

## Fungal isolates from diabetic amputations: Histopathologic spectrum and correlation with culture

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### Abstract

**Objectives:** Fungal infections of diabetic foot ulcers are often not suspected and when neglected are an important cause of non-traumatic diabetic limb amputations. The present study was undertaken to focus upon the diversity of fungal isolates from diabetic limb amputations.

**Materials and Methods:** Thirty-one diabetic patients who underwent limb amputation formed the study group. Potassium hydroxide (KOH) wet mount examination was done on bits of gangrenous tissue sent by the surgeon. Culture was also done. Representative sections from the amputation specimens were studied for histomorphology. Periodic acid-Schiff (PAS) and Grocott special staining was done on the sections to facilitate the microscopic detection of fungus.

**Result:** There were 20 male and 11 female patients. The age bracket was 29 to 90 years (mean: 58±9.32 years). In 13 cases fungus was isolated on KOH/culture. *Candida* (8), *Fusarium* (2), *Trichosporon* (2) and *Aspergillus* (1) were isolated. On PAS and Grocott staining, fungal profiles were visualized in 15 cases. Fungus was typed on histopathology as *Candida* (10), *Trichosporon* (2), *Aspergillus* (2) and *Fusarium* (1). Histopathologic sections revealed fungal elements of *Candida* in 3 culture/KOH negative cases. In 18 cases, one-month of incubation failed to yield any fungus.

**Conclusions:** Use of special stains facilitates identification of fungal profiles on histopathology. Careful scrutiny of slides for fungus however remains quintessential. Culture allows accurate identification of fungal strain which is necessary for therapeutic decision making thereby greatly improving the treatment outcomes. Prompt medical and surgical intervention in fungal diabetic foot ulcers can help salvaging the limb.

**Keywords:** Diabetes, Fungus, Histopathology, Amputation, Culture.

### Introduction

The prevalence of diabetes mellitus has increased by more than 60% from 1990-2001 and the number will further increase by 165% by 2050 as per the estimates.<sup>1</sup> India harbors maximum people with diabetes worldwide.<sup>2</sup> Diabetes tops the list of non-traumatic lower limb amputations.<sup>3</sup> During their lifetime about 15%-25% of diabetic patients will develop lower extremity ulcers.<sup>4</sup> People with diabetes are at an increased risk of developing fungal infections in comparison to the otherwise healthy individuals.<sup>5</sup>

The propensity to develop foot ulcers and gangrene in diabetic patients rises when fungal infections are superimposed by bacterial infections. The clinical findings are seldom diagnostic in fungal foot ulcer infection. Microbiologic and histopathologic examinations together hold the key. Special stains like periodic acid Schiff (PAS) and Grocott facilitate the identification of fungus on histopathology while the culture allows for accurate identification of fungal strain. Diagnostic accuracy in identification of fungus on histopathology/cytology specimens on microscopy to the tune of 79% has been observed.<sup>6</sup>

Effective and timely communication between microbiologists, pathologists and clinicians can often clinch the correct diagnosis of many difficult-to-diagnose cases.<sup>7</sup> There is paucity of studies on the magnitude of fungal infections in diabetic lower extremities.<sup>5,6,8,9</sup> The present study was carried out to discover the histopathologic

spectrum of fungal isolates from diabetic amputations and correlate with findings on culture.

### Materials and Methods

The study was done in the Department of Pathology in collaboration with Departments of General Surgery and Microbiology in a tertiary care teaching hospital on 31 patients who underwent diabetic limb amputation. Informed consent was taken from all the patients included in the study. The material was sent by the surgeon from the ulcer/necrotic area after gently cleaning the surface with saline and sterile gauze. Little amount of specimen was examined under the microscope after partial digestion with 10-20% potassium hydroxide (KOH) as a wet mount. Sabouraud dextrose agar (SDA) tube slants were inoculated with the sample in duplicate, with incubation at both 37°C and 22°C. In addition, the sample was also inoculated in brain heart infusion broth with incubation at 37°C. Culture was examined for growth daily for the first week and twice a week for the next 3 weeks. For histopathology, 10% buffered formalin was used to fix the amputated specimens. Appropriate sections were submitted for tissue processing and paraffin embedding. Sections cut at 3-5 micron thickness were stained with hematoxylin and eosin (H&E). Special stains like periodic acid Schiff (PAS) and Grocott were done in all the cases.

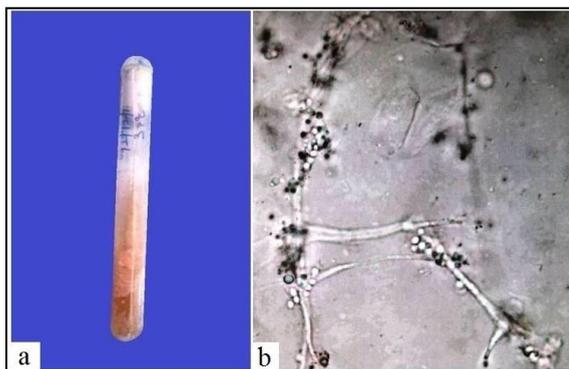
### Results

There were 20 male and 11 female patients. The age bracket was 29 to 90 years (mean: 58±9.32 years). Most of the

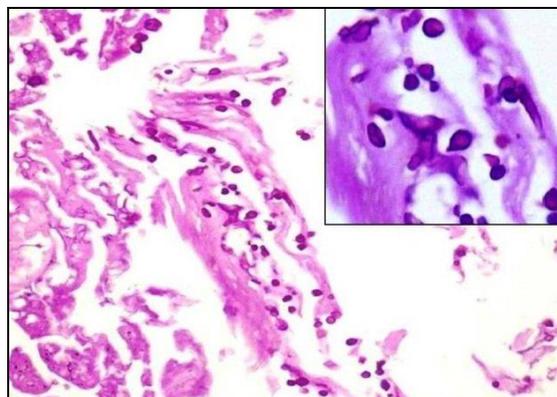
patients (64.5%) were in fifth and sixth decades of life. Of 31 cases, neuropathic ulcers were seen in 18 (58%) cases while neuroischemic ulcers were present in 13 (42%) cases. Below knee amputation (Fig. 1) was done in 21 cases, above knee amputation in 2 cases and transmetatarsal amputation in 8 cases. The cultures were examined for growth. *Candida* species were cultured in 8 cases (Fig. 2), followed by *Trichosporon* and *Fusarium* in 2 cases each and *Aspergillus* in 1 case. In 18 cases, one-month of incubation failed to yield any fungus. On PAS and Grocott special staining fungal profiles were visualized in 15 cases. Fungus was typed on histopathology as *Candida* (10), *Trichosporon* (2), *Aspergillus* (2) and *Fusarium* (1). Histopathologic sections revealed fungal elements of *Candida* in 3 culture/KOH negative cases. One case of *Fusarium* was interpreted as *Aspergillus* on histopathology. *Candida* organisms on H&E and PAS appeared as groups of yeasts measuring 3 to 5  $\mu\text{m}$  in diameter intermingled with pseudohyphae (Fig. 3). The host tissue reaction predominantly comprised of neutrophilic inflammation. In addition, lymphocytes, macrophages, fibrin, and necrosis of coagulative type were also observed. *Trichosporon* on H&E and PAS stains demonstrated an admixture of hyphae, pseudohyphae, and budding yeasts resembling *Candida* spp (Fig. 4). The hyphae were of variable length, had thick walls, but were overall very long with rare branching. *Aspergillus* and *Fusarium* on H&E and PAS were seen as septate, narrow hyphae with acute angle branching (Fig. 5).



**Fig. 1:** Gross photograph showing diabetic foot ulcer with extensive gangrene of the limb



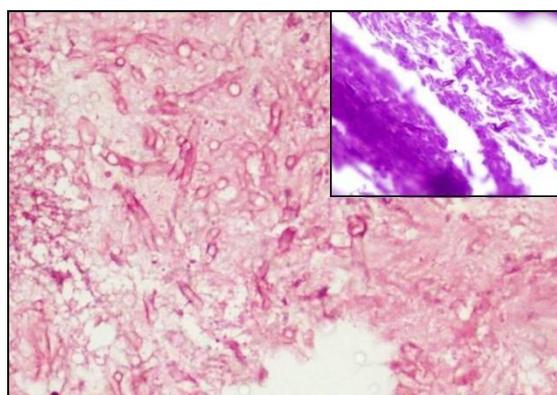
**Fig. 2a:** Yeast like colonies of *Candida* well visualized on Sabouraud dextrose agar slant; **b:** KOH wet mount showing budding yeast forms and pseudohyphae of *Candida* (x400)



**Fig. 3:** Section showing plenty of *Candida* organisms (H&E, x200). Inset shows deeply magenta stained yeasts and pseudohyphae of *Candida* (PAS, x600)



**Fig. 4:** Section showing budding yeasts of *Trichosporon* embedded in keratin flakes (H&E, x400)



**Fig. 5:** Section showing septate, narrow, acute angle branched hyphae of *Aspergillus* (H&E, x400). Inset shows fungal profiles highlighted on periodic acid Schiff stain (PAS, x400)

### Discussion

Diabetes and its complications have shown an exponential rise over the past few decades. Foot ulcers are a significant cause of financial burden in diabetic patients leading to hospitalization in about one-third cases.<sup>10</sup> Osteomyelitis, peripheral vascular disease, neuropathy, duration and depth of ulcer, smoking, diabetic foot infections including fungal

infections are notable risk factors for amputation in patients with diabetes.<sup>11-13</sup> As a first line of treatment, diabetic foot ulcers are managed with antibacterial agents. A fungal etiology is almost never considered or is underestimated; henceforth samples to the mycology laboratory are not usually sent for culture.<sup>14</sup>

Fungal infection of diabetic foot ulcers should be suspected when they fail to heal despite intensive antibiotic therapy and good podiatric care.<sup>15</sup> Deep tissue infected by fungi in isolation or with co-existence of bacteria is seen in about a quarter of patients.<sup>14</sup> *Candida* infections are seen in diabetic patients with poor glycemic control and not uncommonly be the indicators of underlying undiagnosed diabetes mellitus.<sup>16,17</sup> The frequency of fungal infection in our study was 42%. Chellan et al<sup>14</sup> isolated deeply seated fungal organisms in 27.2% cases of diabetic lower limb wounds while Raiesi et al<sup>18</sup> reported a prevalence of 19.1%. In a study from the Indian subcontinent, fungi formed 9% of the total isolates in cases with diabetic foot wounds.<sup>19</sup> Raza et al<sup>20</sup> reported a prevalence of 19%. In our study histopathologic identification of fungi was seen in 48% cases in comparison to microbiologic isolation.

Fungal elements owing to their characteristic shapes and microscopic appearances can be identified on histopathology of tissue from the diabetic foot ulcer. Use of special stains like Gomori-methenamine silver (GMS) and periodic acid-Schiff (PAS) is a useful diagnostic aid.<sup>21</sup> The major growth forms of the fungi that help in histopathologic diagnosis are the yeast cells, hyphae, pseudohyphae, arthroconidia, chlamydoconidia and spherules. The characteristics of each of these (shape, size, location and colour) help in identification of the fungus.<sup>7</sup>

Histopathologic examination serves as a rapid and fairly reliable means to look for infectious fungal organisms and perhaps is the only means when tissue is not sent for culture. Identification of fungus on histopathology along with the tissue reaction is a sure shot indicator of invasive fungal infection.<sup>22</sup> The major advantages of histopathology are speed, low cost and the ability to provide a presumptive identification of the infecting fungus as well as demonstrating the tissue reaction.<sup>23</sup>

To conclude, the results of the present study highlight the usefulness of histopathology in identification of fungal organisms in diabetic limb amputations and correlation with microbiologic culture in our population. The study provides an impetus to future researchers on the subject, given the scarcity of literature available.

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**Conflict of Interest:** Nil.

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