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Report

Prevalence and epidemiology of tinea pedis and toenail onychomycosis and antifungal susceptibility of the causative agents in patients with type 2 diabetes in Turkey

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Abstract

Background Diabetes patients are particularly susceptible to fungal infections because their vascular and immunological systems are compromised.

Objectives The present study aimed to determine prevalences of tinea pedis and onychomycosis, factors predisposing to their development, and antifungal susceptibilities of causative fungal species against fluconazole, itraconazole, and terbinafine in patients with type 2 diabetes mellitus (DM).

Methods Study groups were defined according to hemoglobin A1C rates of ≥6.5% for the diabetes group and ≤5.7% for control subjects. A total of 600 diabetes subjects and 152 control subjects were evaluated. Rates of onychomycosis and tinea pedis in diabetes patients, and associations with age, gender, blood glucose level, duration of diabetes and serum lipid profile were investigated, as were the distribution and antifungal susceptibility of agents isolated.

Results Patients with onychomycosis and/or tinea pedis numbered 85 in the diabetes group and nine in the control group ($P = 0.006$). The development of onychomycosis or tinea pedis was significantly related to increasing age and male gender. Although the most common agents were dermatophytes, non-dermatophyte fungal isolates were not uncommon. Terbinafine was the most effective drug against dermatophytes but was invalid for non-dermatophyte isolates by *in vitro* antifungal susceptibility testing.

Conclusions The development of onychomycosis or tinea pedis was significantly related to type 2 DM, increasing age, and male gender. The most common isolate was *Trichophyton rubrum*. The isolation and identification of the fungus is important to the effective management of tinea pedis and onychomycosis in diabetes patients because non-dermatophyte fungi can cause these infections.

Introduction

According to World Health Organization reports, the global prevalence of diabetes was estimated to be 9% among adults aged ≥18 years in 2014, and hence about 347 million people around the world have the disease.[1] In Turkey, the national prevalence was 7.4%, and the number of adults aged 20–79 years with diabetes was 3.7 million in 2010.[2] Type 2 diabetes mellitus (DM) is the most common form of diabetes and accounts for 90% of all cases of diabetes. Diabetes can cause complications that affect all systems of the body. However, such complications mainly refer to the compromising of the vascular and immune systems and of peripheral neuropathy, and hence diabetes patients are particularly susceptible to fungal infections.[3] Gupta *et al.*[3] showed that patients with diabetes were 2.77 times more likely to develop onychomycosis than those without diabetes, and onychomycosis is associated with tinea pedis in 33% of diabetes patients. These superficial infections not only cause cosmetic problems but also increase the risk for secondary bacterial infections such as lower limb cellulitis.[4] The abrasion or ulceration can increase in size, become chronic, and serve as a portal of infection for bacteria, fungi, or other organisms. Impaired wound healing may result in increased morbidity, the possible amputation of the lower extremity, and even mortality.[3]

The present study aimed to determine the prevalences of tinea pedis and onychomycosis and the factors predisposing to their development in patients with type 2 DM in Turkey. In addition, the causative fungal species and their antifungal susceptibilities were evaluated.

Materials and methods

Participants and sampling

Participants were randomly selected from adult outpatients admitted to the internal medicine clinic for various problems other than dermatological symptoms from January 2014 to January 2015. Hemoglobin (Hb) A1C rates were evaluated in all participants, and study groups were designated according to Hb A1C rates: patients previously diagnosed with type 2 DM and with A1C levels of ≥6.5% were included as patients with diabetes in this study.[5] Subjects without diabetes with Hb A1C rates of ≤5.7% were included as a control group. Local ethics committee approval and participant consent from all subjects were obtained.

Various data and laboratory parameters were obtained in all subjects. These included details of age, gender, duration of diabetes, height and weight measurements (for body mass index), serum glucose, Hb A1C levels, and serum lipid profile. The feet of all participants were examined for any sign or symptom of tinea pedis or onychomycosis in both the diabetes and control groups. When any clinical abnormality resembling superficial mycosis was determined, the area to be sampled was cleaned with 70% alcohol, and skin scrapings and/or nail clippings were collected and sent to the microbiology laboratory in sterile containers for mycological examination.

Microbiological examination

Microscopic examination was performed after treatment with 15% potassium hydroxide for the presence of fungal filaments. All specimens were then inoculated on Sabouraud dextrose agar plates with and without cycloheximide. These plates were incubated aerobically at 26 °C and 30 °C for up to 4 weeks before they could be discarded as negative. Filamentous fungal isolates were identified by colony morphology, microscopic appearance, and biochemical tests. Yeast isolates were identified by germ tube production, microscopic morphology on cornmeal agar, and a commercial identification system based on assimilation of carbohydrates (API 20C AUX; bioMérieux SA, Marcy l'Etoile, France).

For a mycological finding in a specimen to be regarded as significant, it was necessary to observe fungal filaments under the light microscope, except when a dermatophyte was cultured.[3] In the case of a yeast or non-dermatophyte mold, congruous and recognizably non-dermatophytic fungal spores, filaments, or pseudomycelium had to be observed under the microscope, and the culture was required to be positive for these organisms. In instances in which the culture grew a yeast or other non-dermatophyte with negative light microscopic examination, or in which a non-dermatophyte grew as a probable contaminant from specimens positive for filaments consistent with those of a dermatophyte, the non-dermatophyte was regarded as not causative of onychomycosis or tinea pedis and therefore excluded from the list of organisms causing onychomycosis or tinea pedis. It was not within the scope of this study to have patients return for repeat sampling.[3]

Antifungal susceptibility

Antifungal susceptibility testing for all isolates was performed against terbinafine (TRB), fluconazole (FLU), and itraconazole (ITRA) according to Clinical and Laboratory Standards Institute (CLSI) recommendations.[6,7] The following drug concentration ranges were used for non-dermatophyte molds and yeasts: ITRA, 0.0313–16 μg/ml; FLU, 0.125–64 μg/ml, and TRB, 0.0078–2 μg/ml. Drug concentration ranges for testing dermatophytes were: ITRA and TRB, 0.0019–0.5 μg/ml, and FLU, 0.125–64 μg/ml. These antifungal solutions were prepared at 2× dilutions, dispensed into 96-well microtiter plates at quantities of 0.1 ml in each well, and stored at −70 °C until use.

Prior to the initiation of testing, each mold isolate was subcultured on potato dextrose agar at 30 °C for 4–5 days, and each yeast isolate was subcultured on Sabouraud dextrose agar at 35 °C for 24–48 hours. The fungal mold colonies were covered with 1 ml of sterile 0.85% saline and a suspension was prepared by gently probing the colonies and subsequently transferred to a sterile tube. The heavy particles were allowed to settle for 3–5 minutes, and then the upper homogeneous suspension was transferred to a sterile tube. The final fungal suspensions were adjusted spectrophotometrically to be twice as concentrated as the density needed for testing. Final dilutions were made using RPMI 1640 broth. For yeast isolates, a standard 0.5 McFarland fungal suspension was prepared with sterile 0.85% saline and then diluted with RPMI 1640 broth medium to obtain a starting inoculum that provides $1–5 \times 10^3$ colony-forming units (CFU)/ml.

Each well of the microtiter plates containing antifungals was inoculated with 0.1 ml of the 2× fungal inoculum suspension. Thus both inoculum densities and drug concentrations were diluted at half the ranges of the final test concentrations desired. In addition, all plates included growth (drug-free) and sterility (microorganism-free) control wells for each isolate. All plates were incubated at 35 °C for 48 hours in non-dermatophyte isolates and for 4 days in dermatophyte isolates.

Candida krusei ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality controls, according to the CLSI M27-A3 document.[7]

Minimum inhibitory concentrations (MICs) were defined as follows: for FLU, the MIC allowing turbidity corresponding to reductions in growth of approximately ≥50% (non-dermatophyte isolates) or ≥80% (dermatophyte isolates) in comparison with growth in the control well; for ITRA, the lowest drug concentration to prevent any discernible growth (non-dermatophyte isolates) or the MIC allowing turbidity corresponding to a reduction in growth of approximately ≥80% (dermatophyte isolates) in comparison with growth in the control well; and for TRB, the MIC allowing turbidity corresponding to a reduction in growth of approximately ≥80% in comparison with

growth in the control well.[6] The CLSI standards for *Candida* spp. and *Cryptococcus neoformans* were used to evaluate MIC results for *Trichosporon* spp. as there is no antifungal susceptibility reference method for this species; MICs were defined as the lowest concentration at which a prominent decrease in turbidity was observed.[7]

In addition, interpretive breakpoints are not available for any species of filamentous fungi (including dermatophytes) versus any antifungal agent, and the clinical relevance of testing any organism–drug combination remains uncertain.[6] Therefore, susceptibility results were evaluated based on MIC values rather than on the description as susceptible or resistant.

Statistical analysis

Data were evaluated using IBM SPSS Statistics for Windows Version 20.0 (IBM Corp., Armonk, NY, USA). Student's *t*-test was used in instances of normally distributed variables, and the Mann–Whitney *U*-test was used in instances of non-normally distributed continuous variable parameters in comparative statistical analysis. The compliance of continuous variable parameters with normal distribution was assessed using the Kolmogorov–Smirnov test. In the presence of categorical variables, comparative analysis was made using the chi-squared test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated from the coefficients.

Results

A total of 600 diabetes patients (185 males and 415 females) and 152 control subjects without diabetes (40 males and 112 females) were included in this study. The mean ± standard deviation (SD) age was 58.91 ± 10.37 years in the diabetes group and 45.63 ± 12.46 years in the control group ($P < 0.001$). The groups were similar in terms of gender distribution ($P > 0.05$). A total of 459 skin or nail samples were obtained from 328 diabetes patients and 43 control subjects in whom at least one lesion suspicious for superficial mycosis was observed on the foot. In the diabetes group, lesions were observed in the interdigital regions in 127 patients, in nails in 123 patients, and in both the interdigital regions and nails in 78 patients. In the control group, lesions were observed in the interdigital regions in 13 subjects, in the nails in 20 subjects, and in both the interdigital regions and nails in 10 subjects. The frequencies of both onychomycosis and tinea pedis were significantly higher in the diabetes patients than in the control group ($P = 0.006$) according to criteria reported by Gupta *et al.*[3] In the diabetes group, onychomycosis and/or tinea pedis were mycologically detected in 85 (14.2%) subjects, among whom 39 had onychomycosis, 28 had tinea pedis, and 18 had onychomycosis together with tinea pedis. In the control group, onychomycosis and/or tinea pedis were detected in nine (5.9%) subjects, of whom five had onychomycosis, three had tinea pedis, and one had both onychomycosis and tinea pedis (Table 1). All of the tinea pedis cases were of the interdigital form, and all of the onychomycosis cases were of the subungual lateral form in the toenail. The distribution of isolates and results of antifungal susceptibility testing are presented in Table 2.

Table 1 Mycological findings in clinical specimens from type 2 diabetes mellitus patients ($n = 600$) and control subjects ($n = 152$)

Finding	Participants, *n*		
	Diabetes patients	Control subjects	All subjects
Positive for microscopy and culture	43	4	47
Positive for microscopy and no growth in culture	37	5	42
Negative microscopy and positive culture for dermatophyte	5	–	5
Total	85	9	94

A total of 89 specimens were positive for the presence of fungal filaments by microscopic examination, and 52.8% of them were positive for mycological culture (Table 1). As expected, culture positivity was significantly higher among specimens that were positive in microscopy than in those that were negative ($P < 0.001$); 11.6% of microscopy-negative specimens were positive for mycological culture. The agreement between direct microscopy and culture was moderate (Cohen's kappa: 0.43). In addition, using mycological culture as a reference-standard diagnostic method, sensitivity, specificity, positive and negative predictive values of microscopic examination were 59.5%, 64.0%, 31.0%, and 88.4%, respectively.

Various demographic and disease characteristics of the diabetes patients are summarized in Table 3. When patients with and without onychomycosis or tinea pedis were compared in terms of mean age, gender, Hb A1C levels, duration of diabetes, body mass index, and cholesterol and triglyceride levels, a significant difference was found for gender ($P < 0.001$); tinea pedis and onychomycosis were observed more frequently in men. In addition, this frequency significantly increased with age, especially in men (Fig. 1).

Discussion

The findings of the Achilles Foot Screening Project showed that the proportion of patients with foot diseases who visited a dermatologist was high (58%) and that fungal infections (35%), especially onychomycosis (23%) and tinea pedis (22%), were the most commonly clinically diagnosed foot diseases in the total population.[8] Onychomycosis is known to represent the most common nail disease and probably accounts for about 30% of all cutaneous fungal infections.[3,9–12] Although the most common predisposing factors for tinea pedis and/or

Table 2 Distribution and results of antifungal susceptibility testing of isolates from type 2 diabetes mellitus patients and control subjects

Isolate	Total, *n*		Antifungal susceptibility (μg/ml)								
			Fluconazole			Itraconazole			Terbinafine		
	Diabetes patients	Control subjects	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Trichophyton rubrum	33	2	2–8	4	8	0.25–0.5	0.25	0.25	0.0019–1	0.078	0.078
Trichophyton mentagrophytes (*interdigitale*, 4; *erinacei*, 1)	7	2	1–16	4	16	0.015–0.125	0.06	0.25	0.0019–0.0039	0.0039	0.0039
Trichophyton tonsurans	1		4			0.25			0.0156		
Trichophyton thuringiense	1		64			0.06			0.0039		
Trichophyton gloriae	1		8			0.06			0.0039		
Microsporum audouinii	1		4			0.06			0.0039		
Scopulariopsis brevicaulis	2		>64	>64	>64	1	0.5	1	1	0.5	1
Neoscytalidium dimidiatum	2		>64			0.25			1		
Chrysosporium keratinophilum	2[a]		4			0.5			0.0625		
Aspergillus spp.	2[a]	1	>64			0.5			0.25–1		
Fusarium spp. *(solani* and *chlamydosporum)*	2		>64			1			0.25–1		
Alternaria chlamydospora	1		32			0.25			0.5		
Trichosporon spp.	5		2–8	2	8	0.06–0.5	0.25	0.5	0.25–0.5	0.5	0.5
Candida albicans	1		1			0.06			0.5		
Total	61	5									

[a]Both isolates were obtained from the same patients.

onychomycosis are vascular disease and participation in sports, DM may be another important factor because it can also predispose the foot to disease or contribute to the severity of disease in the foot.[3,8,11–13] A total of 69.3% of patients with diabetes-related complications in the feet show at least one of these fungal infections.[13] Therefore, knowledge of the prevalences and epidemiology of tinea pedis and onychomycosis may be important for the appropriate management of these patients.

To the present authors' knowledge, this study is the first investigation to evaluate the prevalences of tinea pedis and onychomycosis and the factors associated with these conditions in patients with diabetes in Turkey. As expected, both fungal infections were significantly more frequent in diabetes patients (14.2%) than in the control group (5.9%). However, a former study found no significant differences between diabetes subjects and control subjects in the frequency of dermatophytosis (4.1% and 6.1%, respectively).[14] In addition, these differences were not found to be associated with the duration or control of diabetes, blood glucose levels, gender, or age. However, these investigators included a control group consisting of subjects admitted to a dermatology outpatient unit, and thus it is unlikely that this control group represented an accurate reflection of the general population. Dogra *et al.*[11] reported the prevalences of clinical onychomycosis in diabetes patients and control subjects to be 17.0% and 6.8%, respectively. It is probable that the present results (9.5% and 3.9%, respectively, for onychomycosis) were lower than those reported by Dogra *et al.*[11] because the latter investigators used clinical data for the diagnosis of onychomycosis, whereas the present cases referred to proven microbiological results. Manzano-Gayosso *et al.*[15] found an incidence of onychomycosis of 28% in type 2 DM patients; these investigators reported that incidence increased with age and gave the average age of their patients as 63.5 years, which is slightly higher than the 58.9 years in the present study groups. In addition, Manzano-Gayosso *et al.*[15] included all forms of onychomycosis, whereas the present study evaluated only the distal lateral subungual form.

Leelavathi *et al.*[12] found a higher incidence of onychomycosis in diabetes patients but used nail abnormalities with a positive culture as their criterion for diagnosis. Probably for this reason, these authors reported that non-dermatophyte molds were the most common fungi isolated (39.7%), followed by yeast (20.5%) and dermatophytes (0.7%).[12] By contrast, in the present study, most isolates were dermatophytes (72.7%), and *Trichophyton rubrum* (53.0%) was the most common agent of onychomycosis and tinea pedis, followed by non-dermatophyte molds (18.2%) and yeast (9.1%). In a study similar to the present investigation, Gupta *et al.*[3] reported that most isolates were dermatophytes (88%), and that *Candida* spp. and non-dermatophyte molds accounted for 3% and 9% of cases, respectively. *Trichophyton rubrum* has been identified as the most common etiological agent of onychomycosis and tinea pedis in many studies.[3,11,14–17] Although *Candida* spp. have been reported as representing the most common yeast pathogen of onychomycosis in several

Table 3 Demographic and disease characteristics of patients with diabetes (n = 600)

Patient characteristics	Diabetes mellitus patients (Hb A1C ≥ 6.5), *n*		
	Without onychomycosis and/or tinea pedis (*n* = 515)	With onychomycosis and/or tinea pedis (*n* = 85)	*P*-value
Patients, *n* (%)	515 (85.8%)	85 (14.2%)	
Age, years, range (mean)	25–84 (58.6)	25–80 (60.7)	>0.05
Gender, female/male, *n*	374/141	41/44	<0.001
Blood glucose, mmol/L, range (mean)	62–614 (208)	85–460 (202)	0.801
Hb A1C, %, range (mean)	6.5–17.6 (8.9)	6.5–13.0 (8.7)	0.659
Patients by Hb A1C level, %			
6.5–7.9%	39.4%	40.0%	
8.0–9.9%	32.8%	40.0%	
10.0–11.9%	17.9%	16.5%	
≥12.0%	9.9%	3.5%	
Duration of diabetes, years, range (mean)	1–35 (8.8)	1–30 (8.0)	0.223
Patients by duration of diabetes, %			
1–10 years	68.3%	71.8%	
11–20 years	24.8%	21.2%	
≥ 21 years	6.9%	7.0%	
BMI, kg/m^2, range (mean)	16.2–53.4 (32)	22.7–45.1 (32)	0.925
Patients by BMI, %			
16–25 kg/m^2	12.1%	10.6%	
26–35 kg/m^2	67.3%	61.2%	
≥36 kg/m^2	20.6%	28.2%	
Total cholesterol, mg/dL, range (mean)	70–511 (216)	120–326 (217)	>0.05
Patients by cholesterol level, %			
50–150 mg/dL	9.5%	8.2%	
151–250 mg/dL	70.0%	71.8%	
≥251 mg/dL	20.5%	20.0%	
LD lipoprotein, mg/dL, range (mean)	28–390 (128.0)	31–207 (128.2)	>0.05
Patients by LD lipoprotein level, %			
1–100 mg/dL	22.5%	16.5%	
101–200 mg/dL	73.9%	82.4%	
≥201 mg/dL	3.6%	1.1%	
HD lipoprotein, range (mean)	23–182 (46.0)	26–79 (44.7)	>0.05
Patients by HD lipoprotein level, %			
20–39 mg/dL	31.0%	35.3%	
40–59 mg/dL	60.0%	54.1%	
≥60 mg/dL	9.0%	10.6%	
Triglyceride level, mg/dL, range (mean)	28–1427 (225.0)	57–1278 (216.2)	>0.05
Patients by triglyceride level, %			
1–200 mg/dL	53.2%	54.1%	
201–400 mg/dL	37.5%	40.0%	
≥401 mg/dL	9.3%	5.9%	

BMI, body mass index; Hb, hemoglobin; HD, high-density; LD, low-density.

studies,[3,11,15,18] the majority of yeast isolates in the current study (five of six yeast isolates; four in onychomycosis, one in tinea pedis) were *Trichosporon* spp. However, Gunduz *et al.*[19] reported a higher rate of *Trichosporon* spp. as an agent of onychomycosis in primary schoolchildren in Turkey. Although the frequencies of causative agents may vary according to geographic and climatic conditions, this emerging yeast should also be considered as a primary agent of onychomycosis.[20]

The present authors observed that the development of tinea pedis or onychomycosis in diabetes patients was significantly higher among males than among females and increased with age. This finding is compatible with those of other similar studies.[3,11,12,15] However, no significant correlation between the presence of onychomycosis or tinea pedis and levels of blood glucose or Hb A1C, duration of DM, body mass index, and serum lipid profile emerged in the present study. Romano *et al.*[14] found no correlations between dermatophytosis and duration or type of diabetes, its complications, or glucose and glycosylated hemoglobin levels. Similarly, Dogra *et al.*[11] found no correlation between the prevalence of onychomycosis and

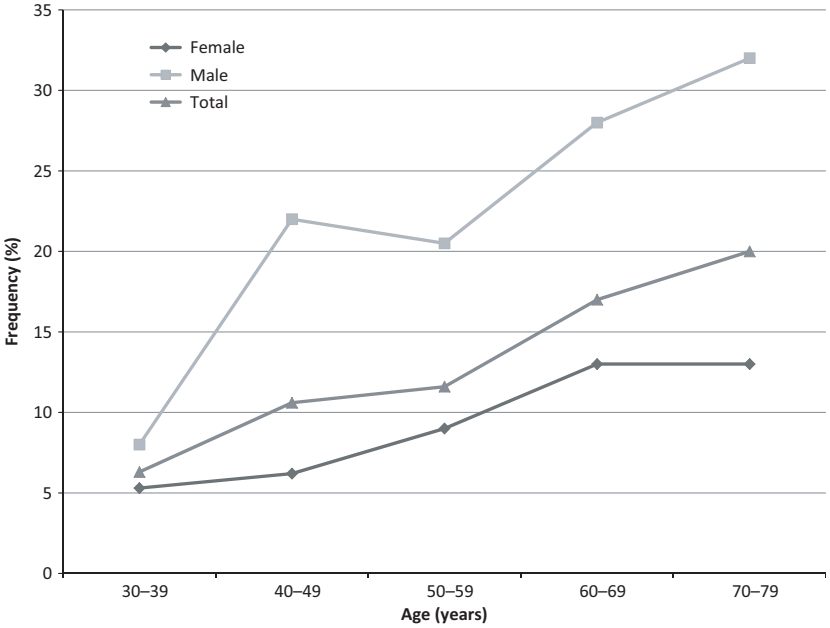

Figure 1 Distributions of onychomycosis and tinea pedis by age and gender

mean blood glucose levels in the previous 6 months; however, both the prevalence and severity of onychomycosis were significantly more often associated with the duration of diabetes.

In dermatophytosis, systemic treatment is generally considered to be the most successful approach; the management of dermatophytic skin and nail infections in diabetes patients is based on oral antifungal therapy.[17,21] Although griseofulvin was the first oral antifungal agent to be approved for use in skin and nail dermatophyte infection, TRB, and ITRA have shown much greater success rates in toenail infections.[21] A Cochrane review revealed that TRB was significantly more effective in tinea pedis than griseofulvin, and TRB and ITRA were more effective than placebo.[22] In addition, TRB achieved higher clinical cure rates than ITRA or FLU in the treatment of onychomycosis.[22] Similarly, *in vitro* studies revealed that TRB had the lowest and FLU had the highest MIC values against dermatophytes.[17,23,24] The present study also identified TRB as having the lowest and FLU had the highest MIC values for all dermatophyte strains tested. However, isolates from the present patients included not only dermatophytes but also non-dermatophyte molds and yeasts at a rate of almost 25%. The MIC values of both TRB and ITRA increased against non-dermatophyte molds and yeasts. Therefore, only TRB or ITRA treatment cannot be acceptable for all of diabetic patients with tinea pedis or onychomycosis due to this diversity of causative agents. Therapy of onychomycosis caused by non-dermatophyte molds is extremely challenging and time-consuming and may require the application of topical amphotericin B, voriconazole, and sometimes combinations of drugs such as ITRA and TRB to achieve synergistic effects.[25] Ultimately, the isolation and identification of the agent are important for the effective management of tinea pedis and onychomycosis in diabetes patients.

In conclusion, fungal skin and nail infections of the feet, such as tinea pedis and onychomycosis, were significantly more frequent in diabetes patients, and the development of onychomycosis or tinea pedis in these patients was significantly associated with increasing age and male gender. However, no correlations were detected between the presence of onychomycosis or tinea pedis, and blood glucose or Hb A1C levels, duration of DM, body mass index, or serum lipid profile. Although the most common agents were dermatophytes, particularly *T. rubrum*, non-dermatophyte fungal isolates were not uncommon. *In vitro* antifungal susceptibility testing revealed that TRB was the most effective drug against dermatophytes but was invalid for non-dermatophyte isolates. For this reason, the isolation and identification of the fungus are important for the effective management of tinea pedis and onychomycosis in diabetes patients.

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