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Clinical Microbiological Study of Fungal Infections in Diabetic Foot Ulcers: The Hidden Hinderance

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KEYWORDS

Diabetic foot ulcer, fungal profiling, *Candida albicans*, polymicrobial infection.

ABSTRACT:

Introduction: In diabetic foot ulcers (DFUs), bacterial infection is a major concern and highly prioritized, while fungal infection is often neglected, yet it contributes to delayed healing and complications. Early diagnosis and treatment of fungal infections could hasten the healing and prevent further complications. This study examined the prevalence of fungal infection in DFUs, conducted fungal profiling and developed effective fungal detection methods.

Methods: A cross-sectional study was conducted on 117 DFU patients after the approval by the Institutional Ethical Committee of SRM Medical College Hospital & Research Centre (Ethics clearance number: SRMIEC-ST0624-1390). The swabs and tissues are collected from the depth of the ulcer and microscopic examination is conducted to identify the gram-positive budding cells and pseudo-hyphae. The sample was cultured on sabouraud dextrose agar (SDA) with chloramphenicol and cycloheximide. The cultures were maintained at 25°C-30 °C and checked twice weekly for 4-6 weeks. Identification of fungal species is done along with fungal profiling.

Results: The analysis shows that 82% of patients were negative for fungal infections, while 18% were positive. Among fungal infections, non-*albicans Candida* (10%) was more prevalent than *Candida albicans* (5%), with Aspergillus species (*A. niger* 2%, *A. flavus* 1%) being the least common. The findings highlight that even though the fungal infections are less frequent in DFUs, *Candida* infections dominate, which highly targets antifungal management.

Conclusions: DFU is polymicrobial and fungal infections are often ignored but become potentially fatal. All long-standing DFUs demand effective diagnosis and treatment of fungal infections.

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1. Introduction

One of the main public health concerns in the world is diabetes mellitus. Diabetes has various repercussions, but foot issues are frequently more concerning to patients and healthcare providers than many other issues. It is estimated that DFU impacts 6.3% of the global population and the lifetime risk of DFU for people with diabetes ranges from 15 to 34% [1]. In DFUs, polymicrobial infections are common and are caused by anaerobic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Deep tissue damage from these infections raises the possibility of gangrene and perhaps amputation [2].

According to the International Consensus on the Diabetic Foot (2021), a foot ulcer is explicitly described as a wound that penetrates the entire thickness of the skin beneath the ankle [3]. Among the elements driving the high frequency are walking barefoot, poor socioeconomic level, lack of education, ignorance about diabetic foot care among patients and primary care doctors and postponing of medical attention [4].

Studies indicate that fungal infections occur in 12–32% of DFUs, with *Candida* species being the most prevalent [5]. Addressing fungal infections in DFUs represents a crucial step toward improving clinical outcomes and mitigating the severe consequences associated with diabetic foot disease [6]. Usually, the key therapies to maintain ideal glycemic control include debriding necrotic or damaged tissue, relieving pressure from the affected area, applying the suitable wound dressings, and treating infected wounds with the appropriate antimicrobial medicine [7].

Thus, the present study aims to detect the prevalence of fungal infections in diabetic foot ulcers, to perform fungal profiling from diabetic foot ulcers and to identify the appropriate methodology for the detection of the fungus in DFUs.

2. Objectives

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3. Methods

The study included a total of 117 patients diagnosed with diabetic foot ulcers (DFUs), between 20 and 89 years old. For each patient, fungal cultures from tissue and swab samples were collected and relevant clinical information was systematically documented.

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Sample Collection

Before sample collection, the ulcer regions of the patients were thoroughly cleansed using 10% povidone-iodine solution and then rinsed with normal saline to get rid of residual antiseptic. The swab samples were collected using sterile cotton swabs and stored in conventional sterile containers. Furthermore, tissue samples were obtained aseptically utilizing punch biopsy forceps. All collected samples were tagged, sealed and transported to the microbiology laboratory within one hour for fungal examination.

Microscopic Analysis:

Prior to cultivation, the obtained tissue samples were subjected to preliminary microscopic evaluations:
The KOH mounting method was performed by placing a small piece of tissue sample on a glass slide and treating it with 10% potassium hydroxide (KOH) solution. Then the slides were incubated at 37°C for 2 h to facilitate tissue clearance and enhance the fungal visibility.

For Gram staining, the tissue sample was gently crushed and smear was prepared on a glass slide. The glass slides were then air-dried, heat-fixed, and stained using the standard gram staining technique. The stained slides were examined under an oil immersion microscope to detect the presence of gram-positive budding yeast cells and pseudohyphae, suggestive of *Candida* species.

Fungal Culture and Identification:

Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol was used as the culture medium. For each sample, four agar slants were prepared with two of them additionally amended with cycloheximide. The tubes were inoculated and incubated at 25°C to 30°C. Cycloheximide inhibited the growth of non-pathogenic saprophytic fungi, while chloramphenicol suppressed the bacterial growth, thereby enhancing the selective isolation of pathogenic fungi. The cultures were monitored every two weeks over a period of four to six weeks.

For microscopic identification, the lactophenol cotton blue (LPCB) staining procedure was followed to visualize fungal filaments and spores. Additionally, slide culture techniques were frequently employed to preserve the native structural integrity of the fungal elements for accurate identification.

4.Results

Among the total 117 patients studied, most of the participants were aged between 50 and 69 years, while the youngest (20–39 years) and oldest (80–89 years) age groups had the lowest representation (Figure 1).

1

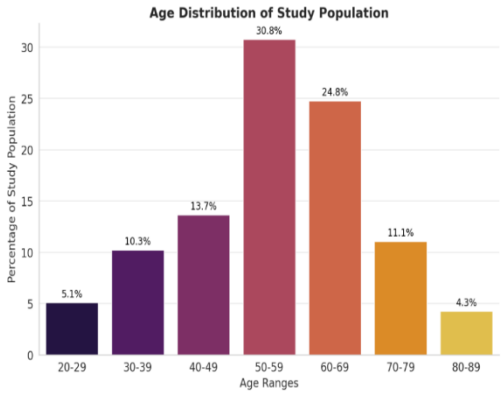

Figure 1: Age distribution profile of DFU patients (ranging from 20 to 89 years).

Table 1 depicts the prevalence of fungal infections in DFUs, in which 82% (96/117) of the samples tested were negative and 18% (21/117) positive for fungal infection. Among the fungal-positive cases, 13.7% were identified from wound swabs, 2.6% from both tissue and swabs, and 1.7% from tissue samples alone. The fungal species isolated from the positive samples are *Candida albicans* (5%), *Candida non-albicans* (10%), *Aspergillus niger* (2%) and *Aspergillus flavus* (1%) (Table 2).

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Table 1. The prevalence of fungal infection in DFUs on the basis of type of culture.

Category	Percentage (%)
Negative Fungal Samples	82
Positive Fungal Wound Swab	13.7
Positive Wound Swab and Tissue	2.6
Positive Fungal Tissue	1.7

Table 2. The causative organisms and percentage of fungal infection in DFU Patients (n=117).

Category	No. of Samples	Percentage (%)
Negative samples	96	82
Candida albicans	6	5
Candida non-albicans	12	10
Aspergillus niger	2	2
Aspergillus flavus	1	1

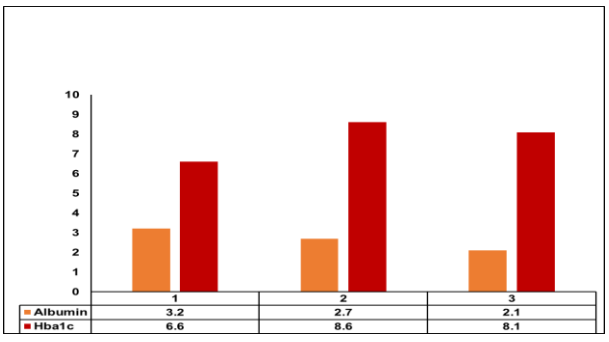

Figure 2: The levels of Albumin and HbA1c in positive *Aspergillus* spp. Samples

All samples that tested positive for *Aspergillus* spp. showed elevated HbA1c levels (>6.5%), suggesting a possible association between poor glycemic control and increased susceptibility to fungal infections (Figure 2). The maximum serum albumin level is found to be 3.2 g/dL among tested samples. In patients positive for *Candida* spp., the majority of them exhibited elevated HbA1c values above 6.5%, reflecting poor glycemic control and their albumin levels were typically within the moderate range (Figure 2). In particular, patient 12 represented a significant outlier with an HbA1c of 11.9%, reflecting highly uncontrolled diabetes (Figure 3).

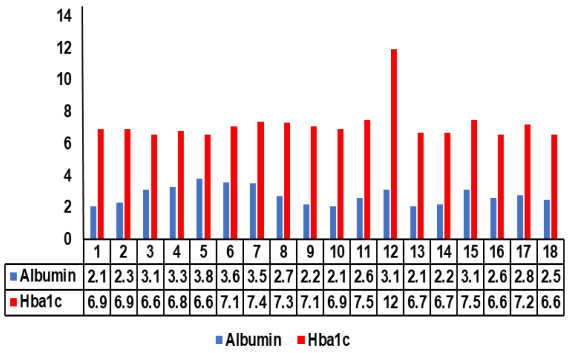

Figure 3: Albumin and Hba1c levels in positive Candida spp. positive samples

4. Discussion

The presence of polymicrobial infections in DFUs crucially complicates treatment and increases the risk of deep tissue invasion and often leads to amputation to save a patient's life. Fungal infections, particularly dermatomycosis affecting interdigital spaces highly facilitate bacterial colonization. These fungal infections sometimes extend into the occlusive webspace by disturbing the stratum corneum, leading to irritation, maceration and enhanced bacterial growth.

In the present study involving 117 DFU patients, individuals aged 50–59 years had the highest incidence, 30.8% (Figure 1). The two cohorts in our research had comparable baseline

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sociodemographic factors, such as age and gender. With 83 males and 34 females in our study sample, the diabetes demographic profile was similar, with more men than women afflicted with the disease [8]. Huang et al. (2016) also reported a male-dominated cohort of 60.5% [9, 10].

DFUs are considered one of the most severe complications of diabetes mellitus, generally resulting from diabetic neuropathy, a frequent microvascular complication [11]. Research suggests that diabetes is the leading cause of non-traumatic lower limb amputations in the United States and approximately 5% of individuals with diabetes develop foot ulcers annually, with 1% progressing to amputation [12].

In our cohort study, *Candida* spp., were the predominantly isolated fungi among the culture-positive samples (15%, 18/117). Specifically, non-albicans *Candida* accounted for 10% (12/117), while *Candida albicans* was found in 5% (6/117) (Table 1). This finding contrasts with some previous studies that reported *C. albicans* as the most prevalent fungal isolate (42.85%) among fungal-positive DFUs [5, 13].

Aspergillus spp., though uncommon in this population, were detected in 3 samples (3%), with *A. niger* in 2 cases and *A. flavus* in 1 case (Table 1). Since *Aspergillus* infections are typically observed in immunocompromised hosts and associated with highly morbidity, low rates of detection are a reason for concern, particularly when clinical presentation is suggestive of invasive disease.

Figure 2 depicts the albumin and HbA1c levels of *Aspergillus* spp., positive patients, in which a notable correlation was observed between fungal infection and poor glycemic control. A decline in serum albumin levels from 3.2 g/dL to 2.1 g/dL was observed among samples, implying a potential connection between systemic inflammation or nutritional deficiencies and fungal infections.

Concurrently, all the samples possess increased HbA1c levels, which are markers of long-term blood glucose control and are observed between 6.6% and 8.6%. This finding that poor control of glycemia could be implicated in diabetes patients susceptibility to and occurrence of *Aspergillus* infections (Figure 2). These findings align with the previous research, which identified HbA1c >9% as one of the risk factors for ulceration [14].

Figure 3 represents a comparative analysis of albumin and HbA1c levels across 18 *Candida* spp. positive samples. HbA1c levels consistently remain elevated across all samples, with most patients exceeding 6.5%, indicative of chronic hyperglycemia. Notably, sample 12 exhibited HbA1c level of 12%, representing an extreme case of poorly managed diabetes. Albumin levels varied across the samples tested, with lower values potentially reflecting poor nutritional status or underlying systemic complications.

Thus, the present study highlights the correlations among serum albumin, HbA1c and fungal infections in DFUs and so far, it has not been widely reported. Our findings suggest this relationship warrants further investigation.

Conclusion

This study highlights the clinical significance of fungal infections in diabetic foot ulcers (DFUs), which are often overlooked and leads to delayed healing, polymicrobial complexity and increased risk of amputation. The study reveals *Candida* spp., particularly non-*albicans* strains are predominant fungal pathogens, while *Aspergillus* spp., found to be less common often coexist with bacterial infection in DFUs and pose serious treatment complications. The study advocates for routine mycological screening, including fungal culture and susceptibility testing, to ensure timely and targeted antifungal treatment. Since conventional antibiotics alone are often ineffective due to the polymicrobial nature of DFUs, a comprehensive microbiological

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evaluation is essential. This study also emphasizes preventive strategies such as proper glucose management, regular foot examinations, wound care, and patient education. The study concludes that a multidisciplinary approach is vital for improving DFU management and patient outcomes.

Ethics approval

The study was approved by SRM Medical College Hospital & Research Centre (SRM MCH & RC) Institutional Ethical Committee (Ethics clearance number: SRMIEC-ST0624-1390) on JULY 3, 2024.

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Declaration of Competing Interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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