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Research Article

Fungal Infection in Diabetic Foot A Clinicomicrobiological Study

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Abstract

Background: Foot ulcers are a frequent complication of patients suffering from diabetes. Secondary infection of these ulcers is by far the leading cause of amputation of feet and the polymicrobial nature including fungi in few cases of diabetic foot infection has been well documented in literature. The present study sought to reveal the fungal etiology of diabetic foot ulcer and associated risk factors and outcomes in the patients presented to S.L. Raheja Hospital, Mumbai.

Method: A 6 month retrospective study of 100 patients with type 2 Diabetes was carried out to analyze the fungal isolates of all patients admitted with diabetic foot infection presented with Wagener grade 2 to 5 ulcers. Clinical profile associated other diabetic complications and relevant investigations observed and treatment outcome analyzed using standard procedures.

Results: 40% patients were found to have fungal etiology with Candida species being most common among them (40%). Growth of fungi from chronic foot ulcer was associated other diabetic complications, poor glycemic control, high Wagener grade, prior antibiotic therapy and polymicrobial flora. These patients required a higher extent of amputation with prolonged hospital stay and healing period. Few of them needed ICU care as a part of treatment. There was no mortality observed during this observation period.

Conclusion: Fungi are frequently isolated from chronic foot ulcers of many elderly diabetic patients and are associated with other diabetic complications and other parameters including high Wagener grade, prior antibiotic therapy, poor glycemic control as well as poor treatment outcome in form of prolonged hospital stay, major amputations, need for postoperative ICU care and prolonged healing period. Identification of such cases on the basis of these characteristics along with early mycological evaluation and consideration of appropriate antifungal therapy may help us to prevent poor treatment outcomes.

Keywords: Fungal Infection; Diabetic Foot; Foot Ulcers

Introduction

Patients with diabetes represent a unique group of individuals who appear more prone to develop infections than others. Lifetime risk to a person with diabetes for developing a foot ulcer could be as high as 25% [1]. The alarming fact is that India has more people with diabetes then any other country [2,3]. The incidence of foot problems and amputation remains very high, accounting for up to 20% of diabetes related hospital admissions [4,5]. Infected foot ulcer is a common cause of morbidity in diabetic patients, ultimately leading to dreadful complications like gangrene and amputations. The decision regarding proper management of diabetic foot infection is a difficult one and still a matter of debate. While optimal therapy is yet to be established, most authors agree that the management of these infections require isolation and identification of the microbial flora, appropriate antibiotic therapy according to the sensitivity pattern, precise selection and identification of the chronic complications and proper surgical intervention for these complications [6]. Most diabetic foot infections are polymicrobial in nature and mixed organisms are frequently encountered including fungi in some cases [6-11]. However, the spectrum of microorganisms depends mainly on microbial flora of the lower limb, metabolic factors, foot hygiene and the previous use of antibiotics [12]. Fungal infection of diabetic foot may be associated with some specific conditions and can affect the patient management and outcome.

In view of above facts, this retrospective study was carried out of 100 diabetic foot patients to determine the incidence of fungal infection in diabetic foot and relevant associated conditions.

Purpose of study

Bacteriology of diabetic foot ulcers has been studied by many investigators, however, there is paucity of reports on the incidence of fungal pathogens in deep tissue. The present study was undertaken to evaluate the incidence of pathogenic fungal species isolation in

diabetic foot ulcer as well as to explore risk factors for the development of fungal infections in foot ulcer and observe the outcome pattern of fungal infected diabetic foot ulcers which may help us to consider appropriate early empirical antifungal therapy.

Materials and Methods

A retrospective study was carried out on 100 type 2 diabetic patients with foot ulcer during the period of August 2008 to January 2009 admitted in S.L.Raheja Hospital and Diabetic Research Centre, Mahim, Mumbai.

The criteria for inclusion were type 2 diabetic patients with foot ulcer Wagner grade 2 or more. Diabetic foot ulcer patients who on antifungal treatment were excluded from study.

A semi structured questionnaire was developed to record the detailed medical history, examination and investigation details. Details specifically regarding duration and treatment of Diabetes, foot ulcer onset and associated complaints, personal habits like smoking and alcohol inquired. Neuropathy was assessed by the ability to sense touch with a 10 gm monofilament (Semmes Weinstein Filament) and tuning fork [13], Lower Limb Ischemia assessed by pulsations of dorsalis pedis and posterior tibial arteries [13], Ankle-Brachial Index and arterial Doppler.

Ulcer was classified according to Meggit Wagner Classification System [14] (mentioned below).

On admission, routine investigations considered in form of CBC, urine routine examination, Creatinine, FBS, PP2BS, Local Xrays and deep ulcer debrided tissue biopsy was collected with sterile method and immediately transported to the microbiology laboratory for further processing. The tissue specimen inoculated to Sabouraud's Dextrose Agar and incubated at room temperature for 4 weeks and evaluated frequently for growth of fungal culture. Fungal culture were identified by microscopic as well as macroscopic appearance of colonies pattern on Hi-Chrome Dish Agar. In this retrospective study, needed surgical intervention as well as stay in hospital and need for ICU care noted. Follow up visit at the end of 4 weeks after discharge considered and healing in form of appearance of complete healthy granulation tissue considered.

The data was collected and analyzed using descriptive statistics. Statistical significance was evaluated using Fisher's Chi-square test.

Wagner's classification

- Grade 0: Pre Ulcer. Skin is intact, no open lesion.
- Grade 1 : Superficial ulcer without penetration of subcutaneous fat layer.
- Grade 2: Full thickness ulcer. Penetrates through fat, tendon or joint capsule.
- Grade 3: Deep ulcer which may or may not probe to bone, with abscess, osteomyelitis or joint sepsis.
- Grade 4: Gangrene of a geographical area of the foot.
- Grade 5: Gangrene to the extent that foot is beyond salvage.

Results

In 100 diabetic foot patients studied, 74 (74%) were men and 26 (26%) were women, male-to-female ratio being 2.84. The age ranged from 42 to 76 years. The mean age for patients having fungal diabetic foot infection was 65 years in our study. Among those having fungal diabetic foot infection, a majority of patients (55%) were in the age group 61 to 70. History of trauma present in 74 patients, 30 patient (40.54%) of them found to have fungal infection in foot. Total 27 patients has history of ulcer for more then 1 month, out of them 25 patient (92.59%) had fungal diabetic foot (p value=0.0001). History of 1 month or less duration found in 63 patients and 15(23.80%) had no growth of fungi. Absence of fever was noted in 58 patients, 34(58.61%) of them detected to have fungal infection in foot ulcer. Long standing Diabetes (≥10 years) found in 55 patients, 32(58.18%) of them had fungal diabetic foot. History of fever was absent in 58 patients, out of them 34 (58.81%) had fungal infection (p=0.0001). Personal habit inquired in form of smoking or alcohol and it was seen that 40 patients were smoker, out of them 15 patients (37.50%) had fungal diabetic foot. History for chronic alcohol use was present in 33 patients and 11 out of them (33.33%) had fungal growth in foot ulcer (Table 1).

For glycemic control, 63 patients were taking Oral hypoglycemic agents, 24(38.09%) of them had fungal infection in diabetic foot. Total 25 patients were on both OHA plus Insulin; 16 (64%) of them grew fungi in ulcer tissue growth. Poor glycemic control in form of HbA1c > 8 was seen in 66 patients and 32patients (48.48%) of them had fungal infection of diabetic foot (p=0.001). Total 68 patients had taken antibiotic course in intravenous or oral form in past for diabetic foot, 38 patients (55.88%) had fungal growth out of them (p=0.001). Majority of patients (72) having Grade 2 ulcer and 18 (45%) of them had fungal diabetic foot, whereas 23 patients were from grade 3 and among them 19 patients (82.6%) had detected to have fungal infection in diabetic foot. Ulcer grade >2 is significantly associated with fungal foot infection (p =0.0001).

Other associated complications of diabetes were also assessed with reliable parameters (Table 2). Neuropathy assessed with filament test (Semmes-Weinstein (10-g) nylon filament test) in all patients. Sensory neuropathy found in 71 patients, out of 71,

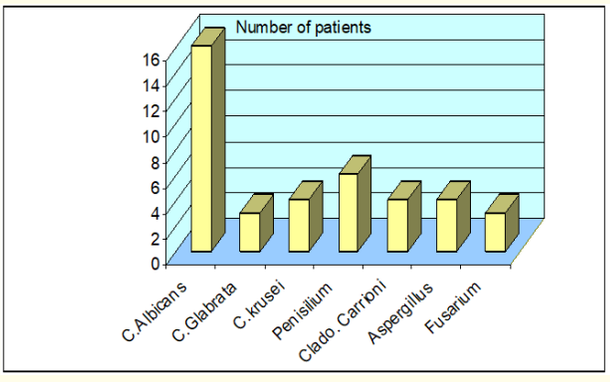

Figure 1: Number of Fungal Isolates.

Clado. Carrion = Cladosporium carrioni, C. Albicans= Candida Albicans, C. Glabrata = Candida Glabrata, C. krusei = Candida krusei

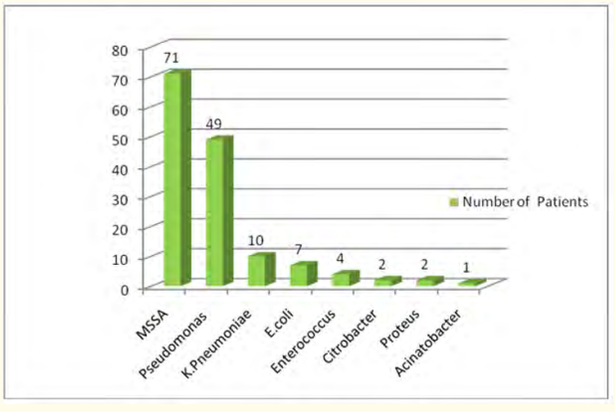

Figure 2: Associated Bacterial isolates.

MSSA = Methicillin resistant staph aureus.

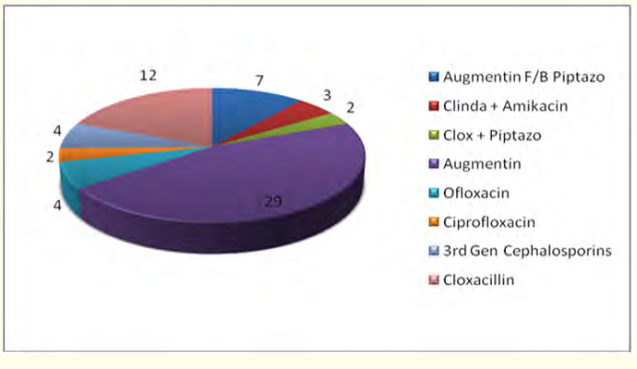

Figure 3: Prior Antibiotics used.

Patient characteristics	Number of patients	Patients with fungal isolation
Age		
< 60 years	43	16 37.2%)
≥60 years	57	24(42.10%) p=0.6829
Sex		
Male	74	31 (41.84%)
Female	26	9 (34.61%) p=0.642
H/O trauma		
Present	74	30 (40.54%) p=1.000
Absent	26	10(38.46%)
Duration of Ulcer		
≤ 1 month	63	15 (23.80%)
>1 month	27	25(92.59%) p=0.001
H/O Fever		
Present	42	6(14%)
absent	58	34(58.61%) p=0.001
Duration of Diabetes		
< 10 years	55	24(43.63%)
≥10 years	45	16(35.55%) p=0.5385
Treatment history		
OHA	63	24(38.09%) p=0.674
Insulin	25	16(64%)
O+I	2	0
H/O Smoking		
Present	40	15 (37.50%) p=0.835
Absent	60	25(43.28%)
H/O Alcohol		
Present	34	11(32.35%) p=0.397
Absent	66	29(43.93%)
Wagner's Grade		
II	72	18(45%)
III	23	19(82.5%) p=0.001
IV	5	3(60%)
HbA1c		
≤8	24	8(33.33%)
>8	66	32(48.48%) p=0.2365
Anemia		
<12 gm %	71	34(47.88%) p=0.0137
≥12 gm %	29	6(20.68%)

Table 1: Patient profile and fungal isolation.

Associated complications	Number of patients	Fungi isolation
Neuropathy		
Present	71	30(42.25%) *p*= 0.508
Absent	29	10(34.48%)
PVD		
Absent	66	19(28.78%) *p*=0.0023
Present	34	21 (61.76%)
IHD		
Present	29	24 (82.75%) *p*=0.0001
Absent	61	16(26.22%)
Retinopathy		
Present	15	4(26.66%) *p*=0.3919
Absent	85	36(42.35%)
Nephropathy		
Present	23	17(73.91%) *p*=0.002
Absent	67	23(34.32%)
Anemia		
<12 gm %	71	34(47.88%) *p*=0.0137
≥12 gm %	29	6(20.28%)
Proteinuria		
Present	87	38(43.69%) *p*=0.489
Absent	13	2(15.38%)
UTI		
Present	35	23(65.71%) *p*=0.0002
Absent	65	17(26.15%)
Microbiology		
Monomicrobial	48	16(33.33%) *p*=0.132
Polymicrobial	52	24(46.15%)

Table 2: Associated complications and fungal isolation.

PVD: Peripheral Vascular Disease; IHD: Ischemic Heart Disease; UTI: Urinary Tract Infection

Associated bacteria	Number of patients
S. aureus	71
Pseudomonas	49
K. pneumonia	10
E. Coli	7
Enterococci	4
Citrobacter	2
Proteus	2
Acinatobacter	1

Table 3: Associated bacteria.

Prior Antibiotics	Number of Patients	Fungal growth
Amoxicilin+clavulanate F/B Pipracillin+tazobactum	7	6 (85%)
Clindamycin+Amikacin	3	3 (100%)
Cloxacillin +pipracillin+tazobactum	2	2 (100%)
Amoxicillin+clavulanate	29	17(58.6%)
Ofloxacin	4	1 (25%)
Ciprofloxacin	2	0
Cephalosporins (3rd Gen)	4	4 (100%)
Cloxacillin	12	3 (25%)

Table 4: Prior antibiotics and Fungal growth.

Patient characteristic	Number	Patient with fungi isolation
Type of surgery required		
Below knee Amputation	41	29(70.73%) P = 0.0001
Ray's Amputation	16	3 (18.75%)
Toe Amputation	1	0
Debridement	42	8 (19.04%)
Hospital stay		
>7 days	56	34(60.71%) P = 0.0262
≤7 days	44	16(28.57%)
ICU care required	10	8(80%) P = 0.0132
Healing Period		
> 4 weeks	42	33 (78.57%)

Table 5: Outcome of Fungal diabetic foot

30 patients (42.25%) had fungal infection in foot ulcer. PVD assessed with ABI (Ankle-Brachial Index) and pulsation of distal lower limb arteries as well as Arterial Doppler, we got the result that 34 patient had PVD, out of them 21 patients (64.70%) having fungal growth in diabetic foot (p=0.0023). Ischemic heart disease was associated in 29 patients, 24 (82.75%) out of them had fungal growth in diabetic foot (p=0.0001). Retinopathy was found in 15 patients and among them 4 patients (26.66%) had fungal infection of foot ulcer. Nephropathy was seen in 23 patients, among them 17 patients (73.91%) had fungal growth detected in foot ulcer. Oral steroid therapy was going on in 5 patients for Rheumatoid Arthritis or Bronchial Asthma, all of them grew fungal species in foot ulcers. Anemia has significant association noted (p=0.0137), 71 patients had anemia with Hb < 12 gm%, out of them 34 (47.88%) fungal growth in diabetic foot, whereas 29 has Hb of ≥12 gm%, in them 6 (20.68%) had fungal infection of foot ulcer. Proteinuria was found

in 87 patients and 38 (43.69%) had fungal growth in diabetic foot tissue culture. Urinary tract infection was observed in form of microscopically visible pus cells and proteinuria in 35 patients, out of them 23 (65.71%) having fungal diabetic foot (p=0.0002).

The outcome of fungi infected diabetic foot patient was assessed in form of type of Surgical intervention, Hospital stay period, any need for post operative ICU care and healing period time (Table 3). All patients screened as a routine protocol for HIV also and all were seronegative. We found that 41 patient required major amputation in form of below knee amputation, of them 29 patient (70.73%) having fungal infection of diabetic foot ulcer (p=0.001). Ray's amputation was needed in 16 patient, 3 (18.75%) of them were having fungal infection of diabetic foot. Surgical debridement was done in 42 patients and among them 8(19.04%) patients had fungal infection. Prolonged hospital stay i.e. > 1 week was observed in 56 patients, 40 patients (71.42%) out of them were having fungal infection of foot ulcer (p=0.001) and 16 patients(28.57%) had no fungal growth but they required prolonged hospital stay. Postoperative ICU care was needed in 10 patients, 8 out of them had fungal infection in diabetic foot (32% of total fungal diabetic foot, p value=0.0132). There was no mortality observed among included cases. Follow up after 4 weeks of hospital discharge considered and observed that 42 patients had taken more than 4 weeks for complete healing, out of them 33 patients (78.57%) had fungal infection. Out of these 33 fungal infected diabetic feet, 7 patients has taken more than 6 weeks for healing and 4 out of them had Aspergillus and 3 had Penisillium marneffei in culture.

Microbiological observation

Fungal growth was found in 40 patients (40%) out of total 100 diabetic foot patients. Fungal species were identified with microscopic examination as well as colony characteristics and it was observed that majority of them were *Candida Albicans* species i.e. 16 patients (40%). *Candida krusei* in 4 patients (10%), *Cladosporium carriani* in 4 patient (10%) and *Aspergillus Niger* cultured in 4 patients(10%). *Penisillium Marneffei* was found in 6 patients (15%). *C. Glabrata* and Fusarium, each has been found in 3 patients (7.5%) associated. Simultaneous pyogenic culture was also done as a part of routine protocol, among them 52 has polymicrobial growth (>1 bacterial species) whereas 48 patients has monomicrobial bacterial growth. We observed that 24 patient (46.15%) had fungal growth associated with polymicrobial growth (p=0.1527). Species of bacteria identified which showed that incidence of *Staphylococci aureus* (MSSA) was maximum (71 patients),followed by Pseudomonas, *K. pneumoniae, E.coli, Enterococci, Proteus, Citrobacter* and *Acinatobacter* as mentioned in table (Table 3). Antibiotic used before admission in 63 patients also considered and we found that 36 patients has grown fungi in foot tissue culture (p=0.0001). In 12 patients more then one broad spectrum antibiotic combinations has been used and among them 11 patients (91.66%)has grown fungi subsequently (Table 4).

Discussion

Foot infections are a major cause of morbidity in people with diabetes [16]. Devitalized tissue is the site where the microorganisms responsible for the non-healing ulcers inflict damage. Diabetic foot infections are usually polymicrobial in nature, includes fungi in few cases [15,16]. Though there are a few reports on the incidence of fungal pathogens in diabetic foot infections [10,17,18], there is paucity of published work on the incidence of fungal infection in deep tissue samples of diabetic foot. Our study was initiated with purpose of to evaluate the incidence of pathogenic fungal species isolation in diabetic foot ulcer and to assess relavant factors, included 100 patient of diabetic foot patients, out of them 40 had grown fungal species and majority of them are candida. The study conducted by Chincholikar and Pal showed similar kind of results [10]. Heald., *et al*. [19] has also reported the association of candida species with diabetic foot which improved after antifungal therapy.

The presence of various species of candida was reported in diabetic foot patients by Missoni*., et al.* [18].This is in agreement with the findings of the present study showing that among the fungal pathogens isolated from deep tissues, 57.5% were Candida species. The most common candida was *C. parapsilosis* in the study by Missoni., *et al.* [18] whereas in present study it is *C. Albicans.* Study by *Chincholikar*., *et al*. [10] has also shown similar results. The presence of Aspergillus flavus and Fusarium soloni in foot ulcers of diabetic patients has been reported by Bander., *et al*. [17] and Lai., *et al.* [20]. Total 6 cases of *Penisillum marneffei* has been isolated in our study which is rarely found in India. *P. marneffei* is more common in immunocompromised patients especially in HIV infected patients from northeastern part of India [21].

Fungal foot infection was seen more commonly in patients with prolonged foot ulcer history in present study. The possible explanation for the same is prolonged wrapping of foot and over application local ointment as a part of dressing. As known from literature, on covering the skin with dressing, selective antibacterial and immunomodulating action of antibiotics favour growth and replication of yeast [14,15]. In Indian scenario, walking bare foot in many patients as well as use of improper shoes which may cause

repeated trauma to the foot and cause ulceration and local infection. This may get Fungi from soil and become chronic non-healing ulcer with fungal infection.

Diabetic foot infection having multifactorial etiology with many risk factors. Foot ulcer and subsequent infection may be the result of microvascular as well as macrovascular complications. In present study, associated risk factors and co morbidities were assessed to correlate with the diabetic foot fungal infection. In study by Fatma Al-Maskari., *et al.* [22] showed well that there is significant association between diabetic foot ulcer and other variables like male gender, old age, prolonged disease duration, poor glycemic control, peripheral neuropathy and vasculopathy. Our results are in agreement with them as in our study also majority of the patients are with mean age of 65 years males, Diabetes duration was prolonged,HbA1c was >8 in majority of the patients as well as most of them are having associated PVD or IHD (Table 1,2).. The foot complication was also significantly associated with other microangiopathic complications such as diabetic retinopathy and presence of microalbuminuria [22]. In present study, similar results were observed. In our study, Nephropathy was associated with fungal foot ulcer and with poor outcome in form of major amputation and prolonged hospital stay. Similar result was demonstrated by Wolf., *et al*. [23] saying that there was a strong association between the degree of renal function impairment and diabetic foot ulcer in their observational study. Data show that Diabetic nephropathy with diabetic foot undergo a higher incidence of amputation. It may be contributed by microneuroangiopathy.

Diabetic foot ulcers were polymicrobial in almost half of the cases in our study, many of them having associated fungi. The most frequent isolated organisms from the lesions are Staph aureus (MSSA), Pseudomonas aeruginosa, Klebsiella and Proteus Most of them invade from local skin flora or soil related. Few of them may get entry into the wound via unsterile local dressings. Similar results were observed in study done by Sharma V.K., *et al* [24]. Those who have received broad spectrum antibiotics before admission shown to grow fungi in most of the cases. It may be because of killing of bacteria which might inhibits growth of some fungi in their presence. It has been also found that deeper ulcer tends to grow fungi, that may be because of prolonged dressings and local wet environmental condition favours fungal growth. Ekta Bansal., *et al.* has also shown polymicrobial flora with fungi in few cases in their study at Chandigarh, India [25] and as Wagener's ulcer grades increased, the prevalence of isolates also increased.

In our study, the outcome of fungal diabetic foot was assessed with type of surgery required, extent of amputation, hospital stay, need for ICU care and healing period. There was no mortalities observed in this study. Poor outcome was demarketed by higher extent of amputation e.g. Below Knee Amputation, Prolonged hospital stay > 7days or need for ICU care. In present study, the poor outcome was seen in associated with many factors apart from fungal infection, like Multiorgan involvement (peripheral neuropathy, vasculopathy, nephropathy, retinopathy, and ischemic heart disease), duration of diabetes and poor glycemic control, higher Wagner's grade, Anemia and associated bacterial flora. In addition to aforementioned factors, delay in healing was also observed in majority of infected diabetic feet in association with certain specific fungi like Aspergillus and Penicilium.

To know the risk factors which may predict the outcome of diabetic foot ulcer with special reference of fungal infection, Binary logistic regression is used from SPSS version 16. After applying logistic regression to know the factors affecting the presence of fungal infection in diabetic foot, it is observed that Duration of ulcer, Wagener's grading, Alcohol consumption and History of antibiotic usage is strongly contributing to it. Out of the all these factors Duration of ulcer contributes 59.7%, Duration of ulcer and Wagener's grading contributes 61.8%, Duration of ulcer and Wagener's grading and alcohol consumption contributes 63.9%, duration of ulcer, Wagener's grading, alcohol consumption and past history of antibiotic usage contributes 66.1%. In other words, we can say that 66% of the variance presence of fungal infection in diabetic foot were explained by duration of ulcer, Wagener's grading, alcohol consumption and past history of antibiotic usage. With help of this parameter we can predict association of fungal infection and may consider proper empiric therapy prior to culture confirmation.

Conclusion

Diabetic foot infections are polymicrobial in nature associated with high incidence of Fungi. Candida species are the predominant isolates among fungal pathogen. In elderly patients with chronic foot ulcer, poor glycemic control, high Wagner's grade, associated micro and macrovascular complications (e.g. neuropathy, nephropathy, retinopathy, IHD, PVD) and prior antibiotic use are significantly associated with fungal isolates from diabetic foot. Most of these patients had poor treatment outcome and prediction of associated fungal foot infection can be done with the help of parameters like duration of ulcer, Wageners grade, alcohol consumption and prior antibiotic usage. The present study signifies the need of identifica-

tion of such cases on the basis of these characteristics along with early mycological evaluation and consideration of appropriate antifungal therapy which may help us to prevent poor treatment outcomes.

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