascular and macrovascular complications of the disease. This difference could be explained by the fact that very few patients in this study had diabetes mellitus for more than 10 years.

Smoking was associated with high mortality in the current study. All of the five patients who expired were smokers. Nonetheless, smoking was not found to increase the incidence of major or minor amputations in the present study. Smoking acts synergistically with other co-morbidities and increases the mortality.

Infection and sepsis lead to uncontrolled blood glucose levels in diabetic patients, which will in turn aggravate sepsis. The presence of foot infections in the cases may have caused high blood glucose levels at presentation, which was evident by the increased total leukocyte counts in 55.7% of Group 1 as opposed to 18.2% in Group 2 (P=0.0001). The studies conducted by Lan and others[11] and Kilpatrick and colleagues[12] yielded the same results. Adler and coworkers[13] proposed that for every 1% increase in HbA1c levels, the rate of amputations increases by 1.26%. This highlights the negative impact of high HbA1c and blood glucose levels on the outcome of diabetic foot infections.

Studies on foot infections in the Indian diabetic population have shown Gram-negative organisms to be most commonly associated with diabetic foot infections. Escherichia coli was the most common organism isolated from patients who required major amputations in many Indian studies.[14]

The presence of co-morbidity has been linked to both the development of diabetic foot ulcers and the increased mortality.[15] The present study did not show any significant association between the presence of co-morbidities and the outcome of foot infections. The fewer number of patients with co-morbidities among the cases may be the reason for this difference.

Peripheral neuropathy was associated with a higher number of major amputations, persistent foot ulcers, and mortality. This was a statistically significant observation. These results chime with the other studies which describe a diabetic foot with neuropathy as a “high-risk foot”, which carries the increased risk of amputation with long-term morbidity and mortality.[16-18] The present study, however, did not find a significant association between the serum vitamin D level and diabetic neuropathy in diabetic foot infections. Evidence supporting the role of vitamin D in peripheral neuropathy has emerged in recent studies. Nevertheless, most of these studies have too small a sample volume to draw any significant conclusion. A recent meta-analysis by Qu and others[19] concluded that Asian type 2 diabetic patients with vitamin D deficiency were 1.22 times more likely to develop diabetic neuropathy. They concluded that vitamin D supplementation must be given to type 2 diabetic patients to prevent the development of neuropathy.

Nephropathy was found to be related neither to vitamin D level nor to the outcome of the diabetic foot infections in our study. In contrast, other studies with larger proportions of diabetics with chronic kidney disease have shown the significant impact of nephropathy on the outcome of diabetic foot infections.[17, 20] Another factor is the proportion of cases with diabetic nephropathy in our study groups, which was clearly low. Ndip and others[21] conducted a cross-sectional study involving 326 diabetics with chronic kidney disease to compare the prevalence of diabetic foot ulcers between them and showed a negative impact of diabetic nephropathy on the outcome of foot infections.

An Indian study conducted by Tiwari and others[22] found a significant difference in the prevalence of vitamin D deficiency between diabetics with and without foot infections. The present study did not find any difference in the prevalence of vitamin D deficiency or insufficiency in diabetics with foot infections. However, on subgroup analysis, the study found lower mean vitamin D levels in the diabetics with foot infections who had a poor outcome. The difference in the results could be explained by vitamin D cutoff values taken in the present study (normal≥30 ng/mL, insufficiency 10–30 ng/mL, and deficiency<10 ng/mL)[3, 6] and those in the study done by Tiwari and colleagues (normal≥30 ng/mL, insufficiency 20–30 ng/mL, and deficiency <20 ng/mL), which resulted in the classification of a higher number of patients with vitamin D insufficiency as deficient.

The merits of the present investigation are that it is a cross-sectional analytical study with a reasonably large sample size (N=176) and it involves Indian patients. The study can be followed up by randomized controlled trials involving larger numbers of patients in the future.

However, there are several limitations to our study. This is an in-hospital study and its results have not been externally validated. As the prevalence of microvascular complications and co-morbidities was low in the study, their impact on the outcome of diabetic foot infections could not be assessed. The follow-up period in the study is too short to assess the long-term outcomes of diabetes mellitus. The study is not interventional and measures only the association between vitamin D level and various outcomes. Other confounding factors which can alter the level of vitamin D like serum calcium levels, parathormone, and albumin were not analyzed in the study.

Conclusion

The study found no significant difference in the serum level of vitamin D between diabetic patients with and without foot infections. However, subnormal vitamin D levels were found to be more common in the diabetic foot patients who underwent major and minor amputations or died of the disease. This possibly explains that the occurrence and prognosis of diabetic foot infection are multi-factorial and the level of vitamin D could play a vital role. Further interventional studies in the future involving larger numbers of patients may substantiate the evidence obtained from this study.

Conflict of Interest: None declared.

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