Onychomycosis in Iran: Epidemiology, Causative Agents and Clinical Features

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Background: Onychomycosis represents the most frequently encountered nail disease, which is difficult to eradicate with drug treatment. Aim: This study was undertaken to document the clinico-mycological pattern of onychomycosis in Iran. Results of mycological tests of nail scrapings collected over a 4-year period were analyzed. Methods: Both direct microscopy and cultures of the nail material were performed to identify the causative agents. Results: The microscopic and/or cultural detection of fungi was positive in 40.2% of samples. The most common clinical type noted was distolateral subungual onychomycosis in 48.4% of cases. Etiological fungal agents were 50% dermatophytes, 46.8% yeasts, and 3.2% saprophytic moulds. The most frequently detected dermatophyte species were *Trichophyton rubrum* (48.4%) and *T. mentagrophytes* (41.9%). Among yeasts, *Candida albicans* (58.6%) was most common, followed by *C. parapsilosis* (17.2%), *C. glabrata* (10.3%), *C. krusei* and *C. tropicalis* (each 6.9%). *Aspergillus niger* and *A. flavus* were the most frequent saprophytic moulds. Females were affected more frequently than males, and in both sexes those most infected were between 40-49 years of age. Fingernails were affected more frequently than toenails. Conclusions: The clinico-epidemiological data collected can serve as reference for future research and may be useful in the development of preventive and educational strategies.

Key words: onychomycosis, *Trichophyton rubrum*, *Candida albicans*, Iran

Introduction

Among nail diseases, onychomycosis is still considered as a major public health problem in many parts of the world. These diseases are relatively common with a universal prevalence and relapsing condition mostly in adult populations, which have increased during the past few decades. Large-scale epidemiological studies performed worldwide have demonstrated different epidemiological results. However, onychomycosis represents up to 50% of all nail disorders and 30% of all superficial skin fungal infections diagnosed.

Clinically, onychomycosis is classified into various types: distolateral subungual onychomycosis (DLSO), superficial white onychomycosis (SWO), proximal subungual onychomycosis (PSO), candidal onychomycosis (CO) and total dystrophic onychomycosis (TDO)

These infections are produced by yeasts, dermatophytes, or saprophytic moulds. The nails infected with yeasts are usually hard and thick, brown colored, and grooved, whereas the nails infected with dermatophytes are fragile. A chronic infection with yeasts can lead to complete destruction of the nail tissues. Candidal onychomycosis is seen in people whose hands or feet are put in water frequently or for long periods in relation with their job, and/or in patients with diabetes, neoplasias, or those under treatment with cytotoxic or immunosuppressive drugs. The impairment of the immune system of the normal host by drugs provides suitable conditions for colonization of *Candida* species. Onychomycosis with dermatophytes is often produced by scratching an infected area of the body, clipping the nail with a contaminated clipper, rasping nails with a contaminated file, or extension of an infection from infected skin to the toenails. Onychomycosis with...
Saprophytic moulds are frequently produced by a trauma to the nail and connection of this injured nail with a polluted source. The most common opportunistic moulds are *Scopulariopsis, Aspergillus*, and *Fusarium*. *Candida albicans* is the most frequent genus of candidas among the yeasts causing onychomycosis, but *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and other candidas can also involve nails.

To the best of our knowledge, this is the first report on different aspects of onychomycosis in Iran and with respect to the importance of the subject; this study was carried out over a period of four years in order to determine the epidemiology, causative agents and different types of onychomycosis among people resident in Qazvin. There is a need to educate the general population concerning the danger of acquiring infections from infected persons and animals.

**Materials and methods**

The study was conducted from June 2004 to June 2007 in Qazvin province, situated northwest of the capital, Tehran. Carried out using an observational method and samples of nail scrapings were taken by scalpel from the fingernail or toenail of patients referred to the Medical Mycology Department of the Medical School of Qazvin.

Microscopic examination of nail clippings or subungal scrapings samples was performed following treatment with an aqueous solution of 20% (w/v) potassium hydroxide (KOH). A portion of each sample was placed on a slide, a drop of KOH added and the slide was stored in a humid chamber for 1–2 h. This incubation period was necessary in order to make the test material transparent. The wet preparation was then examined under low (×100) and high (×400) magnification for the presence of mycelia or arthroconidia, yeast cells, blastoconidia, pseudohyphae and hyphae and even spores in some saprophytic moulds. These microscopic features indicate the presence of a fungal infection but do not reveal the species or genus of the pathogen. All samples from suspected cases were cultured irrespective of the negative or positive examination result. Each sample was cultured on two plates of Sabouraud’s glucose agar (BioMérieux, Marcy-l’Etoile, France), with 0.5 mg/ml cycloheximide and 0.05 mg/ml chloramphenicol (SCC), which was prepared according to the manufacturer’s instructions. The cultures were kept at 28-30°C for 4-6 weeks, and examined macroscopically once a week for evidence of growth. It was also required to do a repeat culture where at least five out of 20 inocula planted demonstrated that the mould was the sole etiologic agent. However, it was not always possible to carry out repeated sampling. After the growth of the dermatophytes was established, a subculture was made on Sabouraud’s glucose agar and potato dextrose agar plates (Oxoid, Basingstoke, UK) for further identification. The dermatophytes were diagnosed on the basis of their gross and microscopic morphologies, especially according to their formation of macroconidia and microconidia in slide cultures. In order to differentiate between *T. mentagrophytes* and *T. rubrum*, additional special methods including a urea medium test, hair penetration, and production of pink color pigments on corn meal agar medium plus 1% glucose were used if needed. In order to determine *T. mentagrophytes* varieties, the isolates were differentiated according to macroscopic and microscopic characteristics and divided into *T. mentagrophytes var. interdigitale* and *T. mentagrophytes var. mentagrophytes*.

Diagnosis of the isolated yeasts was made on the basis of their microscopic morphology on cornmeal agar containing Tween 80 and production of their colors on CHROMagar Candida culture medium (Becton-Dickinson). The saprophytic moulds were diagnosed by their morphological characteristics and confirmation of the diagnosis was made by incubation on Sabouraud’s glucose agar with chloramphenicol (SC) culture medium. Information of age, gender, and injury location were collected and evaluated using descriptive statistics procedures.

**Results**

Samples were taken from 308 patients referred for suspicion of onychomycosis, of which 124 cases (40.2%) were positive by both direct microscopy and culture. Of those, 62 cases (50%) were infected with dermatophytes, 58 (46.8%) with candidas, and 4 (3.2%) with saprophytic moulds. The most common dermatophyte isolated from nails was *T. rubrum* (48.4%), the predominant yeast isolated was *C. albicans* (58.6%) and the sole mould isolated was the genus *Aspergillus* (Table 1). As shown in Table 1, the frequency of dermatophytes isolated was as follows: *T. rubrum* 24.2%, *T. mentagrophytes var. interdigitale* 16.9%, *T. mentagrophytes var. granular or mentagrophytes* 4.0%, *T. verrucosum* 1.6% and *Epidermophyton floccosum* 3.2%. After *C. albicans* (27.5%) the most commonly isolated non-*albicans* species were *C. parapsilosis* (8.1%), *C. glabrata* (4.9%), *C. krusei* and *C. tropicalis* (each 3.2%).

Eighty patients (64.5%) had fungal infection on their hands and 44 (35.5%) on their feet. All patients showed only fingernail or toenail involvement. Of the 58 patients with onychomycosis of candidal origin, 42 (72.4%) were females and 16 (27.6%) males. Of the 62 patients with
onychomycosis of dermatophytic origin, 26 (41.9%) and 36 (58.1%) were females and males, respectively. The prevalence of onychomycosis of dermatophytic origin in toenails and fingernails was 51.6% and 48.4% and of candidal origin was 13.8% and 86.2%, respectively.

Aspergillus niger and A. flavus were the only saprophytic moulds observed in toenails of 4 (3.2%) male subjects (Table 2). In fungal infections of fingernails, after yeasts the most common isolated agent was dermatophytes and no saprophytic moulds were observed.

Table 4 shows the various clinical types. DLSO was the most common clinical type, being encountered in 60 patients (48.4%), followed by PSO with paronychia (33 patients; 26.6%), PSO without paronychia (23 patients; 18.5%) and TDO (8 patients; 6.5%). SWO was observed in none of the patients. Discoloration (yellow-brown to brown-black) was observed in all of the patients and subungual hyperkeratosis leading to detachment of distal part of the plate from its bed with a furrowed base and ragged edge and onycholysis were the common features of dermatophyte infections that were seen in 83.3% of T. rubrum infections. In PSO with paronychia cases, the skin appeared edematous with erythema and sometimes painful and in 75.8% the nails were rough, convex and 24.2% had transverse grooves.
behind the rise in incidents of onychomycosis is the single reason more prevalent in women. According to Alvarez, advancing age of the population is the single reason increases with age and is unusual prior to puberty. Overall, onychomycosis is more common in adults than children and its prevalence was the main etiological agent followed by T. mentagrophytes var. interdigitale (anthropophilic form) and T. mentagrophytes var. mentagrophytes (zoophilic form). In this study we identified these two varieties of T. mentagrophytes, which was a positive point for our study. However, only a small number of the mentioned studies provided different varieties of T. mentagrophytes isolates. In this study, T. rubrum (24.2%) was the main etiological agent followed by T. mentagrophytes var. interdigitale (16.9%) and the presence of the zoophilic variety of T. mentagrophytes (var. granulare or mentagrophytes) was infrequent and isolated only in 4.0% of the diseased nails (Table 1). This finding is in concordance with a Turkish study on onychomycosis, in which T. rubrum (48.0%) and T. mentagrophytes var. interdigitale (26.6%) were responsible for most of the nail infections. But Khosravi et al. found that in Iran T. mentagrophytes var. interdigitale is the most prevalent species.

Irrespective of T. mentagrophytes varieties, comparing our results with those from other Iranian studies, T. rubrum has been the principal or one of the main dermatophyte species responsible for tinea unguium, whit the exception of one Iranian study which found only two cases of T. rubrum infection. Moreover, our results are in accordance with a report in Brazil that 10.4% of toenails were contaminated with T. rubrum (80%) and T. mentagrophytes (20%). Similar results in other studies found that T. rubrum was the most common pathogen causing onychomycosis, whereas Gupta et al. reported that T. mentagrophytes was the main etiological agent followed by T. rubrum in distal and lateral subungual and white superficial onychomycosis in toenails.

The results of the present study showed that onychomycosis was more frequent in fingernails (64.5%), whereas Ilkit reported that toenails were more involved in the Turkish people in his study. This difference might be due to the lifestyle differences between members of the two populations. The

Table 3. Distribution of patients with onychomycosis by age groups

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Suffering from onychomycosis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n (%))</td>
</tr>
<tr>
<td>0–9</td>
<td>10 (8.1)</td>
</tr>
<tr>
<td>10–19</td>
<td>12 (9.7)</td>
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<tr>
<td>20–29</td>
<td>14 (11.3)</td>
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<tr>
<td>30–39</td>
<td>18 (14.6)</td>
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<tr>
<td>40–49</td>
<td>26 (20.8)</td>
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<tr>
<td>50–59</td>
<td>20 (16.1)</td>
</tr>
<tr>
<td>60–69</td>
<td>18 (14.6)</td>
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<tr>
<td>70–79</td>
<td>6 (4.8)</td>
</tr>
<tr>
<td>Total</td>
<td>124 (100)</td>
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Discussion

Because of difficulties in treatment of onychomycosis, determining the identity of the causative organism of the disease is essential in choosing the most appropriate therapy. In our study, 40.2% of the samples were positive in both culture and direct vision. The etiological fungal agents were 50% dermatophytes, 46.8% yeasts, and 3.2% saprophytic moulds. According to Mügge et al., 54% of the nail samples were positive in both culture and by direct vision. The disease is known to occur at any age, but is more common between 40 and 60 years and is unusual prior to puberty; but in our study, however, 12.9% of the patients showed the disease before puberty. Overall, onychomycosis is more common in adults than children and its prevalence increases with age. Investigators assert that the slowly advancing age of the population is the single reason behind the rise in incidents of onychomycosis. In the present study, the majority of subjects (65%) were under 49 years of age and the age group of 40–49 contained the highest prevalence of onychomycosis. This observation is in agreement with other studies and in contrast with the findings of other reports. According to Alvarez et al., onychomycosis has been more prevalent in women and in the current study also 54.8% of people with onychomycosis were females.

From an etiological point of view, in our study, 50% of the causative fungal agents were dermatophytes. This finding is in accordance with a study carried out in Tehran and is in contrast to some other Iranian studies, where Moghaddami and Shidfar and Mahmoudabadi, respectively, found 32.1%, and 13% of the patients had documented yeast species as most common pathogens. In contrast, studies carried out by other investigators have demonstrated a greater prevalence of dermatophytes as etiological agents of onychomycosis. Based on Rubio Calvo et al., Mercantini, Jaannidou et al., Mügge and El Sayed et al., the prevalence of onychomycosis with dermatophytic origin was 14.7%, 23.7%, 52%, 68% and 77.1%, respectively.

Regarding etiological agents, many researchers have reported T. rubrum and T. mentagrophytes as the most important etiological agents of onychomycosis in the populations under their investigation. T. mentagrophytes is a common worldwide dermatophyte species composed of two chief varieties: T. mentagrophytes var. interdigitale (anthropophilic form) and T. mentagrophytes var. mentagrophytes (zoophilic form). In this study we identified these two varieties of T. mentagrophytes, which was a positive point for our study. However, only a small number of the mentioned studies provided different varieties of T. mentagrophytes isolates. In this study, T. rubrum (24.2%) was the main etiological agent followed by T. mentagrophytes var. interdigitale (16.9%) and the presence of the zoophilic variety of T. mentagrophytes (var. granulare or mentagrophytes) was infrequent and isolated only in 4.0% of the diseased nails (Table 1). This finding is in concordance with a Turkish study on onychomycosis, in which T. rubrum (48.0%) and T. mentagrophytes var. interdigitale (26.6%) were responsible for most of the nail infections. But Khosravi et al. found that in Iran T. mentagrophytes var. interdigitale is the most prevalent species.

Irrespective of T. mentagrophytes varieties, comparing our results with those from other Iranian studies, T. rubrum has been the principal or one of the main dermatophyte species responsible for tinea unguium, whit the exception of one Iranian study which found only two cases (7%) of T. rubrum infection. Moreover, our results are in accordance with a report in Brazil that 10.4% of toenails were contaminated with T. rubrum (80%) and T. mentagrophytes (20%). Similar results in other studies found that T. rubrum was the most common pathogen causing onychomycosis, whereas Gupta et al. reported that T. mentagrophytes was the main etiological agent followed by T. rubrum in distal and lateral subungual and white superficial onychomycosis in toenails.

The results of the present study showed that onychomycosis was more frequent in fingernails (64.5%), whereas Ilkit reported that toenails were more involved in the Turkish people in his study. This difference might be due to the lifestyle differences between members of the two populations. The
epidemiological differences are related to several factors, of which lifestyle is an important component. In this regard, the lifestyle of the Turkish people is rather close to the Europeans, but in Iran most of people still follow their traditional lifestyle. For instance, most Iranian women are housewives and wash the dishes by hand, which is one of the most potential risk factors for fingernail onychomycosis. The prevalence of onychomycosis of dermatophytic origin found in this study was higher in toenails (51.6%) than in fingernails (48.4%) (Table 2). Similar results have also been found in Iran\(^{17,27}\), Algeria\(^{20}\), Lebanon\(^{19}\), Canada\(^1\), Italy\(^{20}\), Spain\(^{21}\) and United Kingdom\(^{66}\). Furthermore, in fungal infections of toenails, after dermatophytes the most common isolated agent was yeasts and then saprophytic moulds.

Similar to some Iranian\(^{17,26,27}\) and a Japanese\(^{46}\) study, the frequency rate of tinea unguium was higher in males than females and was rarely seen in children. In fact, this type of onychomycosis is more frequently seen among adults\(^{27}\) and in a study on 382 Iranian children aged < 16 years suspected to have dermatophytic lesions, tinea unguium has not been found\(^{66}\). This could be due to smaller nail plate, diversity in nail plate structure, less exposure than adults to trauma and environments containing fungi and rapid growth of nails in children, which results in the elimination of dermatophytes from their nails. In our study, tinea unguium occurred in age group > 19, most of which (38.5%) was in the 50–59 age group. This finding is somehow in accordance with results described by Pie’rard\(^7\), that 15–80% of elderly people in Europe seem to be affected.

Candida albicans has the greatest pathogenic capacity and has been described as the prevailing species in producing onyxis primary or secondary to paronychia\(^31\). The etiological role of C. parapsilosis, C. guillermondii, C. famata, and C. tropicalis has also been demonstrated in onyxis and perionyxis. In the proximal subungual type of onychomycosis with paronychia, nail lesions are almost always caused by Candida species, mainly C. albicans, but other Candida species such as C. parapsilosis and C. tropicalis may also be implicated. Although most of the researchers have introduced dermatophytes as the most important causative agents of onychomycosis\(^4,7,19-21\), studies carried out by other investigators have demonstrated a greater prevalence of candida\(^17,21,39\). Yeast onychomycosis accounts for nearly half of all nail disorders\(^31\) and 46.8% of the suspected subjects examined in this study for this form of the disease proved to be infected. Incidence of onychomycosis of candidal origin is higher in adults and 2–3 times more in females than in males\(^4\). In our study, similar to the previous Iranian study\(^17\), candidal onychomycosis of fingernails is also more prevalent (about 3–1) in women than in men which could be due to constant immersion of their fingers in water and mechanical trauma.

Although onychomycosis is a very common superficial fungal disease, it is considered rare in children. In children, candidal onychomycosis has been seen in fingernails only due to finger sucking habits\(^66\). In the present study, only one case of candidal onychomycosis in a child the age of one year was seen.

Out of 124 onychomycosis–infected patients, 58 cases (46.8%) were infected with candida spp. of which the most common yeast isolated was C. albicans with other candida species: C. parapsilosis, C. glabrata, C. krusei, and C. tropicalis following. There is a vast variation of the percentage isolation of yeasts reported in diseased nails in Iranian and other publications. According to Moghaddami and Shidfar\(^17\), 66.04% of patients with
onychomycosis were infected with *Candida* spp., of which *C. albicans* and *C. parapsilosis* were the predominant species. The most common species of *Candida* to have caused onychomycosis in our patients were *C. albicans* (27.5%) and *C. parapsilosis* (8.1%), as has also been reported by others. In two studies, *C. parapsilosis* and in another study *C. tropicalis* surpassed *C. albicans* to become the most common species isolated.

The last group of fungi causing onychomycoses is opportunistic moulds. This is the most debated fungal group since the majority of species tend to be found as contaminants in the environment or the skin. Ungual infections caused by moulds account for 1-6% of all nail infections in European countries and up to 50% in certain areas of Africa. Their incidence is increasing in European countries such as Greece and Spain. In Iran, only limited data are available on fungal nail infections, particularly by opportunistic moulds, and according to some restricted conducted studies, saprophytic fungi are one of the least important etiological agents.

The most frequently isolated mould from infected nails is *Scopulariopsis brevicaulis*, a species that has been demonstrated to have an affinity for keratin and has been a etiological agent of onychomycosis in most studies. After *Scopulariopsis*, the most frequent saprophytic agents were *Aspergillus* (A. fumigatus, A. versicolor, A. terreus, A. flavus), followed by *Scytalidium, Fusarium, Acremonium, Cladosporium, Alternaria* and *Curvularia*. In the present study, the incidence of involvement of nails with saprophytic moulds was much less than other agents and was seen in the great toenails of males over 60 years of age. Bonifaz et al. in his study, refers to contact with soil, trauma of nails, and peripheral vascular disease of nails as predisposing factors for their involvement with moulds and introduces the great toenail as the most common place for mould involvement. He stated the average age of his patients with mould involvement was 44 years and the prevalence of onychomycoses in the elderly: a pan-European survey. Dermatology 206: 218-228, 2006.


