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Original Article

A Clinicomicrobiological Study on Incidence of Mycotic Infections in Diabetic Foot Ulcers

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Abstract

Introduction: Diabetic foot ulceration is one of the most common complications of diabetes, affecting about 15% of diabetics during their lifetime. Most of diabetic foot ulcers become infected, and more than 20% of the patients with infected foot ulcers undergo foot amputations. Most of the diabetic foot infections are polymicrobial involving bacteria and fungi. The fungi involved in diabetic foot infections are mainly *Candida* spp.; however, data on prevalence of mycotic infections in diabetic foot ulcers are limited.

Aim of the study: To study the incidence of mycotic infections in diabetic foot ulcers and compare our results with previous studies.

Materials and Methods: A hospital-based prospective study was carried out on 105 consecutive patients who were admitted to the surgery ward due to diabetic foot ulcer from November 2014 to October 2016. After detailed history and clinical examination, soft-tissue samples were taken from the ulcer bed and sent for microbiological examination and fungus culture. Results of the study were compared with previous studies.

Results: Direct microscopic examinations of samples in 10% potassium hydroxide mount were positive for fungus in 30 (28.6%) individual while cultures on Sabouraud's dextrose agar were positive in 21 (20%) individuals. *Candida* species were most common fungal isolate (11.43%), followed by *Aspergillus* sp. (3.81%), *Fusarium* species (2.86%) and *Trichophyton* sp. (1.90%).

Conclusion: The results of this study show that more than a quarter of diabetic foot ulcers harbor fungal infection and *Candida* species is the most common fungal isolate.

Hence, all diabetic foot ulcers should be evaluated for mycosis.

Key words: *Candida*, Diabetic foot ulcer, Fungal infections, Mycosis

INTRODUCTION

Diabetes mellitus is fast gaining the status potential epidemic in India. With 62 million diabetic populations, India is diabetic capital of the world.[1] According to Wild *et al.*, diabetic population is going to nearly double to 366 million worldwide by 2030 from 177 million in 2000.[2,3]

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Foot ulceration is the most common complication of diabetes, affecting approximately 15% of diabetic patients during their lifetime.[4,5]

Globally, diabetic foot lesions are a major medical, social, and economic problem and are the leading cause of hospitalization for patients with diabetes. Diabetic foot ulcers account for more than half of non-traumatic lower limb amputations.[6] This can be attributed to several factors such as barefoot walking, inadequate knowledge of diabetic self-care, poor socioeconomic conditions, poor glycemic control, and peripheral neuropathy.[4,7]

Nearly 56% of diabetic foot ulcers become infected and 20% of these patients with infected foot ulcers land

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up in some type of foot amputation. Therefore, timely intervention of diabetic foot ulcers for prevention and healing is fundamental for foot amputation prevention.[8,9]

Infections in diabetic foot ulcer are mostly polymicrobial, and risk of development of diabetic foot syndrome is more with mycotic infections. However, little data are available on the prevalence of fungal foot infections in patients with diabetes. The fungi involved in diabetic foot ulcers are mainly *Candida* spp. Other species commonly isolated are *Trichophyton* sp. and *Epidermophyton folcculosum*.[10-13]

MATERIALS AND METHODS

This prospective study was carried out in a tertiary care hospital in Central India over a period of 2 years from November 2014 to October 2016. Being a tertiary care and teaching hospital with 900 beds, it attracts patients from different states of Central India. A total of 105 consecutive patients who were admitted for diabetic foot ulcer were enrolled in the study. The study was carried out after obtaining due clearances from the Institutional Ethics Committee. The entire diabetic foot ulcer patients of Wagener's ulcer grade 2-5 admitted in the surgery ward who consented were enrolled in the study. Wagener's ulcer grade 0 and 1 were excluded from the study to avoid inoculation of normal skin flora on culture.

After obtaining written informed consent in local dialect from the patients, detailed history and clinical examination were done. The basic data on age, sex, education, occupation, and socioeconomic status were collected from all patients. Detailed history and clinical examination data were obtained from each study patient. History regarding their present complaint, type and duration of diabetes, and history of ulceration and amputation, treatment history, habits of smoking, alcohol intake, and self-care behavior was noted. After thoroughly cleaning the ulcer with normal saline and debridement of superficial slough, around 5 mm long two tissue pieces were obtained from the ulcer bed. The tissue samples were collected in sterile containers containing normal saline and were immediately transported to microbiology laboratory. The tissue pieces were homogenized. Portions of the tissue specimen were mounted in 10% potassium hydroxide (KOH) and inoculated on Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol and cycloheximide. The samples were incubated at 37°C for 4 weeks. Patients were managed with regular debridement, daily dressing of wound, antibacterial therapy, and glycemic control therapy. Based on macroscopic and microscopic features, growth of colonies was identified and named. The results of this study were compared with other similar studies in literature.

Statistical Analysis

The results of physical examination, clinical tests, microbiological and KOH mount were entered in the master chart using MS EXCEL. Statistical analysis was done using descriptive and inferential statistics using Chi-square test. Software used in the analysis were SPSS (Statistical Product and Service Solutions) 17.0 version and Graph Pad prism 6.0, and $P < 0.05$ is considered as level of significance.

RESULTS

The demographic profile of 105 patients enrolled in our study showed that 75 (71.43%) were males and 30 (28.57%) were females, with a male to female ratio being 2.5:1. The age of the patients ranged from 35 to 84 years, with mean age of 56.06 (±12.46 SD) years. The highest incidence was in the age group 45-54 and 55-64 years accounting for 27.62% each (Table 1). In this study, 20% patients were type 1 diabetics and 80% were type 2 diabetics. 63.81% patients had diabetes for more than 10 years duration and 36.19% patients had diabetes for <10 years duration. 53% patients were hypertensive, 62.8% had peripheral neuropathy, 40% had peripheral vascular disease, 41% had nephropathy, and 43.8% had dyslipidemia. 93 (88.6%) patients had abnormal fasting blood glucose levels while 80 (76.2%) had elevated glycosylated hemoglobin. 40.95% patients had Wagener's grade 2 ulcer while grade 4 ulcers were found in 31.43% (Table 2). There was statistically significant relation between grade of ulcer and duration

Table 1: Details of patients according to their age in years and gender

Age group (years)	Male *n*=75 (%)	Female *n*=30 (%)	Total *n*=105 (%)
35-44	8 (7.62)	9 (8.57)	17 (16.19)
45-54	17 (16.19)	12 (11.43)	29 (27.62)
55-64	25 (23.81)	4 (3.81)	29 (27.62)
65-74	16 (15.24)	5 (4.76)	21 (20)
75-84	9 (8.57)	0 (0)	9 (8.57)
Total	75 (71.43)	30 (28.57)	105 (100)
Mean±SD	50.76±10.17	59.06±12.55	56.96±12.46

SD: Standard deviation

Table 2: Wagner's Grading and its relationship with duration of diabetes

Wagner's grading	Duration of diabetes			χ^2-value	*P* value
	≤10 years	>10 years	Total		
Grade 0	0 (0)	0 (0)	0 (0)	14.82	0.002, S
Grade 1	0 (0)	0 (0)	0 (0)		
Grade 2	17 (16.19)	26 (24.76)	43 (40.95)		
Grade 3	13 (12.38)	20 (19.05)	33 (31.43)		
Grade 4	8 (7.62)	4 (3.81)	12 (11.43)		
Grade 5	0 (0)	17 (16.18)	17 (16.19)		

of diabetes with *P* value 0.02. Weight-bearing area was the most common site (65.71%) of foot ulceration. 54 (51.43%) patients had prior history of foot ulceration while 29 (27.62%) had previous history of major or minor foot amputation. Of the 105 patients, 30 (28.6%) were positive for fungal elements on direct microscopy on 10% KOH mount (Table 3) while fungal cultures on SDA were positive in 21 (20%) patients. Most common fungus isolated was *Candida* sp. (11.43%), followed by *Aspergillus* (3.81%), *Fusarium* sp. (2.86%), and *Trichophyton* sp. (1.90%) (Table 4) No fungal growth was seen in 80% specimen.

DISCUSSION

Diabetes mellitus is a metabolic disease, but it affects nearly all organs of the human body including peripheral nerves, blood vessels, eyes, kidneys, and immune system. Diabetic foot ulcer is a very common complication of longstanding disease. Development of diabetic foot ulcer has many risk factors and has complications such as infection, gangrene, and amputation.[5,14]

Any foot infection is serious in diabetic patients. Diabetic foot infections may vary from superficial paronychia to deep infection involving bone and gangrene. Types of infection include cellulitis, myositis, necrotizing fasciitis, septic arthritis, tendinitis, and osteomyelitis. Diabetic foot infections are associated with increased risk of ulceration. Foot ulceration and infection are leading risk factors for lower extremity amputation.[15-17]

Various risk factors for diabetic foot ulcer have been studied. In a study by Boyko *et al.*,[18] longer duration of diabetes, elevated glycosylated hemoglobin (HBA1c), erythrocyte sedimentation rate and serum creatinine, lower ankle blood pressure, and diagnosed peripheral vascular disease were significant risk factors. In another study by Shahi *et al.*,[4] longer duration of diabetes, tobacco use, insulin use, and rural residence were statistically significant risk factors for the development of diabetic foot ulcers.

Microbiological profile of diabetic foot ulcer patients ranges from chronic bacterial infections to opportunistic fungal infections. Most of the diabetic foot infections are polymicrobial, and microbiology of diabetic foot wounds is variable depending on the extent of involvement.[19,20]

Immunocompromised patients are known to be susceptible for fungal infection and are a major public health concern worldwide. However, fungal infections in diabetic foot ulcers have not been studied extensively. Moreover, hence, treating clinicians focus mainly on bacterial infections in diabetic foot ulcers. This many a times leads to protracted course of illness and longer stay in hospitals.

Our study evaluated prevalence of fungal infections in diabetic foot ulcer patients in Central India. Significant relationships were found between age and sex of the patients. Majority of the patients were male and in age group 45-64 years, with mean age being 56.06 years. In our study, males numbered 2.5 times the females, which is similar to the results of a study by Shahi *et al.*[4] This indicates males with diabetes are more prone to develop foot ulcer and infection than females and it may be attributed to more outdoor activities, poor foot care, and differences in lifestyle.

Most of the patients 67 (63.81%) in our study had diabetes for more than 10 years which is similar to the other studies carried out by Gadepalli *et al.*[21] and Mamo *et al.*[22] Poor glycemic control was observed in majority (76.2%) of our study population, which was similar to results of studies carried out by Zubair *et al.*[23] and Mendes *et al.*[24]

Our study results show that 30 (28.6%) patients were positive for fungus on KOH mount microscopy and 21 (20%) had growth of fungus on SDA culture media. According to few available studies, a variety of fungal infections in diabetic patients with *Aspergillus* species, *Candida* types, and other opportunistic species are found, in which yeasts are more common than others. Opportunistic species are not capable of causing disease in healthy people and only when the host resistance is reduced they can be pathogenic.[11,25] Due to the decreased immunity and increased concentration of glucose in the mucosal membrane and various body tissues and fluids, which increases the proliferation of the normal flora of the body yeast, hence, diabetic patients are prone to develop fungal diseases.[26] Heald *et al.* have reported the association of

Table 3: KOH mount results from 105 diabetic foot ulcer patients

KOH mount	Number of patients (%)
Positive	30 (28.6)
Negative	75 (71.4)
Total	105 (100.00)

KOH: Potassium hydroxide

Table 4: Fungal culture results from 105 diabetic foot ulcer patients

Fungal culture	Number of patients (%)
Aspergillus species	4 (3.81)
Candida species	12 (11.43)
Fusarium species	3 (2.86)
Trichophyton species	2 (1.90)
No growth	84 (80.00)
Total	105 (100.00)

protracted ulceration in diabetic feet with *Candida*, which improved the following systemic antifungal therapy.[27] Misoni *et al.* reported the presence of various species of *Candida* (*Candida parapsilosis*, *Candida albicans*, *Candida tropicalis*, *Candida famata*, and *Candida glabrata*) in interdigital spaces of the same or the other foot of diabetic foot ulcer patients.[28] Our study results showed predominance of *Candida* infection in diabetic foot ulcers with mycotic infections. *Candida* sp. was found in 11.43% patients, followed by *Aspergillus* (3.81%), *Fusarium* sp. (2.86%), and *Trichophyton* sp. (1.90%) Statistically, *Candida* (57.1%) was the most frequently isolated fungus in our study, followed by *Aspergillus* species (19%), *Fusarium* species (14.2 %), and *Trichophyton* species (9.5%).

Similarly, Abilash *et al.*[29] reported very high prevalence of *Candida* spp. (88.8%) and Fata *et al.*[30] reported the *Candida* spp. (52%) as most frequently isolated fungus. Similar to our study, Bansal *et al.*[31] reported *Candida* spp. (50%) as most predominant isolate, followed by *Aspergillus* spp. (35%) and then *Fusarium* spp. In contrast to our study, Wijesuriya *et al.*[32] in Sri Lanka reported *Aspergillus niger* as the most common fungal isolated followed by *C. albicans*.

CONCLUSION

Our study highlights the presence of fungal infection and predominantly candidiasis, in more than a quarter of diabetic foot ulcers, and hence, evaluation of diabetic foot ulcers for mycosis should be a routine. It may help reduce hospitalization, reduce morbidity, and reduce financial burden on the part of the patients. Although some studies have highlighted the benefits of antifungal therapy in diabetic foot ulcer patients, more studies are needed in this field. However, in view of beneficial effects of antifungals on protracted diabetic foot ulcer, mycotic infections should always be evaluated.

Limitations of Study

We could not do subtyping of *Candida* species in the absence of subtyping facility and cost issues.

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