High incidence of onychomycosis due to saprophytic fungi in Yazd, Iran

Hossein Sadeghi Tafti, MSc [1]
Kazem Ahmadikia, PhD Candidate [1]
Sara Rashidian, MSc [1]
Seyed Hossein Hekmatimoghaddam, MD [2]
Zeinab Ayubi Yazdi, MSc [3]

1. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Medical Parasitology and Mycology, School of Medicine, Afzalipour University of Medical Sciences, Kerman, Iran

Corresponding author:
Sara Rashidian, MSc
Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
Email: sararashidian18@gmail.com

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Background: Onychomycosis, the fungal infection of the toenails or fingernails, is caused by three major groups of fungi including dermatophytes, yeasts and non-dermatophyte molds. The objective of the present study was to determine the incidence of onychomycosis and to identify the causative fungi during a one year period in Yazd, Iran.

Materials and Methods: From Apr 2013 to Apr 2014 a total of 273 patients with suspected dermatophytosis were included in this study. Nail-clipping specimens of 71 clinically diagnosed cases of onychomycosis were obtained for mycological examination (KOH preparation and fungal culture). Identification of mycelial isolates was based on morphological appearance and microscopic characteristic of the colony. Supplementary methods for identification of dermatophytes were employed. The species of yeasts were identified by germ-tube and chlamydospore test, as well as colony color on chromogenic CHROMagar Candida medium, and the assimilation profile in API 20C Aux system.

Results: Of the 71 patients affected by nail disorders, 26 (36.6%) patients of onychomycosis including 54.9% male and 45.1% female (20 fingernails, 6 toenails) via direct examination and/or culture methods were diagnosed. saprophytic fungi were the most prevailing causative agents of onychomycosis and account for up to 69.2%(n=18) of cases, yeasts and dermatophytes were identified as causative agents of onychomycosis in 7 (26.9%) patients and 1 patient (3.8%), respectively. Distribution of fungal isolates was as follows: *Aspergillus niger* (26.9%), *A. fumigatus* (19.2%), *Candida albicans* (15.3%), *A. flavus* (11.5%), *C. tropicalis* (7.6%), *Penicillium sp.* (7.6%), *C. dubliniensis* (3.8%), *Trichophyton mentagrophytes* (3.8%) and *Fusarium sp.* (3.8%).

Conclusion: Because of considerable prevalence of onychomycosis, necessity for a careful mycological examination in patients with nail disorders is highlighted.

Keywords: onychomycosis, dermatophyte, *Candida*, *Aspergillus*, yeasts

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INTRODUCTION

Onychomycosis, the general term describing mycotic infection of the nail, is caused by dermatophytes, yeasts, and non-dermatophyte molds (NDMs). The nomenclature of fungal infections proposed by the International Society for Human and Animal Mycology (ISHAM) suggest that tinea unguium, onyxis, ungual candidiasis and ungual mycosis, when the causative agents are dermatophytes, yeasts, *Candida* and NDMs, respectively, should be used instead of the term

onychomycosis [1].

Species from the dermatophyte genera (*Epidermophyton*, *Microsporum*, and *Trichophyton*) are the most frequent agents of onychomycosis worldwide [2]. Generally, NDMs are known to be the less frequent etiologic agents of onychomycosis accounting for about 10% of cases [3]. However, depending on the geographical area and the diagnostic approaches employed, the prevalence of NDM onychomycosis could varies from 1% to 68% of cases [4].

There are several risk factors which could facilitate the occurrence of onychomycosis. Living in tropical and subtropical areas, nail trauma, old age, peripheral vascular diseases, participation in fitness activities, poor personal hygiene, use of communal showers and swimming pools, cross infection with contaminated tools used in pedicure and cosmetic nail treatments, wearing closed shoes and boots as well as some other personal factors like family history, diabetes, immunosuppression and skin disorders are considered as risk factors for onychomycosis [4-7].

There are several reports from Iran describing the epidemiology of onychomycosis. The interesting point is the differences in distribution of causal agents in various studies. Yeasts are reported to be the most frequent isolated fungi in some investigations [7-10], while in other studies, dermatophytes [11,12] or NDMs [13] are presented as the leading cause of onychomycosis.

Also, the investigations on the isolation of fungal agents causing onychomycosis in all over the world have shown the same differences. While in some studies dermatophytes are reported as the major cause of onychomycosis [14,15], other authors reported non-dermatophyte fungi [16,17] as the major etiology.

Given to the increasing prevalence of onychomycosis in the last decades, and considering the effects of climatic, socio-economical, and occupational activities, it is necessary to determine the prevalence and the causative agents of onychomycosis in all regions of country. Therefore, the aim of this study was to determine the incidence of onychomycosis, and to identify organisms recovered from the infected nails in patients admitted to the Yazd Central Medical Laboratory, affiliated with Shahid Sadoughi University of medical sciences in Yazd, Iran.

PARTICIPANTS AND METHODS

Study population

A total of 273 patients suspected of dermatophytosis were referred to the medical mycology laboratory during a one year period from Apr 2013 to Apr 2014.Among them, 71 patients were suspected to have fungal nail involvement and included in the present study.

Mycological investigations

Diagnosis of onychomycosis was made based on direct microscopic examination and culture in addition to clinical findings. The nails were swabbed liberally with 70% ethanol before obtaining the specimen in order to eliminate bacteria that could interfere with growth of fungi. Nail clipping or subungual scraping specimens was collected (after evaporation of alcohol) from the deepest part of the nail and as close as possible to the healthy nail. Part of each specimen was mounted in 20% KOH solution and examined microscopically for the presence of fungal elements (hyphae, arthrospores, yeast cells and pseudo hyphae). The rest of each specimen was inoculated onto agar slants of sabouraud dextroseagar (SDA, Merck, Germany) with chloramphenicol both with and without cycloheximide, and incubated at 28°C for 1-4weeks. The cultures were checked twice weekly for evidence of growth. No growth at the fourth week was considered as a negative culture. Yeast isolates, if any, were then subcultured on SDA in Petri dishes. All yeast isolates were identified by routine laboratory methods, including the germ-tube test, culture on corn-meal agar-tween 80 and also colony color on chromogenic CHROMagar Candida medium(CHROMagar, France), and the assimilation profile in API 20C Aux system(Bio Merieux, France). Identification of dermatophytes and NDMs was based on micro and macro characteristics of the colony. Additional tests such as hair perforation, urease and growth pattern on nutritional test media were employed whenever needed.

Statistical analysis

Data were statistically analyzed using SPSS software version 15. Any $P<0.05$ was considered significant.

Ethical considerations

The written informed consent was obtained from all participants.

RESULTS

In this study, 71 individuals (32 females, 39 males) suspected of onychomycosis were examined. A total of 26 cases comprising 14 females (53.8%) and 12 males (46.2%) were proved to have onychomycosis by mycological examination. Statistical analysis did not show any significant relationship between gender and nail infection (P=0.828).

Patient's age ranged from 4 to 88 years. Although the age groups of 30-39 and 40-49 were the most suspected ages, the highest positive rate of onychomycosis (50% of suspected persons) was observed in age group 40-49 (Figure 1). According to data analysis, this correlation was statically significant (P=0.015).

Among 26 positive cases, 20 (76.9%) had fingernail involvement, and 6 (23.1%) showed toenail onychomycosis. The results of culture showed that 69.2 % of onychomycosis cases were due to NDMs (18 of 26) followed by yeasts (7 of 26). In the study period only one case of dermatophyte onychomycosis due to *Trichophyton mentagrophytes* was recorded. Generally *Aspergillus niger* (7 of 26) was the most commonly isolated species in the present study followed by *A. fumigatus* (5 of 26) and *Candida albicans* (4 of 26). Different fungal species of our study and their frequency in cases of onychomycosis is presented in Table 1.

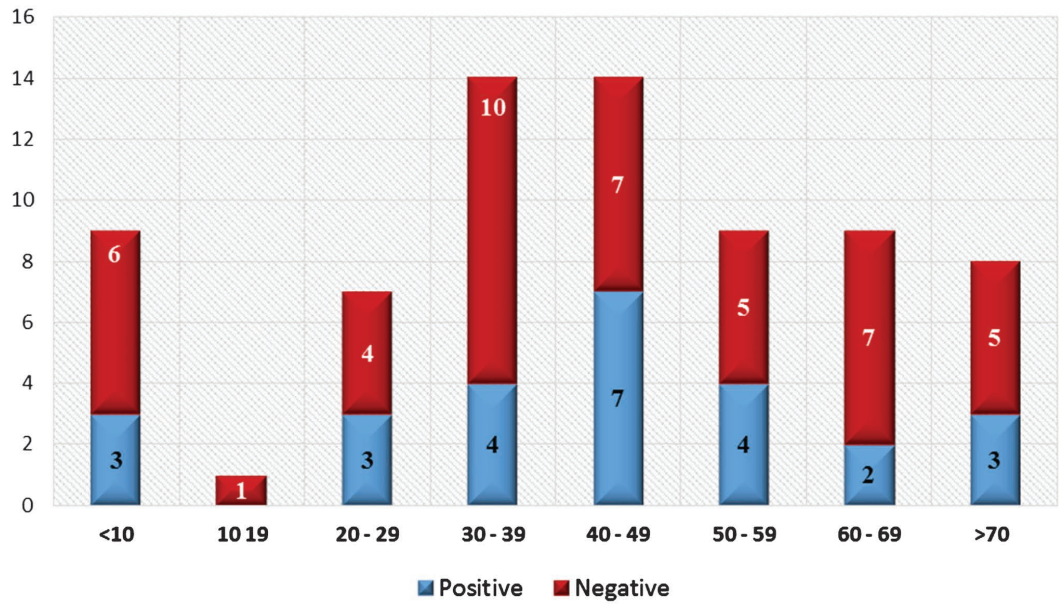

Figure 1. The distribution of 71 patients suspected of onychomycosis and the number of positive and negative cases in different age groups

Table 1. Distribution and frequency of fungal species isolated from 26 cases of onychomycosis in relation to clinical manifestation

Etiologic agent	Clinical form		Total	
	Finger nail (N)	Toe nail (N)	N	% of total (N=26)
Aspergillus niger	7	0	7	26.9
Aspergillus fumigates	3	2	5	19.2
Aspergillus flavus	2	1	3	11.5
Candida albicans	3	1	4	15.4
Candida dubliniensis	1	0	1	3.8
Candida tropicalis	1	1	2	7.7
Fusarium sp.	0	1	1	3.8
Penicillium sp.	2	0	2	7.7
Trichophyton mentagrophytes	1	0	1	3.8
Total	20 (76.9%)	6 (23.1%)	26	100

DISCUSSION

Onychomycosis is a fungal infection of the nails, caused mainly by species of dermatophytes, also yeasts and NDMs may involve in its etiology [18]. Clinical types of onychomycosis including distal lateral subungual onychomycosis, superficial onychomycosis (either white or black), proximal subungual onychomycosis, endonyx onychomycosis, mixed pattern onychomycosis and total dystrophic onychomycosis. The latter is considered as the most advanced form of onychomycosis [19].

While some investigations [8,11] reported that women are affected more than men by onychomycosis, in our study like other studies the reverse has been found [16,20]. However, some studies reported equal involvement of both genders [21]. These differences in gender preponderance from various geographical regions could be due to different cultures, socio-economic status and climatic condition.

In this study, the most common age group affected by onychomycosis was 40-49 years with a prevalence of 50% (7 out of 14) which comprises 26.9% (7 out of 26) of all positive cases. In accordance to our results, Aghamirian *et al.* [11] found the same age range as the most common infected group. Furthermore, Hashemi *et al.* [8], and Zaini *et al.* [10], respectively, reported 40-60 years and 30-50 years as the most affected age group which both cover the age range of our study. However, in contrast to our results, Papini *et al.* [20], Maraki *et al.* [16], found the highest prevalence in the age over 60 years.

Although, in agreement with the results of Nouripour *et al.* [7], in our survey, the frequency of onychomycosis due to NDMs and yeasts exceeds the frequency of nail infections caused by dermatophytes, it differed from another study which reported higher number of onychomycosis due to dermatophytes than NDMs and yeasts in Iran [8]. In the present study, NDMs were the leading cause of onychomycosis. Ungpakorn *et al.* [22] in accordance to our findings, reported NDMs as the most prevalent etiology. Furhtermore, the results of Chadeganipour *et al.* [13] in agreement to our findings, documented the NDMs as the most common causal agent of onychomycosis. However, Chadeganipour *et al.* [23] in another study in a different time, but from the same region reported *Candida* species as the leading cause of onychomycosis. It could be concluded that the epidemiology of onychomycosis could change over time. Other reportes in contrast to our results found dermatophytes [11,12,17,24] or yeasts [9,16] as the most common fungi isolated from cases of onychomycosis. Interestingly, in this study the prevalence of onychomycosis caused by dermatophyte was as low as 3.8% in Yazd where an 18.7% prevalence of dermatophytosis has been documented in the earlier study [25].

Overall, *A. niger* was the most common etiology of onychomycosis in our study and *C. albicans* was the leading C*andida* species. Hilmioğlu-Polat *et al.* [17] reported *A. niger* as the most prevalent NDM but not the leading cause of all cases. *A. flavus* is found to be the major NDM isolated from onychomycosis in studies by Nouripour-Sisakht *et al.* [7], Chadeganipour *et al.* [23] and Hashemi *et al.* [8]. Other fungi are reported as the most common NDMs etiology of onychomycosis, as well. For instance, Ungpakorn *et al.* [22] and Gupta *et al.* [26] indicated *Scytalidium dimidiatum* and *Acremonium* as the most common NDMs. Even though, according to the previous study, *C. parapsilosis* was the predominantly isolated *Candida* species as the normal flora in different sites of the body skin particularly nails, in our study did not recovered as pathogenic agent of onychomycosis [27].

The distribution pattern of NDMs varies based on geographical region. Gupta *et al.* [28] in a systematic review described the *Scopulariopsis brevicaulis*, *Fusarium* species, *Aspergillus* species, *Scytalidium dimidiatum* and *Acremonium* species as the most frequently reported NDMs. Furthermore, according to their review, in South America *Fusarium* species and in European countries *S. brevicaulis*, *Aspergillus* species, *Acremonium* species and *Fusarium* species are more likely to be reported as the most common NDMs. While, *S. dimidiatum* is reported from various counteries and frequently from Thailand. Thus, it is necessary to determine the accurate epidemiology of onychomycosis in different part of the world.

From clinical point of view, the treatment of onchomyosis is important because this condition affects the cosmetic and psychological status of patient. Also, in the cases of onychomycosis caused by dermatophytes, the patient could provide a reservior for contamination of communal places. Furthermore, the risk of cellulitis and diabetic food are consequences of untreated onychomycosis

in immunocompromised and diabetic patients, respectively [29,30].

According to above mentoined data, it is of great importance to accurately diagnose and to use appropriate antifugal drug in treatment of onychomycosis. NDMs are usually complicated to treat and need a combined strategy [26]. Griseofulvin is one of systemic therapies for tinea unguium which is not effective against NDM onychomycosis and misdiagnoses could lead to treatment failure. Azoles (ketokonazole, itraconazole and fluconazole) and terbinafine are other choices in treatment of onychomycosis which are not completely effective against all causal agents of onychomycosis [28,29]. Accordingly, some authors suggested simultaneous application of antifungal drugs and nail plate avulsion [28].

CONCLUSION

The various results from different regions and in different times, highlight the need for continuous epidemiological investigation of onychomycosis and the need for precise mycological examination for all suspected patients in order to determine the causal agents and to employ efficient theraputic strategies.

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