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ORIGINAL ARTICLE/ARTICLE ORIGINAL

A study of onychomycosis in patients attending a dermatology center in Tehran, Iran

Étude sur les onychomycoses chez les patients d'un centre dermatologique de Téhéran, Iran

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Summary
Objective. — To determine the incidence of onychomycosis based on age and sex, morphological pattern of the disease, predisposing factors and identification of fungus by direct microscopy and culture methods.
Methods. — A prospective study was conducted on 140 patients with nail disorders. A detailed history and thorough examination was done in all patients. The samples were taken from patients clinically suspected of fingernails and toenails infections attending a dermatology center in Tehran, Iran. The nails were subjected to potassium hydroxide (KOH) examination and fungal culture on Sabouraud's dextrose agar (SDA) medium.
Results. — Specimens from 79 patients (56.4%) were positive for onychomycosis. The mycological observations showing positive fining with KOH were observed in 79 (56.4%) and culture positive in 35 (25%) cases. Females were more infected than males. The most common age group infected was 41—60 years (40.7%). Toenails were affected more frequently than fingernails and dystrophic onychomycosis was the most common clinical type seen in 39.2% patients. From the culture-positive samples, yeasts were the most common pathogens isolated from 25 (71.4%) patients, followed by non-dermatophytic moulds in 6 (17.1%) and dermatophytes in 4 (11.5%) patients.

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Conclusion. — This study demonstrated that *Candida* species were the main agents causing onychomycosis in our region and accurate diagnosis of onychomycosis was based on direct microscopy and fungal culture.

MOTS CLÉS
Ongle ;
Onychomycoses ;
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Candida ;
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Aspergillus ;
Iran

Résumé
Objectif. — Déterminer l'incidence d'onychomycoses basée sur l'âge, le sexe, l'aspect clinique de la maladie, les facteurs prédisposants et l'identification des champignons par microscopie directe et culture.
Matériel et méthodes. — Une étude prospective a été accomplie sur 140 patients avec onyxis. Une histoire détaillée et un examen consciencieux ont été faits pour tous les patients. Les prélèvements ont été réalisés chez des patients cliniquement soupçonnés d'onyxis des doigts et des orteils consultant dans un centre de dermatologie à Téhéran, Iran. Les ongles ont été examinés sous hydroxyde de potassium (KOH) et la culture fongique réalisée sur milieu gélose-dextrose de Sabouraud (SDA).
Résultats. — Les échantillons des 79 patients (56,4 %) étaient positifs pour une onychomycose. L'examen mycologique direct sous KOH était positif dans 79 cas (56,4 %) et la culture positive dans 35 cas (de 25 %). Les femmes ont été infectées plus que les hommes. La tranche d'âge la plus infectée était 41—60 ans (40,7 %). Les ongles d'orteil ont été infectés plus souvent que les ongles des doigts et l'aspect dystrophique était le type clinique le plus commun vu chez 39,2 % des patients. Parmi les échantillons positifs en culture, les levures étaient le pathogène le plus commun : 25 patients (71,4 %), suivi par les filamenteux non dermatophytes dans 6 cas (17,1 %) et les dermatophytes chez 4 patients (11,5 %).
Conclusion. — Cette étude a montré que les espèces de *Candida* étaient des agents principaux provoquant les onychomycosis dans notre région et le diagnostic exact d'onychomycose a reposé sur l'examen microscopique direct et sur la culture fongique.

Introduction

Onychomycosis is a denomination used to describe nail infection usually caused by dermatophytes, yeast and non-dermatophytic moulds [19]. These fungi may cause onychomycosis particularly as secondary invaders after damage by trauma or disease. Onychomycosis affects approximately 5% of the population worldwide [24] and represents around 30% of all superficial mycotic infection [17] and 50% of nail disorders [11]. It is caused mainly by dermatophytes belonging to the following three genera: *Trichophyton*, *Microsporum* and *Epidermophyton*. Of these, the most common species that affect nails are *T. rubrum* and *T. mentagrophytes* var. *interdigitale*. Dermatophytes are responsible for nearly 90% of toenail onychomycosis and at least 50% of fingernail infections [23]. Yeasts are the source of approximately 5% of onychomycosis [25], the majority of which is caused by *Candida albicans*. The non-dermatophytic moulds account for approximately 4% of onychomycosis such as *Fusarim* spp., *Scytalidium* spp. and *Acremonium* spp. as the most frequently identified mould pathogens [33].

Predisposing factors are immunosuppression, poor peripheral circulation, diabetes, family history, increasing age, occupation, social class, climate, living environment, and skin disorders such as hyperhidrosis, psoriasis, onychogryphosis and nail trauma [31,36]. The prevalence of onychomycosis is 26% in diabetes mellitus patients [14], 23.2% in HIV-positive individuals [15], 36.1% in chronic venous insufficiency patients [27], 24% in systemic lupus erythematosus patients [32] and 20—30% in patients with psoriasis [37]. Clinically onychomycosis is classified into various types; distolateral subungal onychomycosis (DLSO), superficial white onychomycosis (SWO), proximal subungal onychomycosis (PSO), candidal onychomycosis (CO), endonyx onychomycosis (EO) and total dystrophic onychomycosis (TDO) [28]. Although onychomycosis is all too often regarded as merely a cosmetic problem, which is rarely life-threatening, its high prevalence and the associated morbidity makes it an important public health problem. Onychomycosis resembles several diseases in the field of dermatology and medicine, so it is necessary to diagnose the infection with some laboratory evidence before treatment with antifungal agents whose duration of treatment is long and may have some serious side effects. Onychomycosis can be clinically confirmed by direct microscopy of potassium hydroxide (KOH) preparation. However, a fungal culture is required to identify the specific genus and species of pathogens. The incidence of onychomycosis has been well studied in some countries, but few data are available in Iran [20]. The aim of the present retrospective study was to know the incidence of both age and sex, morphological pattern of the disease, predisposing factors and identification of fungus by direct microscopy and culture methods in a 3-year period.

Patients and methods

Patients

A total of 140 patients suspected of onychomycosis i.e. change of the color of nail, deformity of nail, or subungual

hyperkeratosis were included in the study. The patients were attended at the dermatology center of Tehran University of Medical Sciences, Iran during a period of 3 years (from August 2010 to May 2014). A detailed history of patients was taken. It included age, sex, socioeconomic status, trauma, predisposing diseases such as diabetes mellitus, psoriasis, lupus erythematosus, eczema, rheumatoid arthritis, addict and familiar allergy. The clinical pattern and location of disease were documented. We excluded all the patients that did not give their consent to participate in the study as well as patients that had received topical or systemic antifungal treatment in the past 12 months.

Sample collection and mycology investigation

Nail specimens were taken by scrapping and clipping techniques, underside of the nail plate and the hyponychium after cleaning the affected area with 80% ethanol. The samples of each patient were placed in separate sterile Petri dish and transported to the Medical Mycology Center. All samples were examined by direct microscopy for fungal elements such as hyphae or blastoconidia in potassium hydroxide 20%. For fungal cultures, all samples were inoculated on different media:

- Sabouraud's dextrose agar (SDA, Merck Co., Darmastadt, Germany);
- SDA with 5% chloramphenicol and cycloheximide for dermatophyte;
- SDA with 5% chloramphenicol for mould isolation.

The culture tubes were incubated at 25 °C and 37 °C for 30 days. According to the macroscopic and microscopic features, dermatophytes and non-dermatophytic moulds were identified to species levels. Confirmation of *Candida* species were performed based on observation of pseudomycelium under light microscopy with KOH, germ tube test, chlamydospore formation and the API 20 C identification system. The identification of non-dermatophytic fungi was performed by following micro- and macroscopic evaluations of the primary cultures and slide culture in agar potato block, according to De Hoog et al. [7]. All patients were informed, consented, and the protocol was approved by the Ethics Committee of Dermatology Department of Tehran University of Medical Sciences, Tehran, Iran. Results were evaluated by descriptive statistics. Differences were considered significant at $P < 0.05$, two-sided. Onychomycosis prevalence was tested using chi^2 testing.

Results

Among the 140 study patients, the diagnosis of onychomycosis was confirmed in 79 (56.4%) patients who exhibited nail changes according to the results of direct microscopy and culture. Regarding gender, 99 patients (70.7%) with onychomycosis were females and 41 (29.3%) were males. As shown in Table 1, highest prevalence was seen in patients with age varying from 41 to 60 years (40.7%) and lowest prevalence in participants with age varying from 0 to 20 years (6.4%).

Direct microscopic and culture analysis were positive in 79 (56.4%) and 35 (25%) cases, respectively (Table 2). Direct microscopic examination had a higher percentage of positivity than culture ($P < 0.05$). With regard to the positivity of the direct exam and culture, 34 (42.5%) were positive in both exams, 45 (56.2%) had positive direct exams and negative

Table 1 Prevalence of fungal agents in patients according to age.
La prédominance des agents fongiques selon l'âge des patients.

Age	Fungal agents (No)					Total
	Negative	Yeast	Dermatophyte	Non-dermatophyte	Yeast and hyphae	
< 20 years	4	5	0	0	0	9
21—40 years	21	13	2	4	2	42
41—60 years	23	22	4	8	0	57
> 60 years	13	11	6	2	0	32
Total	61	51	12	14	2	140

Table 2 Results of direct microscopic examinations versus cultures based on the isolated fungi.
Résultats d'examens microscopiques directs et des cultures basés sur les champignons isolés.

Test	Fungal agents					Total
	Negative	Yeast	Dermatophyte	Non-dermatophyte	Yeast and hyphae	
Direct						
Negative	54	0	0	0	0	54
Positive	0	51	12	14	2	79
Total	54	51	12	14	2	133
Culture						
Negative	54	28	8	8	2	100
Positive	0	25	4	6	0	35
Total	54	53	12	14	2	135

cultures, and 1 (1.3%) patient had negative direct exam and positive culture.

Toenails were the most frequent anatomic site in 46 patients (58.2%) and fingernail onychomycosis was confirmed in 24 patients (30.4%) ($P < 0.05$). In addition, 9 patients (11.4%) presented infections on fingernails and toenails simultaneously (Table 3).

The distribution of patients showing morphological patterns of onychomycosis was also determined. Dystrophic onychomycosis (No.: 31; 39.2%) was the most common clinical pattern ($P < 0.05$), followed by superficial white (No: 18; 22.8%), dystrophy plus superficial white (No: 12; 15.2%), discoloration (No: 11; 13.9%) and dystrophy plus paronychia (No: 7; 8.9%).

Of 35 culture-positive cases, yeasts were the most common pathogens isolated in 25 (71.4%) patients, followed by non-dermatophytic moulds in 6 (17.1%) and dermatophytes in 4 (11.5%) patients. The etiological yeast agents most frequently found in cases of onychomycosis was *C. albicans* with a total of 10 patients (40%), followed by *C. parapsilosis* (No: 3; 12%), *C. krusei* (No: 3; 12%), *C. tropicalis* (No: 2; 8%), *C. glabrata* (No: 1; 4%) and *Malassezia* spp. (No: 6; 24%). Of the dermatophytic fungi, *T. mentagrophytes* var. *interdigitale* was the most involved, being responsible for 3 samples (75%). Another dermatophytic strain was identified, with *T. rubrum* responsible for 25% of cases of onychomycosis. Regarding non-dermatophytic filamentous fungi, *Aspergillus* spp. was the most frequently isolated, being responsible for 50% of cases, followed by *Penicillium*, *Fusarium* and *Scopulariopsis* species (16.6%).

The frequency of onychomycosis in conjunction with each disease was shown in the Table 4. Concurrent diseases were found in 42 (30%) patients. Of these patients, 17 (12.1%) were diagnosed with corticosteroid therapy, 12 (8.7%) with diabetes mellitus, 5 (3.6%) with psoriasis, 3 (2.1%) with familiar allergy, 2 (1.4%) with lupus erythematosus, 1 (0.7%) with rheumatoid arthritis, eczema and addict whereas 98 (70%) patients had an unknown etiology.

Discussion

Onychomycosis is a chronic infection of the nails, nowadays considered a serious problem for public health, in view of its high occurrence in the worldwide population. This prevalence is probably even higher than is currently thought, as the difficulty in clinical-mycological diagnosis, inappropriate collection of material for analysis as well as ineffective treatment make it hard to ascertain the true profile of such onychopathies.

In this study, from 140 patients with clinical lesions in the nails, 79 (56.4%) had onychomycosis confirmed by culture and direct examination. The results of this research demonstrated value near to those found by Lopes et al. [22] with positivity of 56.6% through exams. Of these patients, 99 cases (70.7%) were females and 41 (29.3%) were males, female to male ratio being 2.4:1. Such data are in agreement with the findings of several authors [10,21]. The susceptibility of the female gender in our region may be explained by the work habits of such patients such as cooks, laundresses, cleaners and prolonged moisture, detergents and cosmetic reasons. Regarding age, patients with age varying from 41 to 60 years old were more affected, representing 40.7% of the positive results in accordance with most of the studies [1,5]. The age group least affected was from 0 to 20 years (6.4%).

Table 3 Prevalence of fungal agents in patients according to sex and anatomic sites.
Prévalence d'agents fongiques chez les patients selon les sites anatomiques et le sexe.

Sex/Fungus		Nail			Total
		Finger	Toe	Finger + toe	
Female					
Negative	Count	14	26	4	44
	%	31.8%	59.1%	9.1%	100.0%
Yeast	Count	14	19	4	37
	%	37.8%	51.4%	10.8%	100.0%
Dermatophyte	Count	1	5	1	7
	%	14.3%	71.4%	14.3%	100.0%
Non-dermatophyte	Count	0	8	1	9
	%	0.0%	88.9%	11.1%	100.0%
Total	Count	29	58	12	99
	%	29.3%	58.6%	12.1%	100.0%
Male					
Negative	Count	8	8	1	17
	%	47.1%	47.1%	5.8%	100.0%
Yeast	Count	8	6	0	14
	%	57.1%	42.9%	0.0%	100.0%
Dermatophyte	Count	1	4	0	5
	%	20.0%	80.0%	0.0%	100.0%
Non-dermatophyte	Count	0	4	1	5
	%	0.0%	80.0%	20.0%	100.0%
Total	Count	17	22	2	41
	%	41.5%	53.7%	4.8%	100.0%

Table 4 Prevalence of fungal agents in patients according to sex and underlying diseases.
Prévalence d'agents fongiques chez les patients selon le sexe et les maladies sous-jacentes.

Sex/Fungus		Underlying diseases						
		Diabetes mellitus	Psoriasis	Lupus erythematosus	Eczema	Corticosteroid therapy	Familiar allergy	Negative
Female								
Yeast	Count	3	1	2	0	6	2	19
	%	8.8%	2.9%	5.9%	0.0%	17.6%	5.9%	55.9%
Dermatophyte	Count	0	0	0	1	2	0	4
	%	0.0%	0.0%	0.0%	14.3%	28.6%	0.0%	57.1%
Non-dermatophyte	Count	0	0	0	0	3	0	6
	%	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%	66.7%
Yeast and hyphae	Count	0	0	0	0	0	1	1
	%	0.0%	0.0%	0.0%	0.0%	0.0%	50.0%	50.0%
Total	Count	3	1	2	1	11	3	30
	%	5.8%	1.9%	3.8%	1.9%	21.2%	5.8%	57.7%
Male								
Yeast	Count	2	1	—	—	2	—	7
	%	14.3%	7.1%			14.3%		50.0%
Dermatophyte	Count	0	0	—	—	0	—	4
	%	0.0%	0.0%			0.0%		80.0%
Non—dermatophyte	Count	0	0	—	—	0	—	3
	%	0.0%	0.0%			0.0%		75.0%
Total	Count	2	1	—	—	2	—	14
	%	8.7%	4.3%			8.7%		60.9%

This is possibly because of a rapid nail growth in children, by the small surface area exposed to fungal invasion and, finally, to the low incidence of *T. pedis* in such individuals.

Direct microscopic analysis was positive in 56.4% cases. This percentage was very similar to 59.3% [12], 61.7% [29] and 63.4% [30] obtained by other studies. However, our result was much higher than the 40.31% obtained by Reisberger et al. [26]. We found 25% positive cultures, which is in agreement with Reisberger et al. [26] who reported 25.8%. This percentage was lower than 44% [9], 52.9% [12], 58.3% [29] and 61.8% [30] obtained by other studies. Direct microscopic examination had a higher percentage of positivity than culture ($P < 0.05$). This is very similar to the results of Weinber et al. [35] who reported the sensitivity of 80% of KOH in comparison with 59% of culture. Consistent with these results, other studies also demonstrated that direct microscopic examination detects more cases of onychomycosis than culture [9,26]. It is important to highlight that the expertise of the examiner is a key factor for interpreting the results of KOH and since our laboratories are specialized in mycological diagnosis, the level of positive responses with KOH is likely to be high when compared to others. With regard to the positivity of the direct exam and culture, 34 (42.5%) were positive in both exams, 45 (56.2%) had positive direct exams and negative cultures, and 1 (1.3%) patient had negative direct exam and positive culture.

Toenails (58.2%) were the most frequently involved sites, followed by fingernails (30.4%) and both were found in 11.4% cases. Ratio of toenail to fingernail infection was 1.9:1. The predominance of lesions in both fingernails and toenails were higher in women than in men. Previous studies reported that toenails are affected more often than fingernails, probably due to their slow growth, which facilitates invasion of the aetiological agent and is perhaps supported by events such as traumas and poor circulation [16]. The major clinical manifestation was dystrophic onychomycosis (39.2%) ($P < 0.05$), followed by superficial white (22.8%), dystrophy plus superficial white (15.2%), discoloration (13.9%) and dystrophy plus paronychia (8.9%).

The most common fungi cultured from infected nails were yeasts (71.4%), followed by non-dermatophytic moulds (17.1%) and dermatophytes (11.5%). This finding is in accordance with many studies which have demonstrated a greater prevalence of yeasts as the etiological agents of onychomycosis [13] and in contrast to others which have found dermatophytes as the most common agents [3]. Such differences may be related to local environmental conditions. The most frequent yeast agents were *C. albicans* being present in 10 (40%) of isolates, followed by *C. parapsilosis* in 3 (12%), *C. krusei* in 3 (12%), *C. tropicalis* in 2 (8%), *C. glabrata* in 1 (4%) and *Malassezia* spp. in 6 (24%). Species of *C. albicans* have been quoted in the literature as being responsible for most cases of onychomycosis worldwide [8,21]. We concluded that yeast species, especially *C. albicans*, might be the most common cause of onychomycosis in our region. In a study conducted by Chowdhary et al. [6], *Malassezia* was an etiologic agent rather than a colonizer in the patient's nails. The most common dermatophyte was *T. mentagrophytes* var. *interdigitale* (75%), followed by *T. rubrum* (25%). Our finding is in accordance with some studies which found *T. mentagrophytes* and *T. rubrum* as the most common dermatophytes responsible for onychomycosis [4,18]. Regarding non-dermatophytic filamentous fungi, *Aspergillus* spp. was the most frequently isolated, being responsible for 50% of cases, followed by *Penicillium*, *Fusarium* and *Scopulariopsis* species (16.6%). According to Afshar et al. [2] and Yaghoobi

et al. [37] reports, the non-dermatophytic filamentous fungus most found in onychomycosis was *Aspergillus* spp., such data being found in our region, where this fungus was the most widespread of all non-dermatophytic fungi, being considered only when direct examination and culture were positive three times consecutively.

In our study, concurrent diseases were found in 30% of patients. Onychomycosis was found to be the most common in patients with corticosteroid therapy (12.1%), diabetes mellitus (8.7%), psoriasis (3.6%), familiar allergy (2.1%), lupus erythematosus (1.4%), and rheumatoid arthritis, eczema and addict (0.7%), whereas 70% patients had an unknown etiology. Tuchinda et al. [34] showed that the prevalence of onychomycosis in patients receiving immunosuppressive therapy was 10.2% when compared to 6.7% in non-immunosuppressive patients. In a multicenter study, Gupta et al. [14] showed that advanced age was associated with infection in diabetic patients. Several studies reported that onychomycosis is an age-related infection. In addition to poorer peripheral circulation and lower immunity, elderly patients are more frequently exposed to fungi over years and then have higher chances of transmission and infection.

Conclusions

In summary, the diagnosis of onychomycosis cannot be performed only clinically, the laboratory studies being extremely important for the purpose of identifying etiological agents involved in the infection. The efficiency of the direct examination emphasizes the importance of the method, when performed by experienced professionals, favoring the speed of diagnosis and treatment of patients. This approach may be considered, together with culture, as an extremely important procedure for the epidemiological study of onychomycosis. This study showed that onychomycosis in population study was proved in 56.4% cases. The most common fungal agents were yeasts, in particular *C. albicans*, followed by non-dermatophytic filamentous fungi and dermatophytes. This survey may be useful in the development of preventive and educational strategies, and consequently in reducing healthcare expenditure.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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