Original article

Mycotic infections in diabetic foot ulcers in Emam Reza hospital, Mashhad, 2006-2008

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Abstract

Introduction and objective: Diabetic foot is the result of uncontrolled diabetes and imperfect sanitary care which leads to necrotic lesions, gangrene and finally amputation. Secondary mycotic infections play a principal role to produce chronic lumpy lesions. This study was designed to investigate the incidence of fungal pathogens in diabetic foot infections.

Materials and methods: The study population included 120 consecutive diabetic patients who were hospitalized in the department of vascular surgery due to diabetic foot during 2006-2008. Direct fresh smear and fungal culture were performed for each patient. Fungal contaminations were confirmed by direct microscopy and/or culture.

Results: The ages of the patients were between 32 to 86 years old. Of those 86(71.7%) individuals were male and 34(28.3%) were female. Direct examinations in 10% KOH were

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positive for fungal element in 25(20.8%) cases, but cultures were positive in 30(25%) cases. *Candida* species were the most predominantly isolated fungi (23 patients). Dermatophytic infection due to *Trichophyton mentagrophytes* was observed in three cases. The isolated opportunistic molds were known as *Acremonium* spp., *Aspergillus fumigatus* and *Scopulariopsis* spp. Significant correlation was found between infection, gender and age of the patients (P=0.0001 and P=0.0001).

Conclusion: This study shows that fungal infection can be observed in about more than 20% of diabetic foot and causes a lesion with poor prognosis. The most common cause of mycotic diabetic foot is different species of *Candida* spp., especially *C. albicans*.

Keywords: Diabetic foot ulcer; Fungal infection; *Candida albicans*

Introduction

Diabetes mellitus is the most common endocrine disorder and takes on pandemic proportions. Worldwide, over~246 million people suffer from the disease in 2007 and estimates for 2025 are depicted at a total of 380 million patients [1]. As a consequence, the impact of diabetic foot disease is on the rise as well. Diabetic foot disease is a poly etiological disease, in the majority of patient peripheral neuropathy plays a central role.

Neuropathy leads to an insensitive and sometimes deformed foot, often with abnormal walking pattern. In patient with neuropathy, minor trauma-cased for example by ill-fitting shoes, walking barefoot, or an acute injury- can precipitate a chronic ulcer [2,3]. Other possible causes of this increased prevalence of infections are defects in immunity, peripheral vascular disease and slower wound healing [4]. Furthermore, some microorganisms become more virulent in high glucose environment [5].

Mycotic infections may increase the risk of developing diabetic foot syndrome. However, little data are available on the prevalence of fungal foot infections in patients with diabetes [6,7]. Ulcers of the foot in diabetes are a source of major suffering and cost. Investing in a diabetic foot can be one of the most cost-effective forms of healthcare expenditure [8]. The risk of toe or lower leg amputation may be increased if ulceration is followed by bacterial and fungal infections [6,9,10].

Bacterial infection of diabetic foot ulcers are polymicrobial and have aerobic and anaerobic origin, which have been characterized in detail [11,12]. However, data on the frequency of fungal isolation from the diabetic patients are rare and heterogeneous. Some studies have reported that some filamentous fungi and yeasts as etiological agents of diabetic foot infection [6,9,13-21]. The fungi involved in diabetic foot ulcers are mainly *Candida* spp. [10,18, 19,22]. The objective of this study was to determine the etiologic agents and frequency of fungal infections in ulcerated diabetic foot tissue samples by conventional mycological technique.

Materials and methods

During the 33 months period, from January 2006 to September 2008, 120 consecutive diabetic patients (86 male, 34 female) with chronic diabetic foot ulcers whose wounds have not already received any antiseptic, antibiotic or surgical treatment were examined for fungal infection. Personal details of patients are in table 1. They were initially presenting with diabetic foot ulcers to the university department of vascular surgery in Emam Reza hospital, Mashhad

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Table 1: Personal details of patients with infected diabetic foot ulcers

Age (year)	Male (%)	Female (%)	Total (%)
<40	4 (3.3)	1 (0.8)	5 (4.1)
41-50	14 (11.7)	4 (3.3)	18 (15)
51-60	29 (24.2)	12 (10)	41 (34.2)
>60	39 (32.5)	17 (14.2)	56 (46.7)
Total	86 (71.7)	34 (28.3)	120 (100)

The patients with diabetic foot ulcers (Figs. 1, 2) that they did not use antifungal agents in the last four weeks before fungal examinations were studied. Information about the basic data include, age, sex, duration of diabetes, currently therapy, presence of vascular insufficiency and/or neuropathy and etc. which were obtained using a questionnaire with their own consent.

Scraping of the lesion surface and nail clipping was performed for all feet with diabetic ulcers. A portion of the material was soaked in 10% KOH and analyzed by direct microscopic mycological examination and the rest was cultured on Sabouraud dextrose agar (SDA, Biomark, Himedia, India) and SDA supplemented with chloramphenicol (SC) and SC medium with cycloheximide (SCC), (Quelab, Canada). Incubation was performed at 30°C and 37°C for four weeks.

The colonies were identified on the basis of their macroscopic and microscopic (slide culture) features. Yeast samples were cultured in Chrom agar Candida (Himedia, India) and Cornmeal agar (Himedia, India) with Tween 80 for isolation and identification of *Candida* spp. The data analyzed using Chi-squared test with SPSS v.18 softwere. A P Value <0.05 was considered statistically significant.

Fig. 1: A typical deep ulceration caused by *Candida albicans* in a diabetic patient

Fig. 2: Simultaneous gangrene of the toe and interdigital space with superimposed infection due to *Trichophyton mentagrophytes* in a diabetic patient

Results

A total of 120 patients were enrolled; 86 male (71.6%) and 34 female (28.4%). Male/female ratio was 1: 0.39. The age of the patients ranged from 32-86 years with the mean of 57.5, and the most common age groups were ≥60 (46.6%), 51-60(34.1%), 41-50(15%) and 32-40(4.1%) (Table 1). Significant correlation was found between infection, gender and the age of the patients (P=0.0001 and P=0.0001).

Among the cases, 115(93.9%) patients had type II diabetes mellitus, whereas only 5(4.1%) of them had type I diabetes mellitus. Foot ulcers were found in 108

patients (90%), and previous toe amputation in 20(16.7%) of patients. The toes were the most common ulcer site (75.81% of patients).

Of these, direct examinations in 10% KOH were positive for fungal elements in 25(20.8%) cases, but cultures were positive in 30(25%) cases. Five cases of direct microscopies were negative but their cultures were positive for *Candida* species. Direct microscopy and culture of all of the the filamentous fungi were positive for fungal elements, and in 90(75%) cases, culture and direct microscopy were negative (Table 2).

Table 2: Mycological culture results from 120 diabetic patient samples

Fungal organism	Number (%) of patients
C. albicans	11(9.1%)
C. tropicalis	5(4.1%)
C. parapsilosis	1(0.83%)
C. glabrata	1(0.83%)
C. krusei	1(0.83%)
Candida spp.	4(3.3%)
T. mentagrophytes	3(2.5%)
Rhodotorula spp.	1(0.83%)
Acremonium spp.	1(0.83%)
Scopulariopsis spp.	1(0.83%)
A. fumigatus	1(0.83%)
Negative culture	90(75%)

The identified species out of 30 cases were: *C. albicans* 11 (9.1%), *C. tropicalis* 5 (4.1%), *C. parapsilosis*, *C. glabrata* and *C. krusei* each 1 (0.83%), *Candida* spp. 4 o (3.3%), *Trichophyton mentagrophytes* 3 (2.5%), *Rhodotorula* spp., *Acremonium* spp., *Scopulariopsis* spp., and *Aspergillus fumigatus* each 1 (0.84%) (Table 2). All of the fungal infections were seen in patient with type II diabetes mellitus.

Discussion

Diabetic foot is the result of uncontrolled diabetes and imperfect sanitary care which leads to necrotic lesions, gangrene and finally amputation. The risk of toe or lower leg amputation may be increased if ulceration is followed by bacterial and fungal infections [6,9,10]. It has already been suggested that fungal infections may be involved in the pathogenesis of diabetic foot ulcers, but this needs more investigation [4]. References on fungal infections of diabetic foot ulcers are rather rare.

Our study evaluated the prevalence of fungal infection in diabetic foot ulcers. Our data are supplemented by the results of questionnaire containing demographic and clinical information of the patients. Our results are surprising and the patients have high level of cooperation. Most of the patients (75%) have severe disease and have been hospitalized however 25% of them were in general practice. In this group, 30 patients (25%) had fungal infections which were confirmed by direct microscopy and/or cultures.

Similar to previous investigations [6, 13], significant relationships were found between gender and age of the patients. Majority of the patients with diabetic foot ulcers were men and older than 40 years and all of the fungal infections were found in men, older than 50 years of age (17 patients older than 60 years and 13 patients older than 50 years). In this study, diabetic foot in men were about 2.5 times more numerous than women, similar findings have been reported by Piérard *et al*. [15]. This indicates that gender-related factors affect the skin and nail structure and it may be due to differences in life style, propensity to micro traumatisms, professi-onal activities, sport practices and etc.

Our study is similar to the other studies by Yosipovitch *et al.* [23] and Sawhney *et*

al. [24]. Majority of the patients with skin lesions had uncontrolled diabetes, because uncontrolled diabetes increase the risk of development of microangiopathy and related complications or sequelae.

Our result showed that *Candida* spp. were the most frequent isolated, including *C. albicans* (36%) and *C. tropicalis* (16%). Similarly, Nair *et al.* [19] reported high prevalence of *Candida* spp. [*C. albicans* (46%) and *C. tropicalis* (27%)]. Whereas, Heald *et al.* [10], Chincholikar *et al.* [22] and Bansel *et al.* [18] reported *C. tropicalis* as the predominant isolate. The presence of other species of *Candida* (*C. parapsilosis, C. guilliermondii, C. krusei, C. tropicalis, C. famata, C. kefyr* and *C. glabrata*) has been reported in diabetic patients by other investigators [6,10,18,20,22].

In contrast to this study Eckhard *et al*. [6] and Romano *et al.* [16] have demonstrated that dermatophytic infection in diabetic foot patients was more common than *Candida* spp. and reported *T. rubrum* as the most isolated fungi in these patients. This conflicting data can be explained by differences in exposure time to infection agents and differences in climatic and socio-economic factors in the respective geographical areas. Furthermore mold species (*Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Scopolariopsis* spp.) were reported as causative agents of fungal infections in the diabetic foot patients [6,19,21]. Honestly, it was very difficult to differentiate fungal infection from fungal colonization. To avoid this problem sampling was performed by scalpel rather than swab and the samples were cultured on serial passages.

Conclusion

We conclude that fungal infection can be observed in more than 20% of patients with diabetic foot ulcers, and *Candida* spp. were the predominant isolates among fungal pathogens. Thus, mycological evaluation of diabetic foot ulcers is necessary in these patients. We believe our results have important implications for the prevention and recognition of mycotic foot disease in diabetic foot and recommend that diabetic foot patients should be examined for fungal infections.

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