

CANDIDA INFECTIONS OF DIABETIC FOOT ULCERS

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SUMMARY

The aims of the study were to determine the incidence of Candida infections of diabetic foot ulcers diagnosed by classic microbiologic and histopathologic methods, to study the influence of coexistent interdigital yeast colonization and dermatophytosis of one or both feet on the incidence of fungal ulcer infections, to propose the criteria for diagnosing fungal ulcer infections, and to describe the effect of Candida species on the clinical presentation of infected foot ulcers and treatment outcome. Over a 3-year period, we identified 22 of 509 diabetic outpatients with Candida foot ulcer infections confirmed by microbiologic and histopathologic methods. These infections had developed in chronically infected ulcers (from eighth week onward, in thirteenth week on an average), with C. parapsilosis as the most common causative agent of infection. Mixed (fungal-bacterial) infections were found twice as often as pure fungal infections, and mixed infections correlated with more severe underlying foot infections. In almost two thirds of the patients, without the help of antifungal therapy, the infected foot tissue had to be amputated. Coexistent interdigital dermatophytosis could not be found in any of the patients. In half of the patients no

simultaneous interdigital colonization with yeasts could be found in either foot. In conclusion, it should be noted that the incidence of Candida infections of diabetic foot ulcers is low (4.3%), the histopathologic and mycologic analyses of ulcer tissue are complementary methods for diagnosing fungal foot ulcer infections, and interdigital colonization with yeasts and dermatophytosis of either foot does not correlate with the frequency of Candida ulcer infections.

INTRODUCTION

Approximately 15% of persons with diabetes will have foot ulcer in their lifetime (1). The pathogenesis of diabetic foot is highly complex, including polyneuropathy, peripheral vascular disease, and compromised immunity, slower wound healing, trauma and infection (2,3). Complications associated with the development of infection and diabetic foot syndrome are the main cause of morbidity, nontraumatic lower extremity amputations, and diabetic patient mortality (2,3). As shown by epidemiologic data, in 85% of diabetic patients foot ulcers preceded amputations of the same extremity; they also increase by as much as 20-fold the risk of minor or major amputation of the same extremity (4,5).

Bacterial infections of diabetic foot ulcers are polymicrobial and mixed aerobic-anaerobic (2,3). While *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., species of *Enterobacteriaceae* and *Pseudomonas* spp. are

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the most common aerobic isolates, *Peptostreptococcus* spp. and *Bacteroides* spp. are the most common anaerobic isolates (2,3). Literature data on the frequency of fungal isolation from the diabetic foot ulcer differ significantly. *Candida* spp. is the most commonly isolated yeast from these ulcers (less than 5%-21%) (6-9).

Given the conditions prevailing in diabetic foot, even low pathogenic yeasts may cause an infection of foot ulcers. These types of yeasts often belong to the normal mycobiota of the skin around ulcers, or may colonize diabetic foot ulcers secondarily, hindering the assessment of the real role of fungal isolates from the ulcer. Because of their typical form and size, fungal elements can be visualized in histopathologic preparations of ulcer biopsy using different staining procedures (10,11). Microscopic demonstration of tissue invasion and host reaction confirms the existence of fungal infection (10,11).

There have been some reports of an increased incidence of fungal infections (dermatophytosis and candidiasis) of interdigital spaces and nails in the toes of diabetic patients, as well as of the association of these infections with the development of severe and deep inflammatory processes in feet (4,5).

In consequence, the aims of the present study were: (i) to describe the incidence of fungal and mixed fungal-bacterial infections of diabetic foot ulcers detected by microbiologic and histopathologic methods, (ii) to identify the incidence of coexistent interdigital colonization with yeasts and dermatophytosis of ulcerated and/or unulcerated foot, (iii) to find out whether the interdigital yeast colonization and dermatophytosis of either foot are predisposing factors for the development of fungal and mixed ulcer infections, and (iv) to determine the most reliable criteria for diagnosing fungal ulcer infections as well as (v) the effect of *Candida* spp. on the clinical presentation of infected foot ulcers and treatment outcome.

PATIENTS AND METHODS

Over a 3-year period, we selected 22 (4.3%) out of 509 consecutively registered diabetic patients with chronically infected foot ulcers, initially presenting with fungal infection of foot ulcers to the University Department of Vascular Surgery, Sestre milosrdnice

University Hospital in Zagreb. An ineffective conservative treatment of foot ulcer (with targeted and longterm antibiotic therapy, standard foot care, and improvements in glycemia control) at the outpatient department of the Internal Medical Ward and/or Family Medicine Service was followed by surgical treatment of ulcers (with necrectomy and drainage). Ulcer tissue samples (sized 10 mm³ on an average) were obtained either by necrectomy or biopsy from all patients simultaneously with interdigital swabs of both feet. Fungi were isolated and identified by the classic mycology methods (12,13), and histopathologic tissue sections were stained by the Periodic Acid Schiff (PAS) method (10,13). During the study, neither local nor systemic antifungal treatment was administered to any of the patients.

The study inclusion criteria for patients were: (i) progression of ulcer infection despite standard antibiotic and surgical-podiatric therapy, (ii) combination of positive fungal culture from ulcer biopsies, and (iii) histopathologic demonstration of fungal elements in both necrotic material and underlying tissue of the same foot ulcers from which biopsy was simultaneously obtained.

RESULTS

Patient characteristics were as follows: 13 (59.1%) males, nine (40.9%) females; age range 53-80 years, mean age 67.1 years; 5 (22.7%) type 1 diabetes and 17 (77.3%) type 2 diabetes cases (Table 1). The mean duration of diabetes from the diagnosis was 11.3 (range 2-20) years. Unlike polyneuropathy, which was present in all patients, peripheral vascular disease was present in 18 (81.8%) diabetic patients. All subjects had a single foot ulcer infected with yeasts or with yeasts and bacteria. According to Wagner classification (14), we classified diabetic foot lesions into five categories according to depth of ulcer, degree of infection, and extent of gangrene. Chronic deep abscess (3°B) was present in eight (36.4%) patients. Moist gangrene of one toe (4°B) was found in seven (31.8%), infected deep ulcer (2°B) in four (18.2%), infected surface ulcer (1°B) in two (9.1%) patients, and whole-foot gangrene (5°) in one (4.5%) patient. The mean duration of foot ulcer infection was 12.3 (range 8-20) weeks, the thumb and plantar-metatarsal area being the most common ulcer sites, found in 11 (50%) and five (22.7%) patients, respectively. Other less common ulcer sites

Table 1. Index patient details

No.	Dg.	Sex	Age	Type of DM	Duration of DM (yrs)	Duration of infection (wks)	Swab of interdigital spaces		Biopsy		
							Ulcerated foot	Ulcer free foot	Mycology	Bacteriology	HPD
1	4°B	F	58	2	6	8	<i>C. albicans</i> <i>G. candidum</i> <i>P. mirabilis</i>	–	<i>C. albicans</i> <i>C. parapsilosis</i>	<i>P. mirabilis</i>	+
2	3°B	M	56	1	17	20	<i>C. parapsilosis</i> <i>P. mirabilis</i>	–	<i>C. parapsilosis</i>	<i>P. mirabilis</i>	+
3	4°B	F	80	2	10	8	<i>C. parapsilosis</i>	–	<i>C. parapsilosis</i>	<i>M. morgani</i>	+
4	4°B	F	79	2	10	8	<i>C. tropicalis</i>	–	<i>C. tropicalis</i>	–	+
5	4°B	F	80	2	10	8	<i>C. parapsilosis</i>	–	<i>C. parapsilosis</i>	–	+
6	2°B	M	57	2	10	13	<i>R. rubra</i> <i>E. coli</i>	–	<i>C. tropicalis</i>	<i>P. vulgaris</i> <i>Enterococcus</i> <i>S. aureus</i>	+
7	4°B	M	70	2	20	10	<i>C. albicans</i>	–	<i>C. albicans</i>	<i>Acinetobacter</i>	+
8	3°B	F	79	2	2,5	10	<i>C. glabrata</i>	–	<i>C. glabrata</i>	<i>Enterococcus</i>	+
9	3°B	M	57	2	10	14	<i>C. tropicalis</i> <i>D. hansenii</i> <i>C. famata</i> <i>S. aureus</i>	–	<i>C. tropicalis</i>	–	+
10	2°B	M	77	2	2	12	<i>C. parapsilosis</i>	–	<i>C. tropicalis</i>	–	+
11	3°B	M	74	2	10	13	–	–	<i>C. lipolytica</i>	<i>P. mirabilis</i> <i>C. freundii</i>	+
12	3°B	M	56	1	17	14	<i>C. parapsilosis</i> <i>S. aureus</i>	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>	–	+
13	3°B	M	57	1	18	12	–	–	<i>C. parapsilosis</i>	<i>P. mirabilis</i>	+
14	4°B	F	79	2	9	12	–	–	<i>C. tropicalis</i>	<i>M. morgani</i> <i>Enterococcus</i>	+
15	5°	F	54	2	4	14	–	–	<i>C. krusei</i>	<i>P. mirabilis</i>	+
16	2°B	F	71	2	15	13	<i>S. aureus</i>	–	<i>C. glabrata</i>	<i>P. mirabilis</i>	+
17	1°B	F	76	1	15	15	–	–	<i>C. kefyr</i>	–	+
18	4°B	M	76	2	20	15	–	–	<i>C. parapsilosis</i>	<i>P. aeruginosa</i>	+
19	1°B	M	53	1	13	12	<i>S. aureus</i>	<i>S. aureus</i>	<i>C. famata</i>	<i>Enterococcus</i>	+
20	3°B	M	62	2	10	12	–	–	<i>C. parapsilosis</i>	<i>P. mirabilis</i> <i>Serratia spp.</i>	+
21	3°B	M	63	2	10	14	–	–	<i>C. parapsilosis</i>	<i>Serratia spp.</i> <i>S. aureus</i>	+
22	2°B	M	62	2	11	13	<i>S. aureus</i>	–	<i>C. parapsilosis</i>	–	+

1°B = infected surface ulcer, 2°B = infected deep ulcer, 3°B = chronic deep abscess, 4°B = humid one-toe gangrene; 5° = whole foot gangrene; F = female, M = male; DM = diabetes mellitus; HPD = histopathologic diagnosis

were the heel and second toe, found in 2 (9.1%) patients each, as well as dorsum of the foot and fifth toe in one (4.5%) patient each.

Microbiologic diagnosis of diabetic foot ulcer infection

The fungal infections of diabetic foot ulcers were caused by eight *Candida* (*C.*) species (Table 1). *C. parapsilosis* was the most common causative agent (45.5% of patients), followed by *C. tropicalis* (22.7% of patients), *C. albicans* and *C. glabrata* (9.1% of patients each). The remaining four species (*C. krusei*, *C. kefyr*, *C. famata* and *C. lipolytica*) were isolated at an equally low rate (4.8% of patients).

While pure fungal infections of diabetic foot ulcer were found in 31.8% of patients, mixed fungal-bacterial infections occurred twice as often (68.2% of patients) (Table 1). The most commonly isolated bacteria from ulcer tissue were gram-negative species of *Enterobacteriaceae* (*Proteus* spp., *Serratia* spp., *Morganella* spp.) and *Pseudomonas* spp.

More severe clinical pictures (moist gangrene of single toe, whole-foot gangrene and deep abscess of plantar space) were found in 80% of patients with mixed ulcer infections, whereas they were significantly less common in patients with pure fungal ulcer infections (57.1%) (Table 1). Milder clinical pictures of foot ulcer infections (infected superficial and deep ulcers) were significantly more common in patients with pure fungal infections (42.9%) than in those with mixed infections (20%).

While coexistent interdigital yeast colonization of ulcerated foot (45.5% of patients) and both feet (4.5% of patients) was confirmed in half of the patients, the colonization of unulcerated foot could not be found in any of the patients (Table 1). The diversity of yeast species obtained from interdigital spaces was much wider than the diversity of those obtained from ulcers, seeing as they were colonized by species from three genera (*Geotrichum*, *Rhodotorula* and *Debaryomyces*) other than *Candida*, while the ulcers were infected exclusively with *Candida* spp. (Table 1). The most frequent colonizer of interdigital spaces was *C. parapsilosis*, colonizing ulcerated feet five times more often (22.7%) than unulcerated feet (4.5%). However, in only one third of the patients (31.8%) the same *Candida* sp. was isolated from interdigital spaces and

ulcer tissue of the same foot. Coexistent interdigital dermatophytosis could not be found in any of the patients.

Complete healing of the ulcer was achieved in 6-8 weeks with radical and repeat ulcer necrectomy in eight (36.4%) of the 22 diabetic patients with histologically demonstrated fungal and mixed ulcer infections without the administration of antimycotics, but with conservative treatment (antibiotics plus standard foot care) in all patients. Thirteen (59.1%) patients underwent amputation of a portion or the entire toe affected with inflammation. In one (4.5%) patient, transmetatarsal amputation of the ulcerated foot was done.

Histopathologic diagnosis (HPD) of diabetic foot ulcer infection

All investigated tissue specimens were representative and included both necrotic material and underlying tissue. In 22 patients with diabetic foot ulcers from which a culture of *Candida* species was obtained, histopathological evidence of deep fungal infection was also present (Figs. 1-3).

Figure 1. *Candida parapsilosis*, histopathology slide of diabetic foot ulcer biopsy, PAS, magnification X1000. Pseudohyphae and septate hyphae are clearly visible as well as the masses of bacteria in necrotic tissue.

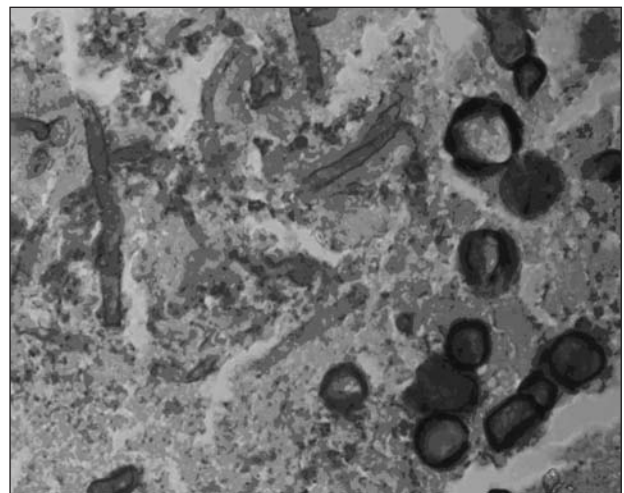


Figure 2. *Candida kefyr*, histopathology slide of diabetic foot ulcer biopsy, PAS, magnification X200. The unbranched fungal hyphae are well demonstrated at this magnification. Pseudophyphae and blastoconidia are not present.

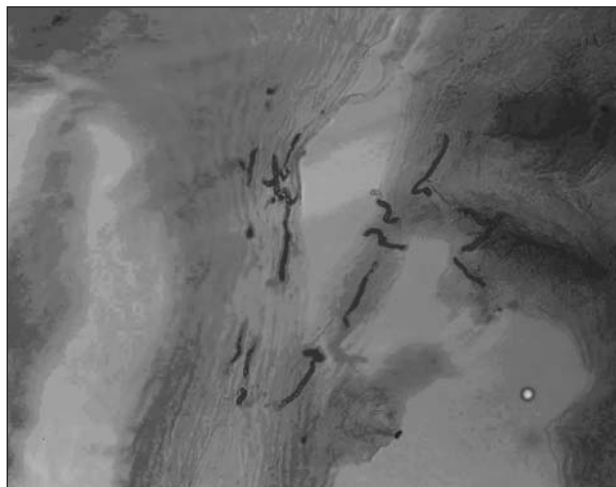
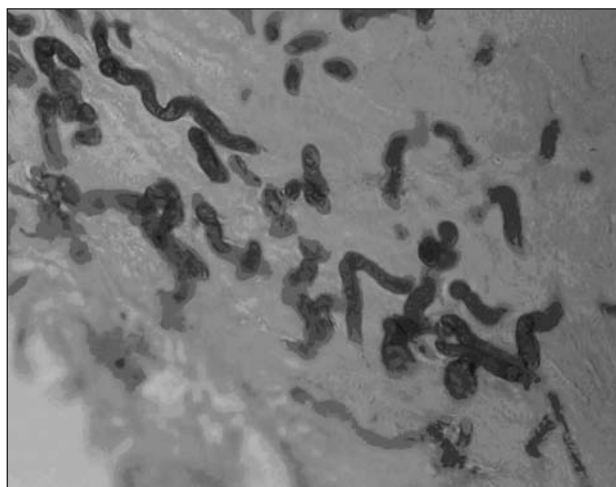


Figure 3. *Candida tropicalis*, histopathology slide of diabetic foot ulcer biopsy, PAS, magnification X1000. Only numerous hyphal fragments are seen. The absence of other fungal elements (pseudophyphae and blastoconidia) suggests that this *Candida* sp. is strongly invasive.



DISCUSSION

It has previously been suggested that fungal infections may be involved in the pathogenesis of diabetic foot ulcers, but this has yet to be explored. Literature references on fungal infections of diabetic foot ulcers are very scarce. Most reports have described low incidence of fungal isolations or of ulcers probably

infected by fungi or of ulceration which improve following systemic antifungal therapy (6-9). In the present study, as well as in those of other authors, *C. parapsilosis* was the most frequently isolated fungal species from diabetic foot ulcers (6). The result of our study concerning yeast isolation from single foot ulcer in all patients is in disagreement with some reports (6) describing positive fungal culture from multiple simultaneous foot ulcers in 59% of examined patients. The high incidence of mixed ulcer infections (in almost two thirds of the patients) and the presentation of these infections by more severe clinical pictures (in 80% of patients) indicate that, through synergy with other microorganisms (mostly enterobacteria), *Candida* spp. increase their pathogenic potential and the ability of causing ulcer infections. These and the results of the low incidence of pure *Candida* ulcer infections (in one third of patients), together with the milder clinical presentation of these infections (in more than 40% of patients), as well as the development of fungal and mixed infections in chronically infected ulcers in all patients (from eighth week onward, in thirteenth week on an average) indicate that *Candida* spp. play a secondary role in initiating diabetic foot ulcers.

A possible explanation for parallel increase in the incidence of ulcer yeast infection with the increased length of foot ulcer infection is long wrapping of the foot and the application of antibiotics during treatment. As known from the literature, on covering the skin with dressings (which stimulates sweating and increase local temperature of the skin), selective (antibacterial) and immunomodulating actions of antibiotics favor the growth and replication of yeasts (12,13,15). The negative finding of interdigital dermatophytosis in our study is explainable by good patient education (mostly urban population and availability of physicians of every specialty) about proper daily foot hygiene and the need of daily foot self-examination. In fact, daily washing of the feet statistically significantly hampers and decelerates the penetration of dermatophytic moulds into the skin and thus the development of dermatophytosis as well (16).

Our results show that coexistent interdigital colonization with yeasts and dermatophytosis has no impact on the incidence of fungal diabetic foot ulcer infections. Neither are these reliable indicators in suspecting *Candida* infection of diabetic foot ulcers. Consequently, carrying out routine mycologic

surveillance of interdigital spaces of either foot in diabetic patients affected with chronically infected nonhealing foot ulcers is unjustified.

As a result of the nonspecific nature of the clinical findings in fungal foot ulcer infection, the diagnosis depends on two basic laboratory approaches: mycologic and histopathologic. It is always preferred that cultural studies, which provide identification of the etiologic agent(s), be done in conjunction with histopathology examinations, which resolve the clinical dilemma whether a fungal isolate is truly pathogenic or merely a superficial colonizer. However, because of the expected low incidence of fungal and mixed infections (less than 5%), there is no indication for routine mycologic and HPD of ulcer infections. The diagnosis of these infections should be directed to the most predisposing diabetic patients (the ones with the progression of ulcer infections despite standard antibacterial therapy and foot care, and those with chronically infected ulcers).

Although some authors have described the benefit of systemic antifungal therapy (flucytosine, fluconazole, itraconazole or terbinafine administered orally at a variable dose and duration), without revascularization procedures during the period of antifungal therapy (6), the influence of antifungal therapy on protracted diabetic foot ulcers is still unclear. Factors that may also impair healing include ischemia, fibrosis, abnormalities in the release and action of growth factors, overexpression of proteases and oxygen free radicals, hypoxia, delayed keratinocyte migration, and metabolic abnormalities (2,3). Nevertheless, we feel that our results of poor treatment outcome without the administration of specific therapy (amputation in almost two thirds of patients) should justify the introduction of systemic antifungal therapy in patients with histopathologically verified fungal ulcer infections.

We believe that our results will make it easier for clinicians to treat fungal and mixed fungal-bacterial infections of diabetic foot ulcers, as well as encourage further research into these infections.

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