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Onychomycosis in Tehran: mycological study of 504 patients

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Summary

Onychomycosis is a common nail disorder resulting from the invasion of the nail plate by a dermatophyte, yeast or mould species and gives rise to some diverse clinical presentations. The purpose of the present study was to isolate and identify the causative fungi of onychomycosis in the population of Tehran, Iran. Nail samples from 504 patients with prediagnosis of onychomycosis during 2005 were examined both by direct microscopical observation of fungal elements in KOH preparations and in culture for the identification of the causative agent. All samples were inoculated on (i) Sabouraud dextrose agar (SDA, Merck), (ii) SDA with 5% chloramphenicol and cycloheximide in duplicate for dermatophyte and (iii) SDA with 5% chloramphenicol in triplicate for mould isolation. The criteria for the diagnosis of onychomycosis caused by non-dermatophytic moulds were based on microscopical observation of fungal elements, growth of the same mould in all triplicate culture and no growth of a dermatophyte or yeast in all the cultures. Of 504 cases examined, 216 (42.8%) were mycologically proven cases of onychomycosis (144 fingernails, 72 toenails). Among the positive results, dermatophytes were diagnosed in 46 (21.3%), yeasts in 129 (59.7%) and non-dermatophytic moulds in 41 (19%). *Trichophyton mentagrophytes* was the most common causative agent (n = 22), followed by *Trichophyton rubrum* (n = 13), *Candida albicans* (n = 42), *Candida* spp. (n = 56) and *Aspergillus* spp. (n = 21). Nearly half of the clinically suspected fungal nail infections are onychomycosis and yeast is responsible for most of the infections in Iran.

Key words: Onychomycosis, nail disorder, Iran.

Introduction

Onychomycosis is a common fungal infection affecting both fingernails and toenails and represents up to 20% of all nail disorders. The worldwide incidence of onychomycosis is increasing and a number of factors such as diabetes, poor peripheral circulation, immunodeficiency, drug treatment, nail trauma and in a small percentage, genetic defect contribute to this rise.[1,2]

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The prevalence rate of onychomycosis is determined by age, predisposing factor, social class, occupation, climate, living environment and frequency of travel.[3] In spite of improved personal hygiene and living environment, onychomycosis continues to spread and persist. In Asia, the prevalence of onychomycosis is relatively low. This was partially confirmed by a large-scale survey in Asia in the late 1990s in which the prevalence of onychomycosis was lower in tropical countries (3.8%) than in sub-tropical countries and countries in the temperate zone (18%).[4]

Dermatophytes are responsible for nearly 90% of toenail onychomycosis and at least 50% of fingernail infections. *Candida* species, particularly *C. albicans*, prevail in fingernail infections.[2–5] Non-dermatophytic fungi or moulds such as *Fusarium* spp. and *Acremonium* spp. have also been described as aetiological agents of

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onychomycosis with an unknown incidence and clinical significance.[5,6]

In Iran, especially in Tehran, there is high awareness of onychomycosis among physicians and dermatologists. The epidemiology of onychomycosis has been well studied in some countries, but only few data are available in Asian countries like Iran. This study, therefore, seeks to improve knowledge of some epidemiological and the mycological features of onychomycosis.

Materials and methods

Over a period of 1 year (2007), samples were obtained from 504 patients with clinically suspected fungal nail infections, who were referred by dermatologists and other physicians to medical mycology department at Tehran University of Medical Sciences. The assessment of participants was carried out and consisted of an interview, clinical examination and collection of specimens for mycological studies. All subjects completed a questionnaire that contained patient history and specific data related to risk factors for onychomycosis (age, gender, occupation and predisposing diseases such as diabetes). The clinical appearance and location of onychomycosis (toenail/fingernail) were documented.

The specimens were obtained from clinically abnormal nails, by a vigorous scrapping of the nail bed, 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 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 each of two isolation media (i) Sabouraud dextrose agar (SDA, Merck), (ii) SDA with 5% chloramphenicol and cycloheximide in duplicate for dermatophyte and (iii) SDA with 5% chloramphenicol in triplicate for mould isolation.

The culture tubes were incubated at 25 °C and 37 °C and examined daily for 4 weeks. Growth in the culture medium was viewed as the confirmation of dermatophytes as aetiological agents. In addition, the identification was confirmed by micromorphological aspects on slide culture and positive urease test, hair penetration and growth on Trichophyton agar media (Hi Media). Confirmation of *Candida* species required positive direct or culture and observation of pseudomycelium under light microscopy with KOH.

The criteria for the diagnosis of onychomycosis caused by non-dermatophytic moulds were based on: (i) microscopical observation of fungal elements in KOH preparations made from nail scrapings, (ii) growth of the same mould in all triplicate culture and (iii) no growth of a dermatophyte or yeast in all the cultures. When the light microscopy of a nail specimen showed filaments, with only a non-dermatophytic growth in culture, a second nail specimen was examined again by light microscopy and culture to confirm non-dermatophytic mould infection.

The identification of non-dermatophytic fungi species was confirmed by direct microscopical examination followed by microscopical and macroscopical evaluations of the primary triplicate cultures and slide culture.

Result

A total of 504 (169 male and 335 female) patients were examined. Of 504 patients with clinical lesions on the nails, 216 (42.8%) had onychomycosis by culture and/or direct examination.

Onychomycosis was the most prevalent in the 40- to 60-years age group and the ratio of male (28.7%) to female (71.3%) onychomycosis patients was approximately 1 : 2.

Fingernails were the most frequent anatomic site in 144 patients (66.7%) and toenail onychomycosis was confirmed in 72 patients (33.3%). In addition, 17 patients (8%) presented infections on fingernails and toenails simultaneously. The predominance of lesions in fingernails was higher in women (53.7%) than in men (15.7%).

Table 1 presents the distribution of causative organism according to patient gender. The organism that was diagnosed most frequently by direct examination and culture was yeast (129; 59.7%), followed by dermatophyte species (46; 21.3%). In 41 (19%) patients, moulds were recovered by direct and culture method. For yeast infections, *C. albicans* was found in 42 (32.8%) patients, whereas the rest 56 (43.4%) were infected by other *Candida* spp. Of the 38 dermatophytes isolated, nine could not be speciated and in the remaining 20, *Trichophyton mentagrophytes* was the most commonly involved, being responsible for positive cultures (9.7%). Other dermatophytic strains identified were seven *Trichophyton rubrum*, one *T. verrocosum* and one *T. tonsurans*. Regarding filamentous non-dermatophytic fungi, *Aspergillus* spp. ($n = 21$) and *Fusarium* spp. ($n = 2$), were the most frequently isolated. Occasionally, *Penicillium* spp. and *Scopulariopsis* spp. were the other fungi that were isolated (Table 2).

Table 1 Onychomycosis distribution based on gender and fungi.

	Sex							
	Male				Female			
	Fingernail		Toenail		Fingernail		Toenail	
	Frequency	percent	Frequency	percent	Frequency	percent	Frequency	percent
Yeast	20	9.3	1	0.5	103	47.7	5	2.3
Dermatophyte	2	0.9	23	10.6	5	2.3	16	7.4
Mould	6	2.8	10	4.6	8	3.7	17	7.9
Total	28	13	29	15.7	116	53.7	38	17.6

Table 2 Non-dermatophytic moulds isolated as agent of onychomycosis.

Moulds	*n* (%)
Aspergillus flavus	9 (25)
Aspergillus niger	4 (11)
Aspergillus fumigatus	3 (8)
Aspergillus spp.	5 (14)
Fusarium spp.	2 (5)
Penicillium spp.	1 (3)
Scopulariopsis spp.	1 (3)
Unknown	11 (3)
Total	36 (100)

Discussion

Onychomycosis is a chronic infection of the nails; presently it is considered a problem for public health, in view of its high occurrence in the worldwide population.[7] Although this disorder is not serious in terms of mortality or physical and/or psychological sequelae, it has a significant clinical consequence given its infectious nature, aesthetic consequences, chronicity and therapeutic difficulties. The prevalence is probably higher than is currently thought, as the difficulty in clinical-mycological diagnosis, inappropriate collection of material for analysis and ineffective treatment make it hard to ascertain the true profile of such onychopathies.[8]

The present study described, and evaluated the prevalence and some risk factors of onychomycosis in individuals representing different strata of population in Tehran, Iran, in an attempt to define the epidemiology of this disorder in general population. In this study, the prevalence of onychomycosis was confirmed in about 40% of the patients analysed, and these data exceeding those published in India, Turkey, Greece and Italy respectively,[6–10] however, lower than the results demonstrated by Lopes *et al.* [11] and Pontes *et al.* [2] of 56.6% and 66.5% respectively. Onychomycosis is more common in the population of 40–60 years of age of positive patients analysed in this study. The increase in cases with age may be justified by repeated nail microtrauma, due to a more prolonged exposure to pathogenic fungi as well as to greater work activity and venous insufficiency.[12] Finally, our data indicate that onychomycosis is uncommon in children in our country thus corroborating the epidemiological reports of other countries.[13,14] In the present study, onychomycosis affected more females (71.3%) than males (28.7%). Fingernails were affected more often than toenails in females, which can be explained by sexual hormonal difference and the work habits such as those of housewives, who generally perform domestic chores.[15] On the other hand, this observation was based on the group of patients examined by the dermatologists and we must consider that some male patients with toenail onychomycosis were not examined, as they did not ask for medical advice or mycological examination. Thus, hands remained wet for most of the day. This fact is mainly due to onycholysis and paronychia of the fingernails caused by *Candida* spp. (75.2%).[1,9] However, in our study, onychomycosis of toenails only due to dermatophytosis was prevalent in men ($n = 25$), whereas the infection of toenail was prevalent in 21 women. The increased prevalence of onychomycosis of toenails in men compared with women could be the result of more traumas in the nails and the more common use of occlusive footwear. Despite previous reports of greater susceptibility of females to this infection,[11] our study demonstrated no such significant difference in the occurrence of onychomycosis on the basis of gender. Yeast have been quoted in the literature as being responsible for the most cases of onychomycosis worldwide;[10,13,16] as in the literature, yeast were the aetiological agents most widely found in our study population, being responsible for 59.7% of cases evaluated.

In addition to the causative dermatophytes and yeast, the present data show that non-dermatophytic moulds can also be the potential cause of onychomycosis. As some of these moulds are common laboratory and nail

Table 3 Onychomycosis distribution based on gender and nail.

	Dermatophyte		Yeast		Mould		
Onychomycosis	Male	Female	Male	Female	Male	Female	Total(%)
Finger	2	5	20	103	6	8	144 (66.7)
Toe	23	16	1	5	10	17	72 (33.3)
Total	25	21	21	108	16	25	216 (100)
	46 (21.3%)		129 (59.7%)		41 (19%)		

contaminants, onychomycosis caused by the moulds is not well understood. This study reflected that 19% of ungual mycosis was due to non-dermatophytic moulds (Table 3), which is similar to some reports from countries such as India (22%) and Spain (17.2%).[8,14] The incidence of onychomycosis caused by non-dermatophytic mould has increased dramatically in the past few years.[17]

The two conventional methods for the identification of fungi are direct microscopy under potassium hydroxide and fungal culture. The microscopical method is more sensitive for showing the presence of fungi, but the identification of the fungal pathogen to the genus and species level requires fungal culture. The positivity from direct microscopical examination in our case was 88%. This may be considered high when compared with the work of Kam *et al.* and El sayed *et al.*[18,19] However, it was similar to the positivity found in certain other studies.[2] Culture was positive in 192 cases including 26 (12%) with negative direct examination and 166 (77%) with positive direct examination. Hence, both tests are complementary to each other. The sensitivity of these diagnostic tests depends on the method of sampling, preparation of sample, failure rate of microscopy and culture and the final interpretation of results. Nail clipping/scraping alone would have a lower yield than curette alone. It was suggested that a combination of curette and clipping would improve the yield.[20] Moreover; histopathological examinations are more accurate tools to confirm the diagnosis of onychomycosis,[21] but are seldom used.

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