

## INCIDENCE OF MYCOTIC INFECTIONS IN DIABETIC FOOT TISSUE

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### Summary

*The objective of the study was to investigate the incidence of fungal pathogens in diabetic foot infections. A total of 74 Type II diabetic patients with non-healing diabetic foot infections were recruited for the study. Among the diabetic patients 65 % (48/74) had yeast and mold infections. Pathogenic yeasts were noted in 77 % of the patients of which Candida species was predominant (93 %). The major Candida species isolated were C. albicans (49 %), C. tropicalis (23 %), C. parapsilosis (18 %), C. guilliermondi (5 %) and C. krusei (5 %). The other yeast species isolated were Trichosporon cutaneum and T. capitatum. Trichophyton spp. was the only dermatophytic fungus found. Molds were isolated from 38 % of the infected patients of which Aspergillus species predominated (72 %). The other molds isolated were Fusarium solani, Penicillium marneffeii and Basidiobolus ranarum. The results of the study indicate the need for mycological evaluation of the non-healing diabetic foot tissues and appropriate antifungal therapy.*

**Key words:** *diabetic foot, yeasts, fungi, infection, pathogenic.*

### Introduction

Patients with diabetes represent a unique group of individuals who appear more prone to develop infections than others. Several mechanisms have been proposed to explain the association between diabetes and infections. However, few conclusive studies exist and a considerable debate is going on regarding the evidence for this predisposition. Diabetes mellitus is a chronic disorder that affects a large segment of the human population and is a major public health problem. Diabetes and foot

problems are almost synchronous [4, 8, 20]. Diabetic foot infections frequently result in morbidity, hospitalization and amputations. The bacteriology of diabetic foot ulcers has been studied by numerous investigators [2, 9, 12, 15, 19, 21]. However, there is a paucity of reports on the incidence of fungal pathogens in deep tissue samples.

The present study was undertaken in order to evaluate the incidence of pathogenic fungi in ulcerated deep tissue samples.

### Materials and Methods

**Sample collection.** The study was carried out over a period of 10 months, from December 2004 to October 2005. Seventy four people with diabetes and a foot ulcer of grade 2 – 4, atten-

ding the Podiatry surgery clinic were enrolled in the study. Grades were defined as: Grade 2 - deep ulcer often infected but no bone involvement; Grade 3 - deep ulcer, abscess formation

and bone involvement and Grade 4 - localized gangrene. Patients with superficial ulcer or mere abrasions were excluded from the study.

Tissue specimens were obtained from the depth of the wound (taking aseptic precautions) after debridement. Samples were transferred to the laboratory within an hour in sterile containers. The necrotic areas of the tissues were mounted on KOH and also inoculated to Sabouraud Chloramphenicol Agar (SCA) and Sabouraud's Dextrose Agar (SDA) (Himedia Ltd, Mumbai). The samples were incubated both at room temperature ( $28 \pm 2$  °C) and 37 °C for one month and evaluated daily for growth of fungal cultures.

**Characterization and identification of the cultures.** Yeast like growth on SCA was evaluated for germ tube formation, urease production, sugar fermentation (dextrose, maltose, sucrose, lactose), assimilation of sugars (dextrose, lactose, raffinose, sucrose and trehalose), Tetrazolium reduction, and microscopic and macroscopic appearance in slide culture and corn meal agar with Tween 80 [4, 7].

Fungal cultures were identified by microscopic (LCB) and macroscopic appearance [3, 5].

Velvety colonies on SDA with red pigment on reverse, tear drop microconidia and long pencil shaped macroconidia were identified as belonging to *Trichophyton* species. *Basidiobolus ranarum* shows a flat, yellowish-grey, glabrous, radially folded colonies covered by a fine, powdery, white surface mycelium on SDA after 17 days incubation at 26 °C. They have globose, one-celled conidia from a sporophore. The sporophore has a distinct swollen area just below the spore that actively participates in the discharge of the spore. *Penicillium marneffeii* (thermally dimorphic) shows hyaline, smooth-walled conidiophores bearing terminal verticils of 3 to 5 metulae, each bearing 3 to 7 phialides. Conidia are globose to subglobose, 2 to 3 µm in diameter, smooth-walled and are produced in basipetal succession from the phialides. The colonies of *P. marneffeii* are rapid growing, flat, filamentous, with wrinkled, folded surface and velvety, woolly, or cottony in texture. The colonies are initially white and become blue green or gray green. The plate reverse is usually pale to yellowish. At 37 °C the organism grows as a yeast like colony.

## Results

Among the 74 patients with diabetic foot infections from surgical units, 49 were male and 25 female patients, aged between 48 to 69 years. The fungal species isolated from diabetic foot ulcers were *Candida* spp, *Trichosporon* spp, *Trichophyton* spp, *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp, and *B. ranarum*. Fungal pathogens were not detected in 26 patients. The incidence of yeast and/or mold infections was 65 % out of the 74 patients studied. Among the fungal cultures identified, 66 % were yeast isolates and 34 % mold like cultures.

Table 1 depicts the identification scheme for yeast cultures adopted in this study. *Candida* was the major isolated species (82%) (Table 2). Among the *Candida* species, *C. albicans* (46 %), *C. tropicalis* (27 %), *C. parapsilosis* (17 %), *C. guilliermondi* (5 %) and *C. kru-*

*sei* (5 %) were observed. The other yeast species isolated were *Trichosporon cutaneum* and *T. capitatum*.

The mold species were identified on the basis of their microscopic and macroscopic appearance as described by Chander [5]. The highest incidence was *Aspergillus* spp (65 %) (Table 3). Among the *Aspergillus* sp., *A. flavus* (60 %) and *A. fumigatus* (40 %) were observed. The other molds isolated were *Fusarium solani* (9 %), *P. marneffeii* (9 %), and *B. ranarum* (4 %). *P. marneffeii* was unique due to its dimorphic nature at different temperatures and also its rare occurrence. Out of these 13 % showed only sterile growth. *Tricho-phyton* is a dermatophytic fungus which might have appeared in the deep tissue sample due to surface contamination

Table. 2. *Candida* species isolated from diabetic foot tissues.

Sl. no.	<i>Candida</i> species	Percentage of samples
1.	<i>Candida albicans</i>	46
2.	<i>Candida tropicalis</i>	27
3.	<i>Candida parapsilosis</i>	17
4.	<i>Candida guilliermondi</i>	5
5.	<i>Candida krusei</i>	5

Table 1. Identification scheme for yeast cultures [4, 7].

Yeast species	Germ tube	Urease	Sugar fermentation				Sugar assimilation					Tetrazolium reduction	CMA + Tween 80
			Glu	Suc	Lac	Mal	Glu	Suc	Lac	Tre	Raf		
<i>Candida tropicalis</i>	-	-	+	+	-	+	+	+	-	+	-	Maroon	No arthroconidia. Blastoconidia. Produced randomly along hyphae or pseudohyphae. Hyphae or pseudohyphae branched.
<i>Candida albicans</i>	+	-	+	-	-	+	+	+	-	+	-	Pale pink	No arthroconidia. Spherical clusters of blastoconidia at regular intervals on pseudohypae. Chlamydoconidia present on hyphae.
<i>Candida krusei</i>	-	-	+	-	-	-	+	-	-	-	-	Pink and dry	No arthroconidia. Elongated clusters of blastoconidia occur at septa of pseudohyphae. Branched pseudohyphae.
<i>Gandida guilliermondi</i>	-	-	+	+	-	-	+	+	-	+	+	Pink and pasty	-
<i>Candida parapsilosis</i>	-	-	+	-	-	-	+	+	-	+	-	Rose pink	No arthroconidia. Blastoconidia present but not characteristic. Giant hyphae with sage brush appearance.
<i>Trichosporon spp</i>	-	-	+	+	+	-	+	+	+	+	+		Numerous arthroconidia. Blastoconidia may be present, septate hyphae present.

Legend: glucose (Glu), sucrose (Suc), lactose (Lac), maltose (Mal), trehalose (Tre), raffinose (Raf).

Table. 3. Mold species isolated from diabetic foot tissues.

Sl. no.	Mold species	Percentage occurrence
1.	<i>Aspergillus</i> spp	65
2.	<i>Fusarium solani</i>	9
3.	<i>Penicillium marneffeii</i>	9
4.	<i>Basidiobolus ranarum</i>	4
5.	Sterile hyphae	13

## Discussion

Foot infections are a major cause of morbidity in people with diabetes. Devitalised tissue is the site where the microorganisms responsible for the non-healing ulcers inflict damage. Numerous investigations have been carried out on the bacteriology of diabetic foot ulcers [2, 9, 12, 15, 19, 21]. Most diabetic foot lesions are known to have a polymicrobial aetiology [13, 18, 21].

Though there are a few reports on the incidence of fungal pathogens in diabetic foot infections [1, 6, 11, 14], there is a paucity of published work on the incidence of fungal pathogens in deep tissue samples. The study conducted by Chincholikar and Pal [5] showed the presence of various fungal pathogens in diabetic foot ulcer tissues, among which *Candida* species preponderated. Heald et al. [10] has also reported the association of *Candida* spp with protracted ulceration in diabetic feet which improved the following systemic anti-fungal therapy.

The presence of various species of *Candida* (*C. parapsilosis*, *C. albicans*, *C. tropicalis*, *C. famata* and *C. glabrata*) was reported in diabetic patients with ulcer and in interdigital spaces of the same and/or the other foot by Misoni et al. [14]. This is in agreement with the findings of the present study showing that among the fungal pathogens isolated from deep tissues, 93 % were *Candida* spp. The investigators could also identify *Candida* species like

*C. parapsilosis*, *C. albicans*, *C. tropicalis* and *C. glabrata* from the infected tissues.

The presence of *A. flavus* and *F. solani* has been reported by some workers on diabetic foot ulcer [1, 11]. In our study, among other mold species we also isolated *A. flavus* and *F. solani* from the infected tissues. Reyes and Rippon have reported a case of simultaneous aspergillosis and mucormycosis complicating diabetic foot gangrene [17]. *P. marneffeii* has been rarely reported in India. *P. marneffeii* infections have been documented in HIV-infected individuals from the northeastern part of India [16]. This infection has been predominantly reported from Southeast Asia [7] where it has been found to be the third most common illness that specifies the Acquired Immuno Deficiency Syndrome (AIDS). *P. marneffeii* is pathogenic particularly in patients with AIDS and its isolation from blood is considered an HIV marker in endemic areas. The present study signifies the need of a mycological evaluation of non-healing diabetic foot and prudent antifungal treatment based on the culture results rather than depending on broad spectrum antifungals for cure.

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## РАЗПРОСТРАНЕНИЕ НА ГЪБНИ ИНФЕКЦИИ В ТЪКАНИ НА ДИАБЕТНО БОЛНИ

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### Резюме

*Целта на работата е да се изследва разпространението на гъбни патогени при диабетни инфектирания по краката. В проучването са включени 74 болни от диабет тип II пациенти с неизлекувани диабетни инфекции по краката. От диабетно болните пациенти 65 % (48/74) имат инфекции от дрожди и плесени. Патогенни дрожди са установени при 77 % от тях, като преобладава родът *Candida* (93 %). Основните видове *Candida* са *C. albicans* (49 %), *C. tropicalis* (23 %), *C. parapsilosis* (18 %), *C. guilliermondi* (5 %) и *C. krusei* (5 %). Другите изолирани видове дрожди са *Trichosporon cutaneum* и *T. capitatum*. *Trichophyton spp.* е единствената установена дерматофитна гъба. Плесени са открити при 38 % от заразените пациенти, като преобладава род *Aspergillus* (72 %). Другите изолирани плесени са *Fusarium solani*, *Penicillium marneffeii* и *Basidiobolus ranarum*. Резултатите от изследването показват необходимостта от микологична оценка на незаздравяващите тъкани по краката на диабетно болни и подходяща антигъбна терапия.*