

Onychomycosis in patients with diabetes mellitus: Etiology, clinical features, and treatment response

Alma Ileana Molina-Hernandez¹, Hassiel Aurelio Ramírez-Marín¹, Alexandro Bonifaz², Judith Guadalupe Dominguez-Cherit¹

¹Dermatology Department, Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán”, Mexico City, Mexico,

²Mycology Department, Hospital General de México “Dr. Eduardo Liceaga”, Mexico City, México

Corresponding author: Judith Guadalupe Dominguez-Cherit M.D, E-mail: Dominguez.judith@gmail.com

ABSTRACT

Background: Onychomycosis accounts for 30% of all superficial mycoses and 50% of all nail diseases. One of the most studied predisposing factors is diabetes mellitus, with a frequency of onychomycosis of 31.5% in these patients. Many show resistance to standard therapeutics and have “polypharmacy”, which represents a risk for pharmacological interactions. **Objective:** The objective was to assess the clinical response to therapy, evaluate with histopathology, direct examination with KOH and white-calcofluor, and culture the most frequent etiologic agents associated with the development of onychomycosis in patients with diabetes mellitus. **Materials and Methods:** A non-randomized, uncontrolled, open-ended, prospective cohort study was conducted on 46 patients with onychomycosis and diabetes mellitus. Treatment was assigned according to clinical findings and specific indications for treatment. **Results:** From the samples taken for direct examination with KOH and calcofluor-white, culture, and histopathological study, positive results were: 39 (84.1%) patients to the direct examination, 32 (69.6%) to the culture, 27 (65.2%) with a positive histopathological study, and 17 (54.86%) to the calcofluor-white. On clinical evaluation, we found no treatment response in 8 patients (20%), a partial response in 14 patients (25%), and a complete response in 18 patients (45%). Out of the 46 patients evaluated initially, 25 persisted with onychomycosis after six months of follow-up. **Conclusion:** The prevalence of onychomycosis is increasing and requires correct diagnosis since there are other non-fungal diseases of the nails that resemble onychomycosis. Presumably, the immunosuppression of diabetes, its systemic affection, and the foot abnormalities of a diabetic patient cause more nail dystrophy, an increased fungal load, and treatment resistance.

Key words: onychomycosis; diabetes mellitus; mycoses; dermatophytes; non-dermatophytes molds; nails; immunosuppression; fungi; polypharmacy

INTRODUCTION

Onychomycosis affects 5.5% of the general population [1] and accounts for 30% of all superficial mycoses and 50% of all nail diseases [2,3]. The estimated prevalence is over 10% in the general population and about 40% in older adults [2]. The main causative agents of onychomycosis are dermatophytes; however, in those that do not respond to standard therapeutics, non-dermatophyte molds should be considered associated or causal agents [2].

One of the most studied predisposing factors is diabetes mellitus, with a frequency of onychomycosis of 31.5% in these patients [2,4]. It is now accepted that diabetes increases the relative risk of developing onychomycosis (from 1.5 to 2.8 times) compared to non-diabetic patients [2]. In a study on 400 patients in India, the prevalence of onychomycosis in diabetics was found to be 17%, being 6.8% in the control group [4]. Other factors related to an increased incidence of onychomycosis include old age, male sex, repeated nail traumas, genetic predisposition and underlying diseases

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such as immunodeficiency, and peripheral vascular disease [4]. Nail thickness has been positively associated with higher HbA1C levels [5], accelerating the typical subungual keratinization induced by fungal infection. Fungal infections exceed 50% of all types of infections among diabetic patients [6]. Diabetic patients with onychomycosis exhibit a higher percentage of gangrene and foot ulcers (12.2%) compared to those without onychomycosis (3.8%) [7,8].

Onychomycosis represents a risk for the development of the diabetic foot due its association with difficulty in nail clipping, causing the patient to injure themselves, producing an access route for bacterial agents [9]. Many of these patients show resistance to standard therapeutics (in some cases associated with incomplete previous treatments); they also have “polypharmacy,” which represents a risk of pharmacological interactions in systemic management, or have contraindications for systemic management, hence they are candidates for topical treatment, after culture.

This study aimed to assess the clinical response to therapy and to evaluate with histopathology, direct examination with KOH and white-calcofluor, and culture the most frequent etiologic agents associated with the development of onychomycosis in patients with diabetes mellitus, both susceptible and resistant to standard treatment.

MATERIALS AND METHODS

A non-randomized, uncontrolled, open-ended, prospective cohort study was conducted on 46 patients with onychomycosis and diabetes mellitus treated at the Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán,” regardless of the time of evolution. Treatment was assigned according to clinical findings and specific indications for treatment, according to the type of onychomycosis and the number of affected nails (Table 1).

All patients were treated after their first dermatology evaluation with a follow-up at three months (in the case of systemic treatment) and at six months (in the case of topical treatment). Nails were photographed for follow-up and the samples were taken for direct examination with KOH 10% and calcofluor-white, and for mycological culture with agar for dermatophytes, SDA and potato dextrose agar. Furthermore, the distal edge of one of the affected nails was cut for histopathological study. Treatment adherence

Table 1: Treatment assigned according to clinical findings and specific indications according to the type of onychomycosis and the number of affected nails

Terbinafine 250 mg every 24 hours for 3 months	Patients with the indication of systemic treatment without liver failure, creatinine clearance greater than 50 ml/min or those who had no interacting drugs.
Itraconazole 200 mg every 24 hours for 3 months	Patients with the indication of systemic treatment, without liver failure, with or without renal failure, or those who had no interacting drugs.
Fluconazole 150 mg every 7 days, for 3 months	Patients with the indication of systemic treatment, with superficial white onychomycosis.
Urea 40% and bifonazole 2% topical	Patients with the indication of topical treatment, who presented subungual hyperkeratosis or those who had contraindications for systemic treatment.
Amorolfine lacquer	Patients with the indication of topical treatment, without subungual hyperkeratosis.

evaluation was conducted by interrogation during patient evaluations.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, were adhered to and the appropriate ethical review committee approval was received.

RESULTS

46 patients with diabetes and a clinical suspicion of onychomycosis were evaluated, 24 males (52.2%) and 22 females (47.8%), with an age ranging from 30 to 90 years, with a median of 57. Two patients were eliminated from the final analysis for death, and no sample was obtained for study from four other patients as they were lost on the last consultation.

Among the patients evaluated, a total of 25 (54.3%) were clinically found with distal subungual onychomycosis, 29 (63%) with total dystrophic onychomycosis, 2 (4.3%) with superficial white onychomycosis, 4 (8.7%) with lateral subungual onychomycosis, among whom the most affected finger was the first left toe, with 41.3%.

Among the treatments indicated, 2 received itraconazole 200 mg daily (4.3%), 7 received itraconazole in pulses, 13 received oral terbinafine daily (30.4%), 2 applied amorolfine lacquer, and 15 were treated with topical bifonazole at 2% plus urea 40% (37.5%). We found global healing of 73%, with topical management with urea 40% + bifonazole, and a response of 9% with

systemic terbinafine, 17% with itraconazole in pulses, and 50% with itraconazole daily (Table 2).

All nails at follow-up were photographed. Samples were taken for direct examination with KOH and calcofluor-white, culture and histopathological study. The positive results were: 39 (84.1%) patients to direct examination, 32 (69.6%) to culture, 27 (65.2%) with positive histopathological study, and 17 (54.86%) with calcofluor-white. At the end of the study, the patients underwent a new clinical evaluation and new samples were taken for direct examination with KOH and calcofluor-white, culture, and histopathological study. Positive results were found in 10 patients (25%) with direct examination, 18 (45%) patients with positive culture, and 20 patients (54.28%) with positive histopathology.

Table 2: Treatment and mycological cure in patients with onychomycosis and diabetes mellitus

Treatment	Mycological Cure				Total	
	Yes		No		No.	%
	No.	%	No.	%		
Terbinafine	1	9	10	91	11	100
Terbinafine + urea and bifonazole	0	0	2	100	2	100
Itraconazole pulses	1	17	5	83	6	100
Itraconazole pulses + urea and bifonazole	1	100	0	0	1	100
Itraconazole 200 mg daily	1	50	1	50	2	100
Urea + Bifonazol	11	73	4	27	15	100
Cyclopyroxolamine lacquer	1	100	0	0	1	100
Amorolfine lacquer	0	0	2	100	2	100
Total	16	40	24	60	40	100

The most frequent histopathological findings before treatment were as follows: compact keratin (93%), parakeratosis (88%), septate short filaments (63%), and bacterial colonies (45%). In biopsies after treatment with positive results, parakeratosis was found in 95%, other frequent findings were septate short filaments (18%), serum (48%), and bacterial colonies (60%) (Table 3) (Fig. 1).

In 40 patients evaluated at the end of the study, 2 showed no treatment adherence (5%), 9 patients (22.5%) showed 50% adherence, and 29 patients (72.5%) showed 100% adherence. It was considered 100% adherence in those who applied their treatment from the beginning, 50% in those who began the treatment late and 0% in those who failed to begin the treatment. Greater adherence was found in patients with systemic treatment (17 of the 29; 58.62%), compared to 13 patients with topical treatment (13 of the 29; 44.82%).

Four patients received systemic treatment, with a minimum of one month of treatment (late onset) and a maximum of five months (prolonged treatment to clinical cure), with a median of 3; and among the 18 patients with topical treatment, the minimum treatment duration observed was two months and the maximum was six months, with a median of four; with a patient with indicated topical treatment which was not initiated. On clinical evaluation, we found no response in 8 patients (20%), a partial response in 14 patients (25%), and a complete response in 18 patients (45%) out

Table 3: Histopathological findings at the beginning and end of the study in nail biopsies of patients with onychomycosis and diabetes mellitus

Before Treatment	N	%	Histopathological Finding	Before Treatment		After Treatment		
				N	%	N	%	
Positive	28	70	Compact keratin	37	93	Positive 17 (42%)	38	95
			Melanin pigment				1	3
			Hematoma in the stratum corneum			1	3	
			Parakeratosis	35	88	37	93	
			Septate short filaments	25	63	7	18	
			Non-septate short filaments			1	3	
			Gram-positive cocci bacteria	18	45	24	60	
			Serum	13	33	19	48	
			Microabscesses of neutrophils	4	10	8	20	
			Arthrospores	3	8	2	5	
			Pseudohyphae	2	5			
			Laminar keratin	2	5			
			Septate filaments, thick or long	2	5	3	8	
			Yeast	1	3	4	10	
			Branched and angled septate filaments at 15–40°			8	20	
Septate short filaments with vesicular dilatations			4	10				
Negative	12	30			23 (58%)			
Total	40	100			40 (100%)			

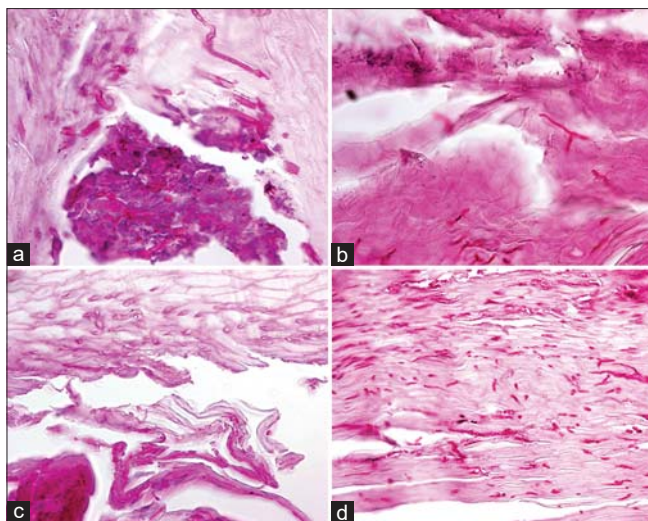


Figure 1: (a) Compact keratin sheets associated with bacterial colonies, septate filaments. (b) Compact keratin sheets associated with bacterial colonies, septate and angled filaments. (c) Compact keratin sheets and thick filaments. (d) Compact keratin sheets associated with bacterial colonies, spores, hyphae, and pseudohyphae.

of the total of 40 patients evaluated. Finally, considering the clinic, and the control studies by histopathological study, direct examination, and culture, it was found that, out of the 46 patients evaluated initially, 25 persisted with onychomycosis. Among these, a late onset of treatment was found in 13 patients, and among the causes evaluated, 11 patients reported economic causes, 1 apathy, and 1 gastrointestinal alterations, such as heartburn, after the beginning of systemic treatment.

Considering the patients who completed their treatment in a consistent manner, completing the minimum treatment required for systemic (three months) or topical (six months) treatment and who, with or without clinical resolution, continued with positive diagnostic studies (direct examination, culture, or histopathological study), 4 (10%) were considered resistant to conventional treatment, without being able to consider the remaining 21 within this group, since they had not completed the indicated treatment by the late onset of the same.

DISCUSSION

Onychomycosis in diabetics is more than just a cosmetic problem and may be a limb-threatening infection if managed inappropriately [7]. A prospective study of 1285 diabetic patients found that the presence of onychomycosis is a significant predictor for the development of foot ulcers [8]. Onychomycosis in diabetic patients is more frequently observed in the

presence of poor glycemic control and peripheral vascular disease [10]. A study found that the presence of onychomycosis in patients with diabetes is associated with subclinical atherosclerosis, which in diabetic patients represents the leading cause of death. [11]. The thickened, brittle nails typical of this infection are capable of causing damage to the surrounding skin that may go unnoticed due to coexistent neuropathy. Enlarged, dystrophic toenails may also put increased pressure on the underlying toe, compromising its tenuous vascular supply and causing pressure ulcers [11,12]. The pressure required to clip thickened toenails may cause inadvertent damage to the surrounding skin. If the patient is unable to perform appropriate foot hygiene, overgrowth of thickened toenails may cause damage to the surrounding skin as well. The subungual debris typical of distal subungual onychomycosis may become a reservoir for molds and bacteria [11,13], which may represent potential invaders of the compromised skin barrier. According to the Achilles Foot Screening Project, the most commonly clinically diagnosed foot diseases in the total population of patients who visited a dermatologist were fungal infections (35%), especially onychomycosis (23%) and tinea pedis (22%) [14]. Its etiology includes dermatophytes, yeasts, and fungi, in order of frequency. Regarding this, in a retrospective study lasting for 14 years on onychomycosis by molds, conducted at the Hospital General de Mexico by Bonifaz et al. [15], it was found that the most frequent clinical presentation was lateral and distal subungual in 69% of the cases, with *Scopulariopsis brevicaulis* in 34/78 patients and *Aspergillus niger* in 13/78 (16.6%) of the patients studied [15] Mixed infections and those caused by non-dermatophyte molds are more prevalent than previously thought, especially in warmer climates [16].

The results found in this study are consistent with the rest of the literature, since most of the patients presented total dystrophic onychomycosis, followed by distal subungual onychomycosis, and the most affected finger was the first of the left foot, with 41.3%.

Dermatophytic onychomycosis, predominates in male patients in a relation of 2:1. The global prevalence falls between 2–15% in adults and represents 50% of all unguinal conditions [12].

Indications for systemic treatment include having three or more affected nail plates or having the condition on one nail at the level of the nail matrix [15]. The main difference in treatment is due to the fact that

some of the patients have chronic renal disease with creatinine clearance less than 50 ml/min, which contraindicates the use of terbinafine. Although it is the safest drug with the fewest drug interactions, there are no controlled studies in patients with creatinine clearance below 50 ml/min. For these, itraconazole may be used safely, although most of our population has polypharmacy, which sometimes makes management difficult because of drug interactions. It also requires greater vigilance with baseline and follow-up liver function tests.

Direct examination is performed after adding potassium hydroxide (KOH). KOH preparations have been reported to have a false-negativity rate of 5–15% [15]. Direct examination with KOH, although operator-dependent, correlates with culture results and with the number of positive histopathological studies, the latter being the gold standard in the diagnosis of onychomycosis, and further supports the positivity of cultures and rules out their contamination. Fungi may be better visualized by adding chlorazol black, periodic acid–Schiff (PAS), or calcofluor-white stain [17]. The use of the latter significantly increases the sensitivity and specificity of this technique for the detection of dermatophytes, yeasts, and non-dermatophyte fungi [18].

Among the main limitations of the study was the lack of confirmation through molecular biology studies, due to the lack of equipment in the institute. One of the key difficulties in interpreting this study is that the majority of fungi identified by culture are either not known to be nail pathogens or are highly rare pathogens. Despite diminution of immunity in some diabetics, the pathogens cited, such as *Fusarium*, cause onychomycosis in the face of profound neutropenia, which is rather different from that in diabetics. Another explanation might be that a dermatophyte in the nail has not grown due to the presence of contaminating fungi. Another is that the fungi found are secondary to some other cause of onychodystrophy.

The presence of bacterial clusters is interesting. It might be due to space under the nail, and the growth of bacteria such as *S. aureus*, which is key to the development of diabetic ulcers, is favored.

The increase in the number of histopathological findings in the control biopsies is thought to have been due to the fact that, initially, some of the patients were managed by “podiatrists,” and, in addition to having

been treated with bad nail cutting, they were given excessive filing of the nails, which made it difficult for some patients to take samples from their nails. When taking control samples, filing and cutting were intentionally suspended for a week prior to the evaluation for facilitation.

Diagnosis of onychomycosis is difficult because of the difficulty in isolating fungi from infected nails. The classical technique is performed with a curette. However, Shemer et al. showed in a study on 194 patients that, with a technique in which a channel 1–2 mm in size is perforated with an unguial drill, a medial, distal, and proximal sample can be taken, being effective for culture and therefore for diagnosis [19] (Fig. 2). Nail sampling is, in fact, a delicate procedure that requires the mycelium front to be reached by the instrument in order to provide positive cultures [20,21]. Histopathology of nail clippings stained with PAS also referred to as histomycology with PAS is the most sensitive method of diagnosing onychomycosis [22], while others point out that it is not more effective than direct examination [23,24]. Confocal laser-scanning microscopy is an emerging diagnostic technique. The aspect of dermatophytes appears as a network of lengthy structures with high reflection and the typical shape of hyphae [25,26]. Other interesting new tools in the diagnosis of onychomycosis are the dermatophyte test strip and Raman spectroscopy [22].

Immunosuppressed patients show variation in clinical presentation, as well as in causative agents. Additionally, fungi traditionally considered as non-pathogenic may be found as pathogens, often associated with high mortality, as in the case of *Fusarium* species that cause perionyxis and may provide a gateway for a disseminated infection [27]. As for the isolated agents in the cultures for this study, a large number of opportunistic fungi was found (*Acremonium* sp. *Candida* sp. (Fig. 3), *Alternaria* sp. *Aureobasidium* sp., *Aspergillus* sp., *Candida guilliermondi*, *Candida albicans*, *Rhodotorula* sp., *Cryptococcus albidus*

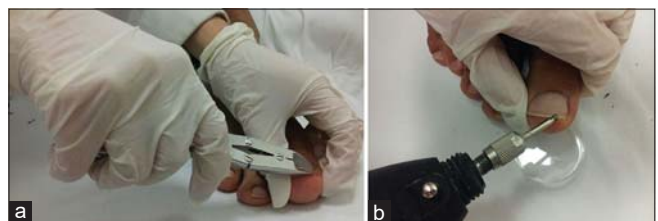


Figure 2: (a) Nail cutting technique for histopathological study. (b) The technique to obtain the sample with a drill for direct examination with KOH, calcofluor white, and culture.



Figure 3: (a-b) *Candida parapsilopsis*. Positive on direct examination and culture. (a) Initial photograph with total dystrophic onychomycosis. (b) Final photograph after six months of topical treatment with 40% urea and bifonazole. Clinical and mycological cure. (c-e) *Acremonium sp.*, *Candida sp.* Positive direct examination and histopathological study. (c-d) Initial photograph. (e) Final photograph after six months of topical treatment with 40% urea and bifonazole. Clinical and mycological cure. (f-g) *Cladosporium sp.*, *Candida parapsilopsis*. Negative initial direct and histopathological examination. (f) Photograph after two months of systemic treatment (total dystrophic onychomycosis). (g) Final photograph after five months of systemic treatment. Clinical cure.

(Fig. 4), *Fusarium sp.* (Fig. 5), among others), which is probably associated with diabetes-associated immunosuppression (Table 4) [28,29].

In general, greater therapeutic failure was seen in onychomycosis caused by non-dermatophytes molds, followed by yeasts. Although the clinical improvement in these patients, both partial and total, was highly evident and larger than expected, the mycological and histological cure was far below from what was expected, hence we consider that a longer treatment time is required, either with the same medication or with topical and systemic combination therapy, and wait for a new culture and histological study. Approximately 20–25% of patients do not respond to the initial therapy and often switch to different treatments. An undetermined percentage of patients do not respond to any of the therapies [30]. The non-dermatophyte molds such as *Fusarium spp.*, are the most resistant fungi to standard treatment for onychomycosis either topical or systemic [31], non-responders are defined as those patients who received treatment for six and three months without improvement in more than 50% of the nail unit [32,33].



Figure 4: (a-c) *Cryptococcus albidus*. Positive direct and histopathological examination. (a-b) Photograph at the onset of topical treatment (total dystrophic onychomycosis). (c) After six months of topical treatment. Clinical and mycological cure. (d-e) *Fusarium sp.* Positive direct examination and histopathological study. (d) Initial photograph (total dystrophic onychomycosis). (e) After five months of systemic treatment with itraconazole 200 mg per day and 40% topical urea. Clinical and mycological cure. (f-g) *Cryptococcus albidus*, *Cladosporium sp.* Positive direct examination. (f) Initial photograph (total dystrophic onychomycosis). (g) Final photograph (one month of systemic treatment with itraconazole in pulses of 400 mg). 10% improvement.



Figure 5: (a-d) *Fusarium sp.* Negative direct examination. Positive histopathological study. (a) Initial photograph (total dystrophic onychomycosis). (b) After three months of systemic treatment with itraconazole pulses of 400 mg per day and topical urea at 40%. Clinical cure without mycological cure. (c) Left foot. (d) Left foot control.

Table 4: Comparison of culture isolates before and after treatment.

	Isolates Before Treatment (n = 26)	Mycological Cure (n = 15)	Mycological Failure After Treatment (n = 11)
Onychomycosis by yeasts	9 (34.6%)	7 (46.6%)	2 (18%)
<i>Candida sp.</i>	1 (3.8%)		
<i>C. albicans</i>			
<i>C. parapsilopsis</i>	5 (19.2%)		2 (18%)
<i>C guilliermondi</i>	3 (11.5%)		
Other yeasts	4 (15.3%)	1 (6.66%)	3 (27%)
<i>Rhodotorula sp.</i>			
<i>Rhodotorula glutini</i>	1 (3.8%)		
<i>Rhodotorula mucilagi</i>			1 (9%)
<i>Cryptococcus albidus</i>	3 (11.5%)		
<i>Cryptococcus neoformans.</i>			1 (9%)
<i>Cryptococcus unigutt.</i>			1 (9%)
Filamentous fungi			
Dermatophytes	1 (3.8%)		1(9%)
<i>Trichophyton sp.</i>	1 (3.8%)		1 (9%)
Non-dermatophytes	12 (46.15%)	7 (46.6%)	5 (45.4%)
Filamentous hyaline fungi:			
<i>Acremonium</i>			
<i>Fusarium sp.</i>	1 (3.8%)		1 (9%)
<i>Aspergillus sp.</i>	3 (11.5%)		2 (18%)
Dematiaceous	2 (7.6%)		
<i>Alternaria sp.</i>			
<i>Cladosporium sp.</i>	2 (7.6%)		
Mucorales (zigomycetous).	1 (3.8%)		1 (9%)
<i>Absidia sp.</i>			
<i>Epicoccum sp.</i>			
<i>Penicillium sp.</i>			
<i>Aerobasidium sp.</i>			
<i>Trichoderma sp.</i>	2 (7.6%)		1 (9%)
	1 (3.8%)		

It would be worth conducting a study on patients without diabetes mellitus to compare whether it is the immunosuppression of diabetes and its systemic affection as well as the foot abnormalities of the diabetic patient that cause more nail dystrophy, an increased fungal load, and treatment resistance.

Recently, a reproducible and objective system called the Onychomycosis Severity Index (OSI) was created to describe the extent and involvement of distal subungual onychomycosis, with points are given based on the area (%) of nail involvement, the proximity of the disease to the matrix, and the presence of dermatophytoma or subungual hyperkeratosis [16].

In conclusion, onychomycosis is a common ungual disease that affects a large part of the global population. Its prevalence is increasing, influenced in part by its association with older age, lifestyle, and concomitant diseases. It may cause disability and lead to mild to severe complications, among which the most important is the diabetic foot, which may condition osteomyelitis

and sepsis, which justifies its early treatment. In addition, it requires a correct diagnosis, which includes clinical evaluation, direct examination, culture, and a histopathological study, since there are other non-fungal diseases of the nails that resemble onychomycosis and are over-treated, generating expensive treatments with potential adverse effects, which makes it mandatory to confirm the clinical diagnosis with direct examination, culture, and, if possible, histopathological study. The treatment of onychomycosis is one additional facet of diabetic care that promises to further improve outcomes in these patients.

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Statement of Human and Animal Rights

All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 2008 revision of the Declaration of Helsinki of 1975.

Statement of Informed Consent

Informed consent for participation in this study was obtained from all patients.

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