Original Article

Frequency and Antifungal Susceptibility of Candida Albicans and Non-albicans Candida Isolates from Diabetic Foot Ulcer at Tertiary Care Hospitals of Peshawar

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ABSTRACT

OBJECTIVE: To study the frequency and antifungal susceptibility of the Candida albicans and non-albican species in diabetic foot infections from samples collected in a tertiary care hospital of district Peshawar.

METHODOLOGY: This cross-sectional study was conducted from March 2020 to February 2021 in Khyber Teaching Hospital and Hayatabad Medical Complex, Peshawar. Specimen for culture swabs or tissue from diabetic foot ulcers was taken using aseptic methods. If pus was absent in the wound, ulcer scraping was collected. One tissue sample was soaked in 10% KOH for microscopy, while the second sample was used for fungal culture and sensitivity using Sabouraud dextrose agar.

RESULTS: Of the 600 samples, 200 patients had diabetic foot ulcers with positive fungal culture from Males 102(51%) and Females 98(49%). The age range was 40-78 years. The frequency of C. albicans, C. dubliniensis, C. famata, C. glabrata, C. parapsilosis, and C. lusitaniae was 23(11.5%), 27(13.5%), 19 (9.5%), 19(9.5%), 88(44%) and 24(12%). C. albicans was the most common fungal species. Antifungal susceptibility testing was done, and resistance to drugs like Amphotericin, Caspofungin, Fluconazole, Flucytosine, Itraconazole, Micafungin, Voriconazole which was 6.5%, 16%, 25.5%, 5.5%, 3%, 22.5%, 21% respectively. Resistance to Fluconazole, Micafungin and Voriconazole was the highest among all commonly used antifungal drugs.

CONCLUSION: Fungal infection in diabetic foot ulcers usually does not respond to antibiotics. Candia albicans and non-albicans spp are also associated with diabetic foot ulcer infection and inflammation, and these fungi have the highest resistance to commonly used antifungal agents.

KEYWORDS: Diabetes mellitus, fungal susceptibility, antifungal, diabetic foot ulcer.

INTRODUCTION

Diabetes is one of the most challenging public health problems of the 21st century, with its epicentre being in Asia. [1] Pakistan has the fourth highest number of diabetic patients in the world. The prevalence of diabetes in Pakistan is approximately 6.8% [2]. The cases are expected to rise to 26.2 million by 2030 and 37.1 million by 2045[3]. Diabetes-affected people are more prone to fungal infection and have marked morbidity and mortality. Due to hyperglycemia, infections are more common in diabetic patients as their immunity is badly affected[4]. Diabetic foot ulcer (DFU) is a common complication in diabetic patients, which could lead to amputations[5]. The prevalence of DFU is reported to be approximately 13%. In a study from Kenya, DFU has a prevalence of 7.3%, while in Tanzania, the prevalence is reported to be 4.6%, while in Egypt, it is 6.2% [6,7]. Significant risk factors for DFU are impaired host immunity, prolonged diabetic wounds, traumatic ulcer, peripheral vascular disease, neuropathy, previous amputation, low socioeconomic status, and lack of personal hygiene and education[8]. The amputation rate in DFU is 17%, while the recurrence rate of DFU is more than 40%, leading to higher mortality in patients with DFU [9]. Foot ulcers are prone to infection by various microbes[10], and timely diagnosis and management with antimicrobial therapy are crucial[11].

Since the last 20 years, the frequency and occurrence of T2DM (Type II-DM) have increased manifolds in developing countries, as a result of which more individuals are becoming prone to diabetic foot ulcers, consequently resulting in complications[12,13].

The pathogenic fungus that causes DFI (Diabetic Foot Infection) include *Candida, Aspergillus, Zygomycetes Dermatophytes, Fusarium & Malassezia* [14]. Almost 90% of infections are caused by five *Candida* spp. named as Candida albicans, *Candida glabrata, Candida tropicalis, Candida parapsilosis* & *Candida*

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krusei. C. albicans mainly occurs in the GI tract, mouth, vagina, mucosal membrane, skin, foot & oropharynx. *C. albicans* is a commensally harmless but opportunistic pathogen. [5] There are many previous studies in which antifungal resistance is reported among Candida albicans and non-albicans Candida spp species. The resistance pattern is confirmed by culturing wound samples collected from the patients.

The mycology of diabetic foot lesions has not been given much importance compared to bacterial infections. In cases of non-healing diabetic foot ulcers, the causative agent was found to be an underlying fungal infection, so this study was done to rule out the frequency and antifungal sensitivity of Candida and non-candida albicans species and to have an accurate management of non-healing diabetic foot lesion.

The current study focused on the neglected pathogen *Candida spp*. in DFI. The treating physicians commonly ignore the fungal involvement of infections. Therefore, the ongoing study highlights the prevalence of *C. albicans* and *non-albicans* in DFI, explaining their antifungal sensitivity pattern to set up the best empirical therapy for DFI and to reduce the patients' financial burden on DFI due to prolonged hospital stays & excess use of antibiotics, which eventually would reduce the frequency of lower limb amputation.

METHODOLOGY

This cross-sectional study was conducted from March 2020 to February 2021 in Khyber Teaching Hospital, Peshawar and Hayatabad Medical Complex, Peshawar, Pakistan. This study was designed for hospital-based data collection. Patients enrolled from outpatient departments and inpatient Departments having any diabetes and presenting with acute and chronic non-healing foot ulcers were admitted to the two tertiary care hospitals of Peshawar. Chronic foot ulcers were defined as wounds that did not heal within three months. The patients were categorized according to age groups, gender, marital state, residence and profession.

All diabetic patients who had foot ulcers were included. Diabetic foot ulcer patients taking the following treatments: antifungal therapy, antibiotics, chemotherapy, and corticosteroids were excluded from the study. The sample size was calculated using the WHO sample size calculator. Non-probability convenient sampling was used for this study. Simultaneously, two tissue samples from the foot area of DFU patients were taken. Deep tissue samples were collected from each patient using sterile cotton swabs after debriding and cleansing the wound with normal saline 0.9%. Then, they transferred to the microbiology laboratory at Hayatabad Medical Complex – Khyber Girls Medical College within 2 hours. Aseptic methods were used to collect swabs and ulcer tissue samples from diabetic patients. If pus was absent in the wound, ulcer scraping was collected. Collected tissues were examined microscopically in the laboratory. The two collected samples were used for two different protocols such as; one tissue sample was soaked in 10% KOH, and a microscopic examination was done, while fungal culture and sensitivity were analyzed in the second sample using sabouraud dextrose agar (SDA) (**Table I**) supplemented with chloramphenicol and cycloheximide. The samples were incubated at 30°C-37°C during the examination period. The plate was observed after 18 to 20 hrs. The appearance of the colony was studied, and further identification was based on microscopy. Fungal growth was further sub-cultured on CHROM agar *Candida*, and the species of Candida were identified. Disk diffusion testing was performed strictly according to CLSI standards. Thermo Scientific, Oxoid, UK, provided antifungal-impregnated paper. Zone diameters were read using a ruler, and values were rounded to the closest millimetre.

Data Analysis: SPSS 22 was used for data analysis. Mean and standard deviation were calculated for numerical variables, i.e., the age of the patients. At the same time, frequencies and percentages were used for categorical variables, i.e., the gender of the patient and resistant cases.

RESULTS

Out of 600 samples of patients having diabetic foot ulcers, only 200 samples were positive for fungal infection of diabetic foot. All of these patients presented with type II diabetes. Of these, 51% (102) were males and 49% (98) were females.

The age range of these patients was from 41-78 years. No case of diabetic foot ulcers aged below 41 years was reported in either of these tertiary care hospitals in Peshawar. The highest number of diabetic foot infections were found in the age group 60-70 years, which had a total no of 85 patients, followed by the age group 51-60 years (62) patients, 70-78 years (45) patients and 41 to 50 years had (18) patients. It was found that the development of diabetic foot increases with the duration of the disease. Most of the patients with diabetic foot infections had long-standing diabetes for more than 10-15 years.

Patients were also checked for blood parameters: blood sugar, complete blood counts, and HBA1c. Results showed that most patients had higher levels of each test than normal. However, the difference between normal and higher blood sugar levels was much more significant. About 121 (61%) patients had higher fasting blood sugar levels, while 187 (94%) had a higher Hb A1C.

The growth of *Candida albicans* was observed on CHROM agar (**Figure Ia**). Antifungal agents in various fungal species were observed on SDA (**Figure Ib**). The positive fungal culture samples were further analyzed for the determination of fungal species; multiple species were found, including *C. albicans*, *C. dubliniensis, C. famata, C. glabrata, C. parapsilosis,*

and *C. lusitaniae* with frequency 23, 27, 19, 19, 88 and 24, respectively. Results of frequency and percentages of various strains of *Candida* are shown in **Table I**.

Table I: Fungal species and their frequency in diabetic patients

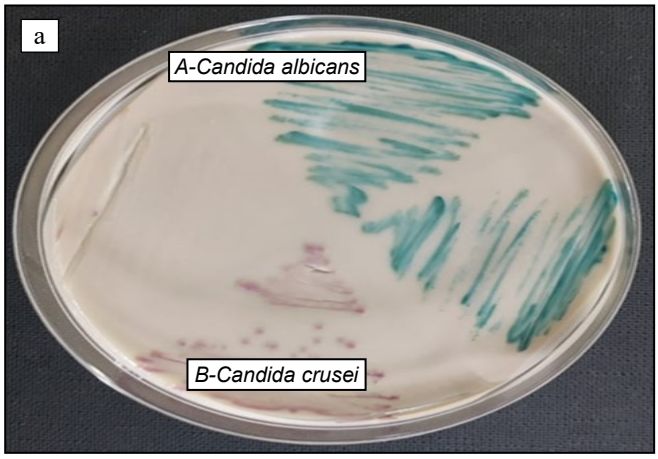
Fungal Species	Frequency	Percentage
C. albicans	88	44%
C. dubliniensis	23	11.5%
C. famata	11	5.5%
C. glabrata	31	15.5%
C. parapsilosis	23	9.5%
C. lusitaniae	24	12%
Total	**200**	**100%**

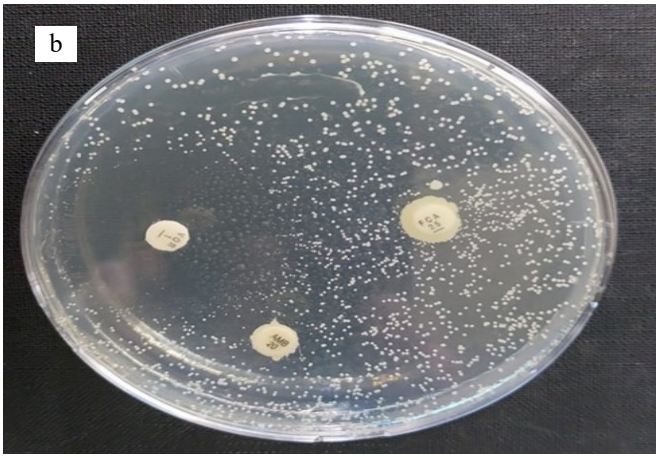
Antifungal susceptibility testing was done. Resistance to drugs like Amphotericin, Caspofungin, Fluconazole, Flucytosin, Itraconazole, Micafungin, and Voriconazole was observed. Its frequency and percentages are shown in **Table II**. Resistance to Fluconazole, Micafungin and Voriconazole was the highest among all commonly used antifungal drugs.

Table II: Antifungal agents and their resistance in diabetic patients with infected DFU

Antifungal Agents	Frequency	Percentage
Amphotericin	13	6.5%
Caspofungin	32	16%
Fluconazole	51	25.5%
Flucytosine	11	5.5%
Itraconazole	6	3%
Micafungin	45	22.5%
Voriconazole	42	21%
Total	**200**	**100%**

Figure 1a: A and B show growing colonies of Candida spp. on CHROM agar, Figure 1b: Antifungal susceptibility test





Among *Candida spp, Candida Albicans* was highly prevalent among diabetic patients. Results of all the fungal species and their sensitivity among various antifungal agents are shown in **Table III**. The highest sensitivity was found against Micafungin and Voriconazole, followed by Flucytosine and Amphotericin. Moreover, *Candida lusitaniae* was the second most prevalent species of *Candida* in diabetic patients, and it was found to be susceptible to Flucytosine and Amphotericin.

Table III: Fungal species and resistance to antifungal agents in diabetic patients

	Ampho tericin	Caspo fungin	Flucon azole	Flucyt osine	Itracon azole	Mica fungin	Voricon Azole
C. albicans	14	4	8	16	0	22	22
C. dubliniensis	2	2	2	6	2	7	2
C. famata	2	1	1	9	1	6	5
C. glabrata	2	2	0	3	0	4	7
C. parapsilosis	4	2	0	3	1	4	5
C. lusitaniae	8	2	0	8	2	2	1

DISCUSSION

The current study assessed the frequency of patients with fungal infections of diabetic foot ulcers in Peshawar, Pakistan. Data was collected from diabetic patients under treatment or management in the district tertiary care hospitals. This study aimed to find the prevalent fungal species and their antifungal resistance in diabetic foot infections.

Out of a total of 600 samples, 200 samples had fungal etiology. *Candida albicans* (44%) was the most prevalent species of fungus in foot ulcers, followed by various non-albicans species such as; *C. dubliniensis* (11.5%), *C. famata* (5.5%), *C. glabrata* (15.5%), *C. parapsilosis* 9.5%) and *C. lusitaniae* (12%).

Knowing the antifungal susceptibility has a crucial role in managing patients with diabetic foot. The highest antifungal agent susceptibility was recorded against *Candida* species, including Micafungin, Voriconazole, Flucytosine and Amphotericin B [5].

The SENTRY international fungal surveillance program also suggested that most Candida species

are susceptible to three main antifungal agents: Echinocandins, Amphotericin B, and Triazoles[15].

Most of the antifungal drugs are highly resistant to Candida species. Flucytosine is one of the common antifungals that generally inhibit pyrimidine metabolism and DNA synthesis in fungus cells and **is** usually used in combination with other agents. Our study showed higher sensitivity to Flucytosine in *C. albicans* species and *non-albicans* species[16].

Amphotericin is the most commonly used antifungal drug for treating fungal infections of diabetic foot. In our study, *Candida albicans* and *C. lusitaniae* were resistant to Amphotericin in 07% and 4% of the patients.

In another study conducted in India, Pakistan, South Africa, and Venezuela, 93% of isolates were resistant to fluconazole, 35% to amphotericin B, and 7% to echinocandins; 41% were resistant to 2 antifungal classes, and 4% were resistant to 3 classes [17].

In a study done in India, approximately 21% were found to be resistant/intermediate, and 20% resistance was observed against voriconozole and 5% against fluconazole.

In non-albicans, 16% resistance was observed against flucytosine, 14% resistance was observed against fluconazole, 03% against voriconazole and 3% against caspofungin[18].

In another study, fungal culture was positive in 17.38% of patients, of which 75% had *Candida* species. In another study, there was 9.3% resistance against fluconazole. The most typical organism with resistance to fluconazole was *Candida auris*[19].

In a study done on DFU, fungal etiology was present in 48% of patients. *Candida* species were found in all the isolates. Other species found were *Candida tropicalis* (34.6%), *Candida albicans* (29.3%), *Candida krusei* (16.0%) *Candida parapsilosis* (10.6%) and *Candida glabrata* (9.33%). All the species were found to be susceptible to amphotericin B [20].

In a study done, 64.7% of *Candida* spp. were found to be susceptible to antifungal susceptibility, while 23.5% were found to be resistant[21].

In another study, *Candida* species showed more resistance to clotrimazole (82%), fluconazole (64%) and miconazole (44%)[22].

The most typical species in all age groups was *C. albicans* (65%), followed by *C. glabrata* (19%) and *C. parapsilosis* (10%). In older people, *C. glabrata* was the most common, while *C. parapsilosis* was found mainly in young children[23].

In a study done in Egypt, the antifungal susceptibility showed resistance rates of *Candida* spp. to fluconazole and voriconazole were 13.1% and 9.8%. Only 4.1% were resistant to caspofungin[24].

Uncontrolled fungal infection can cause prolonged pyrexia and other serious consequences[25].

In routine practice, antifungal treatment is not usually given in diabetic foot ulcers, and patients are given high doses of antibiotics. However, some infections do not respond to antibiotic therapy, and a low-grade inflammation with mild fever remains. So, this study shows that fungal involvement is present in diabetic foot. The limitation of this study was a smaller sample size. Further research needs to be carried out on larger sample sizes.

CONCLUSION

There is fungal involvement by both *Candida albicans* and *non-albicans* species in diabetic foot ulcers. There is a need to consider and explore fungal infections in the differential diagnosis of DFU infections. We must develop a testing and treatment protocol for fungal infections and find effective ways to control drug-resistant fungi.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Ali H: Experiment conduction and data collection.
Mushtaq N: Conceived and designed experiments, prepared figures and tables, and final approval.
Khaliq S: Statistical analysis.
Abbasi SA: Final draft of the study approval.

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