

A PROSPECTIVE STUDY OF THE PREVALENCE OF VARIOUS FUNGAL INFECTIONS IN DIABETIC FOOT ULCERS IN THANJAVUR MEDICAL COLLEGE HOSPITAL

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ABSTRACT

Background: To determine the proportion of diabetic foot ulcers yielding fungal growth, identify the species isolated, and examine whether isolation was related to clinical characteristics. **Study Design:** Prospective observational study with cross-sectional analysis. **Place and Duration of Study:** Department of General Surgery, Thanjavur Medical College and Hospital, Thanjavur, Tamil Nadu, India, over 18 months commencing June 2022. **Materials and Methods:** Fifty patients aged over 30 and under 80 years with diabetic foot and diabetes of at least six months' duration were enrolled. Ulcer swabs underwent microscopy with 10% potassium hydroxide and culture on Sabouraud dextrose agar for one month, with speciation of Candida on corn-meal and chromogenic agar. Pus was cultured for bacteria and ulcers graded by the Wagner classification. Fisher's exact and unpaired t tests were used. **Results:** Mean age was 55.5 ± 9.4 years and 45 patients (90.0%) were men. Fungi grew in seven (14.0%, 95% CI 7.0–26.2): Candida albicans in six and Rhizopus species in one. Bacteria grew in 44 (88.0%), most often Klebsiella species (26.0%). Wagner grade IV predominated (38.0%). All patients underwent surgery, including major amputation in three (6.0%). Fungal isolation was unrelated to sex, ulcer grade, age, admission blood glucose, duration of diabetes, ulcer duration or hospital stay ($P = 1.00, .86, .16, .34, .65, .76$ and $.18$, respectively). **Conclusion:** Fungi were isolated from about one in seven ulcers, almost always Candida albicans, with no distinguishing clinical feature. Mycological culture merits consideration alongside bacterial culture in ulcers failing to respond to antibacterial therapy.

INTRODUCTION

Diabetes mellitus affects an estimated 11.4% of Indian adults (Anjana et al., 2023).^[1] Approximately 18.6 million people worldwide develop a diabetic foot ulcer each year; 50% to 60% become infected, and ulceration precedes about 80% of lower-extremity amputations (Armstrong et al., 2023).^[2] Diagnostic effort in these wounds is directed almost entirely at bacteria, and international guidance on diabetes-related foot infection is framed around bacterial pathogens, with mycological culture forming no part of the routine pathway (Lipsky et al., 2020).^[3] Yet sustained hyperglycaemia impairs neutrophil function and promotes candidal adherence (Casqueiro et al., 2012),^[4] and targeted antifungal therapy accelerated wound healing in a randomised trial (Chellan et al., 2012).^[5] Reported isolation rates vary widely, from 38% (Nair et al., 2006) to 23.3%

(Azharuddin et al., 2023) and 19.4% (Saud et al., 2020), and Indian data remain limited. This study determined the proportion of diabetic foot ulcers yielding fungal growth in a south Indian surgical inpatient population, identified the species isolated, and examined their relationship with clinical characteristics.^[6]

MATERIALS AND METHODS

This prospective observational study with cross-sectional analysis was conducted in the Department of General Surgery, Thanjavur Medical College and Hospital, Thanjavur, Tamil Nadu, India, over 18 months commencing June 2022, and is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations. Patients admitted with a clinical diagnosis of diabetic foot were eligible if aged over

30 and under 80 years, with diabetes of more than six months' duration, and if they gave written informed consent; those with venous or trophic ulcers, receiving or allergic to antifungal agents, and pregnant or lactating women were excluded. For estimation of a single proportion, assuming an anticipated prevalence (p) of 15%, absolute precision (d) of 10% and 95% confidence ($Z = 1.96$), $n = Z^2 \times p(1 - p) / d^2 = 48.98$, rounded to 50 participants. A structured history, clinical examination and baseline investigations including admission random blood glucose were recorded, and ulcers graded by the Wagner classification (Mehraj & Shah, 2018). Specimens were obtained by sterile swab, examined by direct microscopy with 10% potassium hydroxide and inoculated onto Sabouraud dextrose agar, with duplicate slants incubated at room temperature and at 37 °C for one month. Isolates were identified by morphology using lactophenol cotton blue mounts and slide culture, and *Candida* was speciated on cornmeal and chromogenic agar by germ tube formation, urease testing and sugar fermentation. Pus was cultured separately for bacteria. Amputations proximal to the ankle were classified as major and ray and forefoot amputations as minor; participants were followed until discharge only. Continuous variables

are presented as mean $\pm$ standard deviation and categorical variables as frequencies with percentages, proportions carrying Wilson 95% confidence intervals. Categorical comparisons used Fisher's exact test (two-tailed) and continuous variables the unpaired t test with mean differences and 95% confidence intervals (CI). Statistical significance was set at a P value of .05. Analyses used IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). The protocol was approved by the Institutional Ethics Committee of Thanjavur Medical College and the study conducted in accordance with the Declaration of Helsinki.

RESULTS

All 50 enrolled patients completed the study; their characteristics are shown in Table 1. Before admission 43 patients (86.0%) were taking oral hypoglycaemic agents alone, whereas 48 (96.0%) required insulin afterwards; all had received antibacterial therapy before admission, oral agents alone in 37 (74.0%), and all received intravenous therapy during admission

Table 1: Patient, diabetes and ulcer characteristics (N = 50)

Characteristic	n (%)	Characteristic	n (%)
Age group (years)		Duration of diabetes (years)	
30–39	1 (2.0)	≤ 1	6 (12.0)
40–49	13 (26.0)	>1–5	13 (26.0)
50–59	15 (30.0)	>5–10	17 (34.0)
60–69	18 (36.0)	>10–15	8 (16.0)
70–79	3 (6.0)	>15	6 (12.0)
Sex		Duration of ulcer (months)	
Male	45 (90.0)	≤ 1	24 (48.0)
Female	5 (10.0)	>1–5	24 (48.0)
Site of ulcer		>5–10	2 (4.0)
Left foot	23 (46.0)	Operative procedure	
Right foot	14 (28.0)	Wound debridement	24 (48.0)
Left leg	7 (14.0)	Ray amputation	15 (30.0)
Right leg	5 (10.0)	Forefoot amputation	4 (8.0)
Right ankle	1 (2.0)	Split-skin grafting	4 (8.0)
Wagner grade		Below-knee amputation	2 (4.0)
I	1 (2.0)	Above-knee amputation	1 (2.0)
II	15 (30.0)		
III	9 (18.0)		
IV	19 (38.0)		
V	6 (12.0)		

Mean age 55.5 ± 9.4 years; mean duration of diabetes 7.8 ± 5.7 years; mean duration of ulcer 2.0 ± 1.7 months; mean admission random blood glucose 273.3 ± 64.0 mg/dL (15.2 ± 3.6 mmol/L); mean hospital stay 21.9 ± 8.9 days. To convert glucose values to mmol/L, divide by 18. Ray and forefoot amputations were classified as minor and below-knee

and above-knee amputations as major. Bacteria were isolated from 44 patients (88.0%, 95% CI 76.2–94.4) and fungi from seven (14.0%, 95% CI 7.0–26.2). *Candida albicans* accounted for six fungal isolates (12.0%, 95% CI 5.6–23.8) and *Rhizopus* species for one (2.0%); no mixed fungal growth and no other species were encountered. [Table 2]

Table 2: Organisms isolated from pus and fungal culture (N = 50)

Bacterial isolate	n (%)	Fungal culture	n (%)
<i>Klebsiella</i> species	13 (26.0)	No fungal growth	43 (86.0)
<i>Escherichia coli</i>	12 (24.0)	<i>Candida albicans</i>	6 (12.0)
<i>Pseudomonas</i> species	8 (16.0)	<i>Rhizopus</i> species	1 (2.0)
<i>Proteus</i> species	6 (12.0)		
<i>Acinetobacter</i> species	4 (8.0)		

Enterococcus species	1 (2.0)		
No bacterial growth ^a	6 (12.0)		

^a One of these six specimens grew *Candida albicans* on pus culture.

Fungi were isolated from seven of 45 men (15.6%) and from none of the five women ($P = 1.00$). Isolation by Wagner grade was 0 of 1 for grade I, 2 of 15 (13.3%) for grade II, 2 of 9 (22.2%) for grade III, 2 of 19 (10.5%) for grade IV and 1 of 6 (16.7%) for

grade V ($P = .86$). Patients with fungal growth were on average older, had had diabetes for longer and stayed in hospital longer, but no difference reached statistical significance and every confidence interval included zero. [Table 3]

Table 3: Comparison of continuous variables by fungal culture status

Variable	Culture negative (n = 43)	Culture positive (n = 7)	Mean difference (95% CI)	t	P
Age (years)	54.7 ± 9.7	60.1 ± 5.2	-5.4 (-13.0 to 2.2)	1.44	.16
Random blood glucose (mg/dL)	276.8 ± 65.4	251.7 ± 53.1	25.1 (-27.4 to 77.5)	0.96	.34
Duration of diabetes (years)	7.6 ± 5.8	8.7 ± 5.3	-1.1 (-5.8 to 3.6)	0.46	.65
Duration of ulcer (months)	2.0 ± 1.8	1.8 ± 1.0	0.2 (-1.2 to 1.6)	0.31	.76
Hospital stay (days)	21.3 ± 8.2	26.1 ± 12.0	-4.9 (-12.1 to 2.3)	1.37	.18

Values are mean ± standard deviation. Unpaired t test, $df = 48$; t values are absolute. The mean difference is the culture-negative mean minus the culture-positive mean. CI, confidence interval. To convert glucose to mmol/L, divide by 18.

DISCUSSION

Fungi were recovered from 14.0% of diabetic foot ulcers in this cohort, almost always *Candida albicans*, while bacterial growth was far more frequent at 88.0% and dominated by aerobic Gram-negative bacilli. This isolation rate sits at the lower end of the published range, below the 19.4% reported from Nepal (Saud et al., 2020),^[7] the 23.3% from a south Indian tertiary centre (Azharuddin et al., 2023) and the 38% of Nair et al. (2006).^[8] Much of that variation is likely methodological, since studies sampling deep tissue recover more organisms than those relying on surface swabs, a difference well documented for bacterial culture (Malone et al., 2013),^[9] and likely to apply at least as strongly to fungi; cohorts drawn from long-standing non-healing ulcers would also be expected to yield higher rates than an unselected surgical admission cohort. The predominance of *Candida albicans* accords with most published series (Kandregula et al., 2022),^[10] and the single *Rhizopus* isolate deserves note, since Mucorales are recognised opportunists in poorly controlled diabetes and are capable of angioinvasion and rapid necrosis. The bacteriological profile, led by *Klebsiella* species and *Escherichia coli*, mirrors the tropical pattern and contrasts with the staphylococcal predominance of temperate countries (Zubair et al., 2015),^[11] with direct implications for empirical antimicrobial policy. No clinical characteristic distinguished culture-positive from culture-negative patients, in contrast to Kandregula et al. (2022),^[12] who related fungal infection to the duration of diabetes. The study was, however, designed to estimate a proportion rather than to detect associations; with only seven events, confidence intervals were wide and a clinically meaningful effect cannot be excluded, and ulcer duration was compressed, 96.0% having been present for five months or less.

The relevance of these findings lies less in the precise rate than in the recovery of a fungus from about one in seven ulcers where mycological culture is not

routinely requested, supporting its selective rather than universal use. Several limitations apply. Surface swabs rather than deep tissue were sampled, probably underestimating fungal burden, and a single specimen was taken without confirmatory culture or histopathology, so colonisation cannot be distinguished from invasive infection. Potassium hydroxide microscopy findings were not systematically recorded, glycaemic control was assessed by a single random blood glucose rather than glycated haemoglobin, and neuropathy, peripheral arterial disease and osteomyelitis were not documented.

No antifungal susceptibility testing or treatment was undertaken and there was no post-discharge follow-up, leaving healing, recurrence and delayed limb loss unknown. Larger multicentre studies using deep tissue sampling with histopathological correlation and adequate follow-up are required.

CONCLUSION

Fungi were isolated from 14.0% of diabetic foot ulcers in this prospective single-centre cohort, almost all being *Candida albicans*, alongside bacterial growth in 88.0%. Fungal isolation was not significantly related to any demographic, glycaemic or ulcer-related characteristic examined, although the study had limited power for such comparisons. Mycological culture merits consideration alongside routine bacterial culture, particularly in ulcers failing to respond to appropriate antibacterial therapy.

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