The epidemiology and etiology of onychomycosis in 2 laboratory centers affiliated to Tehran university of medical sciences during 2019-2020

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ABSTRACT

Background and Objectives: Onychomycosis is caused by dermatophyte species, non-dermatophyte moulds (NDMs), and accounts for roughly 50% of all nail diseases. As the prevalence of onychomycosis is increasing, new epidemiologic documents may help with treatment and prevention. The present investigation aims to determine the epidemiological profile of onychomycosis in 2 mycology laboratories.

Materials and Methods: A cross-sectional study conducted during eight months (2019-2020) on 169 patients with positive nail mycology tests referred to two mycological laboratory centers affiliated with Tehran University of Medical Science. The nail clippings were examined by direct smear and culture. Also, molecular assays were performed if needed.

Results: 10% of nail lesions referred to Razi Hospital (RH), and 30% of nail lesions referred to TUMS mycology laboratory were positive. Middle age (40-60) suffer more from onychomycosis. Aspergillus flavus, Trichophyton mentagrophytes, and Candida albicans were the most common etiologic agents in each of the three main classes of fungi causing onychomycosis. Females were more infected. NDMs were the predominant etiologic agents, and toenails were the most common site of onychomycosis.

Conclusion: The pattern of etiologic agents and clinical signs of onychomycosis differs according to geographical region and age, so repeated epidemiological surveys of onychomycosis seem to be fundamental.

Keywords: Onychomycosis; Epidemiology; Dermatophyte; Saprophyte; Yeast

INTRODUCTION

The term "onychomycosis" is originally a Greek word derived from the terms "onyx," meaning nail, and "makes," meaning fungus (1-3). Onychomycosis is the most common disease affecting the nail unit and accounts for at least 50% of all nail disease (3).

It can cause by dermatophytes, non-dermatophyte moulds (NDMs), and yeasts. Based on recent published epidemiologic researches, the prevalence of onychomycosis is about 5.5% globally (2, 4). Because the nail unit does not have effective cell-mediated immunity, it is vulnerable to fungal infection (3). Studies demonstrated that toenail involvement...
is the more common clinical form of onychomycosis in males, while Candida fingernail type is more common in females (5-6). The other predisposing factors are fungal infection elsewhere on the body (in particular, tinea pedis), chronic paronychia, previous onychomycosis, wearing of occlusive and tight shoes, hyperhidrosis, participation in sports or fitness activities, nail trauma, poor nail grooming, use of commercial swimming pools, communal bathing, living with family members suffering from fungal infection, poor health, genetic factors, immunodeficiency (in particular, acquired immune deficiency syndrome and transplant patients), diabetes mellitus, obesity, Down syndrome, psoriasis, smoking, peripheral vascular disease, venous insufficiency, hallux valgus, and asymmetric gait nail unit syndrome (2, 4, 5-7). It should be noted that the etiologic agent of onychomycosis varies in different countries and different provinces of a country like Iran. The present study tries to identify the etiologic agents of onychomycosis and assess the current epidemiology of this infection based on age, gender, and site of illness in patients referred to a mycology hospital center and a faculty laboratory in Tehran province, Iran.

MATERIALS AND METHODS

Ethics statement. The Research Ethics Committee approved the study of Tehran University of Medical Sciences (the number of Ethics Committees protocol: IR.TUMS.SPH.REC.1398.197). The project was founded on the ethical principles and the national norms and standards for conducting Medical Research in Iran.

Study design, patients and setting. The experimental interventional study was carried out for eight months from 2019.07.10 to 2020.03.09. Sampling was arranged on patients who presented nail changes compatible with a clinical diagnosis of onychomycosis, including paresthesia, pain, onycholysis, nail discoloration, brittleness, subungual hyperkeratosis, splitting of the nail plate, and nail plate destruction. The exclusion criteria were patients using topical and/or systemic antifungals at the time of sampling or up to 15 days before collecting the specimen and patients whose clinical samples were insufficient for complete analysis. Nail scrapings were gathered from outpatients referred to the mycology laboratories of Razi Hospital (RH) and Tehran University of Medical Science (TUMS). Both mycology laboratories were not private and were subdivisions of TUMS. The demographic and clinical data were documented in the patients’ sheets.

Culture and phenotypic examination. One sample was used to perform a potassium hydroxide (KOH) mount. The second was inoculated in three separate areas into Sabouraud dextrose agar (SDA-Merck, Germany) supplemented with 0.5% chloramphenicol and incubated at 26°C for 20 days and checked daily. Any growth obtained was identified by its characteristics include colony morphology, growth rate, and colony pigmentation. For obtaining pure single colonies and preliminary identification of yeasts, the grown yeast isolates were sub-cultured on CHROMagar Candida medium (CHROMagar, Paris, France). Identification of isolated dermatophytes and NDMs was made based on colonial morphology and microscopic characteristics using lactophenol cotton blue and slide culture. The diagnosis was based on similar growth in three separated sample growth and positive microscopic examination for identification saprophytic fungi from environmental saprophytic contamination. In this study, the correct determination of samples that were not detectable at the species level by mycological techniques, DNA sequencing was performed.

Molecular technique: DNA extraction, PCR conditions and sequencing. DNA was extracted by using the High Pure PCR Template Preparation kit (Roche, Germany) according to the manufacturer's recommended instructions. PCR assay was performed using the three µL of test sample as a template, in a total volume of 25 µL (1 µL of each of forward and reverse primers, ten µL of PCR MasterMix (Amplicon, Denmark) and, nine µL of deionized distilled water based on the following protocol: 10 min of primary denaturation at 95°C, 40 cycles of denaturation for 20 sec at 95°C, annealing for 20 sec at 62°C, an expansion for 20 sec at 72°C, and a final extension for 5 min at 72°C. Eventually, the products were run on a 2% agarose gel. The amplification of Aspergillus isolates was conducted by the β-tubulin primers (BT- forward (5'-GGTA ACC AA ATCGTG CTGTT0TTC-3') and BT- reverse (5'-ACCCCTCAGTGTAGTAGCA CC CTGTCGC-3'). Also, other fungal species were identified to the species level using the universal primers: ITS1

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RESULTS

In the present study, in the mycology laboratory, RH nail mycology requests calculated 10.5% of all mycology tests. Also, in the mycology laboratory of TUMS, nail mycology requests calculated 34.7% of all mycology tests. Furthermore, positive results for onychomycosis in RH and in TUMS were estimated at 41.9% and 30% of total nail mycology tests, respectively. The woman to men ratio in RH was 5:1(89/16) and in TUMS was 2:1(85/43). The average age of referred patients to two investigated centers was about 40-50 years that means onychomycosis is a middle-aged disease in both genders (Table 1).

Table 1. Relative and cumulative frequency of onychomycosis based on gender and age.

| Gender | Male | Female | Total |
|--------|------|--------|-------|
|        | Per  | No     | Per   | No   | No   |
| Age    |      |        |       |      |      |
| 0-17   | 1.8  | 3      | 0.6   | 1    | 4    |
| 18-25  | 0    | 0      | 7.1   | 12   | 12   |
| 26-35  | 3.6  | 6      | 8.9   | 15   | 21   |
| 36-45  | 5.3  | 9      | 13.6  | 23   | 32   |
| 46-60  | 11.8 | 20     | 18.9  | 32   | 52   |
| 60≤    | 14.2 | 24     | 14.2  | 24   | 48   |
| Total  | 36.7 | 62     | 63.3  | 107  | 169  |

Abbreviations: Per: percent, no: number

A total of 169 patients with onychomycosis claimed for culture. The results indicate that laboratory confirmation was reached through 121 (71.6%) positive direct smear and culture tests, 42 (24.8%) positive direct smear and negative culture tests, and 6 (3.6%) negative direct smear and positive culture tests. The age range of patients was between 2 and 87 years (mean age, 46.7 years), 62 male (13 with fingernail involvement, 49 with toenail involvement) and 107 females (56 with fingernail involvement, 51 with toenail involvement) (Table 1). Three patients (1.8%) had fingernail and toenail onychomycosis simultaneously, and the etiologic agents of fingernail and toenail involvement in 2 of them (66.6%) were the same.

Seventy-five non-dermatophyte spp. (44.4%), fifty-seven Candida spp. (33.7%), and thirty-seven dermatophyte spp. (21.9%) were isolated from nail infection in this investigation.

The anatomical sites of onychomycosis in the present study were toenails (n=100, 59.2 %) and fingernails (n=69, 40.8%), respectively. Also, 56 (81.1%) of fingernail onychomycosis belonged to females, while 13 (18.9 %) of fingernail lesions belonged to men. A statistically significant relation was found between gender and the site of infection (P-value<0.05) (Table 2).

The most frequent toenail onychomycosis was seen in 46-60 and over 60-year-old age groups equally (13.6%). The lowest frequency of onychomycosis is due to dermatophytes in the 0-17-year age group (0%). Also, the highest frequency of onychomycosis due to dermatophyte was found in the 46-60-year age group (47.3%), and the highest frequency of onychomycosis due to yeasts was found in the over 60 years (31.6%) age group. Furthermore, the highest frequency of onychomycosis due to NDMO was found in the 36-45- and the 46-60-year age group (24.3%) equally (Table 3).

There was a significant relationship between the etiologic agent of onychomycosis and the site of infection (P-value <0.00006) (Table 4).

Dermatophytic onychomycosis was more frequent in males (64.8%) than females (35.2%), and there was a significant relationship between the patient's gender and dermatophytic onychomycosis involvement (P-value <0.05), but no significant correlation was found.

Table 5 shows the relative and cumulative frequency of etiologic agents of onychomycosis diagnosed by mycological techniques in the present study. Also, for a correct determination of isolates which were not detectable by mycological techniques, DNA sequencing was performed. All of the sequences had been deposited in GenBank under the accession number reported in Table 6.

DISCUSSION

Treatment of onychomycosis needs a long-time treatment protocol, and an accurate diagnosis is essential. Precise diagnosis is made on the basis of clini-
Table 2. Frequency of etiologic agents of onychomycosis based on gender and the site of involvement

|                  | Female |        | Female |        | Total |        |
|------------------|--------|--------|--------|--------|-------|--------|
|                  | Fingernail | Toenail | Fingernail | Toenail |       |        |
| Yeasts           | 10     | 5.91%  | 6      | 3.55%  | 36    | 21.3%  | 5      | 2.95%  | 57    | 33.7% |
| Dermatophytes    | 1      | 0.591% | 23     | 13.6%  | 2     | 1.183% | 11     | 6.50%  | 37    | 21.8% |
| Moulds           | 2      | 1.183% | 20     | 11.8%  | 18    | 11.83% | 35     | 20.71% | 75    | 44.4% |
| Total            | 13     | 7.7%   | 49     | 29%    | 56    | 33.1%  | 51     | 30.0%  | 169   | 100%  |

Table 3. Relative and cumulative frequency of different etiologic agents of onychomycosis based on age groups

| Age groups (yrs) | Yeasts | Saprophytic fungi | Dermatophytes | Total |
|------------------|--------|-------------------|---------------|-------|
|                  | Number | Number | % | Number | % | Number | % | Number | % |
| 0-17             | 3      | 75     | 1 | 25     | -  | 4      | 100|
| 18-25            | 1      | 8.3%   | 9 | 75     | 2  | 16.7   | 12 | 100   |
| 26-35            | 7      | 33.3%  | 11| 52.4   | 3  | 14.3   | 21 | 100   |
| 36-45            | 12     | 37.5%  | 19| 56.25  | 1  | 6.25   | 32 | 100   |
| 46-60            | 16     | 30.8%  | 18| 34.6   | 18 | 34.6   | 52 | 100   |
| ≥60              | 18     | 37.5%  | 17| 35.4   | 13 | 27.1   | 48 | 100   |
| Total            | 57     | 33.7%  | 75| 43.8%  | 37 | 22.5   | 169| 100%  |

Table 4. Relative and cumulative frequency of different etiologic agents of onychomycosis based on infected situation

| Site of infection | Onychomycosis etiologic agents | Total |
|-------------------|--------------------------------|-------|
|                   | Non-dermatophyte moulds | Dermatophyte | Yeast | 69 (100%) |
| Fingernail        | 20 (29%) | 3 (4%) | 46 (67%) | 100 (100%) |
| Toenail           | 55 (55%) | 34 (34%) | 11 (11%) | 169 (100%) |
| Total             | 75 (43.8%) | 37 (22.1%) | 57 (33.7%) | 271 |

Table 5. Relative and cumulative frequency of etiologic agents of onychomycosis diagnosed by mycological techniques

| Fungal pathogen                     | Fungi                          | Number | Percent (in each fungal group) | Percent (in total) |
|-------------------------------------|--------------------------------|--------|--------------------------------|--------------------|
| Non-dermatophyic moulds (n=75, 44.4%) | Aspergillus flavus         | 30     | 40                             | 17.8               |
|                                     | Aspergillus niger           | 20     | 26.7                           | 11.8               |
|                                     | Aspergillus terreus         | 7      | 9.3                            | 4.1                |
|                                     | Fusarium spp.              | 4      | 5.3                            | 2.3                |
|                                     | Cladosporium spp.          | 2      | 2.6                            | 1.2                |
|                                     | Penicillium spp.           | 3      | 4                              | 1.8                |
|                                     | Mucor spp.                 | 1      | 1.3                            | 0.6                |
|                                     | Black fungus               | 1      | 1.3                            | 0.6                |
|                                     | Alternaria spp.            | 1      | 1.3                            | 0.6                |
| Dermatophytes (n=37, 21.9%)         | Saprophytic sterile mycelium | 6      | 8                              | 3.5                |
|                                     | Trichophyton mentagrophytes | 8      | 21.6                           | 4.7                |
|                                     | Trichophyton rubrum        | 6      | 16.2                           | 3.6                |
Table 5. Continuing...

| Yeasts                  | (n=55, 33.7%) |
|-------------------------|--------------|
| Candida albicans        | 17           |
| Candida parapsilosis    | 15           |
| Candida glabrata        | 8            |
| Candida tropicalis      | 2            |
| Trichosporon spp.       | 2            |
| Rodotula spp.           | 2            |
| Candida spp.            | 9            |

Table 6. The results of molecular identification and GenBank accession numbers of DNA sequences included in this study

| Isolate          | Molecular identification (ITS gene) | GenBank accession number | Isolate          | Molecular identification (ITS gene) | GenBank accession number |
|------------------|-------------------------------------|--------------------------|------------------|-------------------------------------|--------------------------|
| SUB10203970 seq1| Neoscytalidium dimidiatum           | MZ882261                 | SUB10203970 seq26| Cladosporium pseudoocladosporioides | MZ882286                 |
| SUB10203970 seq2| Candida albicans                    | MZ882262                 | SUB10203970 seq27| Trichophyton mentagrophytes         | MZ882287                 |
| SUB10203970 seq3| Candida parapsilosis                | MZ882263                 | SUB10203970 seq28| Penicillium glabrum                 | MZ882288                 |
| SUB10203970 seq4| Candida albicans                    | MZ882264                 | SUB10203970 seq29| Mucor circinelloides                | MZ882289                 |
| SUB10203970 seq5| Penicillium glabrum                 | MZ882265                 | SUB10203970 seq30| Aspergillus flavus                  | MZ882290                 |
| SUB10203970 seq6| Fusarium fujikuroi                 | MZ882266                 | SUB10203970 seq31| Aspergillus flavus                  | MZ882291                 |
| SUB10203970 seq7| Aspergillus flavus                  | MZ882267                 | SUB10203970 seq32| Trichophyton mentagrophytes         | MZ882292                 |
| SUB10203970 seq8| Alternaria infectoria               | MZ882268                 | SUB10203970 seq33| Trichophyton tonsurans              | MZ882293                 |
| SUB10203970 seq9| Trichophyton tonsurans              | MZ882269                 | SUB10203970 seq34| Rhodotorula mucilaginosa            | MZ882294                 |
| SUB10203970 seq10| Trichophyton rubrum                 | MZ882270                 | SUB10203970 seq35| Trichophyton mentagrophytes         | MZ882295                 |
| SUB10203970 seq11| Trichophyton rubrum                 | MZ882271                 | SUB10203970 seq36| Trichosporon asahii                 | MZ882296                 |
| SUB10203970 seq12| Aspergillus niger                   | MZ882287                 | SUB10203970 seq37| Fusarium fujikuroi                 | MZ882297                 |
| SUB10203970 seq13| Trichophyton mentagrophytes         | MZ882273                 | SUB10203970 seq38| Fusarium fujikuroi                 | MZ882298                 |
| SUB10203970 seq14| Penicillium polonicum               | MZ882274                 | SUB10203970 seq39| Trichophyton mentagrophytes         | MZ882299                 |
| SUB10203970 seq15| Aspergillus niger                   | MZ882275                 | SUB10203970 seq40| Trichophyton rubrum                 | MZ882300                 |
| SUB10203970 seq16| Candida albicans                    | MZ882276                 | SUB10203970 seq41| Trichosporon asahii                 | MZ882301                 |
| SUB10203970 seq17| Aspergillus flavus                  | MZ882277                 | SUB10203970 seq42| Trichophyton mentagrophytes         | MZ882302                 |
| SUB10203970 seq18| Candida parapsilosis                | MZ882278                 | SUB10203970 seq43| Trichophyton rubrum                 | MZ882303                 |
| SUB10203970 seq19| Cladosporium herbarum               | MZ882279                 | SUB10203970 seq44| Trichophyton mentagrophytes         | MZ882304                 |
| SUB10203970 seq20| Candida parapsilosis                | MZ882280                 | SUB10203970 seq45| Fusarium fujikuroi                 | MZ882306                 |
| SUB10203970 seq21| Aspergillus flavus                  | MZ882281                 | SUB10203970 seq46| Trichophyton rubrum                 | MZ882306                 |
| SUB10203970 seq22| Candida parapsilosis                | MZ882282                 | SUB10203970 seq47| Trichophyton mentagrophytes         | MZ882307                 |
| SUB10203970 seq23| Rhodotorula mucilaginosa            | MZ882283                 |                  |                                      |                          |
| SUB10203970 seq24| Candida tropicalis                  | MZ882284                 |                  |                                      |                          |
| SUB10203970 seq25| Candida parapsilosis                | MZ882285                 |                  |                                      |                          |
Epidemiology and Etiology of Onychomycosis in Tehran

...ical manifestation and laboratory confirmation using microscopy, culture, and molecular tests (2). Also, laboratory confirmation of the clinical diagnosis of onychomycosis prior to initiating treatment is cost-effective and is recommended (2). Treatment assessment, including drug of choices, device treatments, and treatment duration, depends on accurate identification of etiologic agents of onychomycosis, too. In this study, NDMs were the predominant etiologic agent of onychomycosis, followed by yeasts and dermatophytes. But onychomycosis etiologic agent in a study conducted by Aghamirian was dermatophytes, yeasts, and saprophytic moulds, respectively (1). Also, in another study by Halvaei, dermatophytes were diagnosed as the most common etiologic agents of onychomycosis, followed by yeast and NDMO (2).

Previously a study in the Khuzestan province of Iran hypothesized that the causative agents of onychomycosis have shifted from dermatophytes to yeasts (3). But a meta-analysis that reviewed all published studies about the epidemiology of onychomycosis from Iran did not confirm it (4). This difference may be due to the predominant middle-aged population group in this study.

It should be noted in the present study onychomycosis caused by NDMs in toenails was more frequent than fingernails. This finding is similar to the results of previous studies (2, 7-9). The most frequent NDMs isolated from nail samples in the present study were *A. flavus* (40%) and *A. niger*. The finding is consistent with the results of other studies in Iran (10), Nepal (11), and India (12). Studies showed that *A. flavus* was the most distributed species among genus *Aspergillus* in indoor and outdoor environments in Iran. More distribution of *A. flavus* in the environment can facilitate exposure and increase the risk of colonization and infection with this species (13). In this study, a case of toenail onychomycosis caused by *Neoscytalidium dimidiatum* was reported and confirmed by molecular sequencing (The obtained sequence with accession number MZ3377100 was 100% compatible with *Neoscytalidium dimidiatum* clone URF_Pt01) as the first one in Iran.

Furthermore, in this study, the most frequent isolated dermatophytes were *T. mentagrophytes* (21.6%) and *T. rubrum* (16.2%), which was in accordance with the results of studies previously conducted in Iran (2, 14), Germany (15), and Canada (16). Although, results of studies conducted in Tehran (14), and India (17) indicated that the most frequent isolated dermatophyte moulds were *T. rubrum*, *T. mentagrophytes*, *T. violaceum*, and *Epidermophyton floccosum*, subsequently. Also, *C. abidance* (30.9%) and *C. parapsilosis* (27.3%) subsequently were the most frequent yeasts isolated of infected nails. This finding was similar to other studies (18-20).

In the present investigation, the prevalence of onychomycosis was 41.9% in RH and 30% in TUMS mycology laboratories. The difference between the two studied centers might be due to the patients referred to each center because one was a dermatology hospital and the other was a single-specialized laboratory center. The prevalence of onychomycosis in both centers was lower than those previously reported in Dakar (Senegal) (48.4%) (21), Ethiopia (60.4%) (22), and North-East of Iran (56.8%) (23), and higher than those previously reported in Isfahan (13.1%) (24), South Greece (27.99%) (25), Northwestern Greece (28.9%) (26), and similar to those previously reported in other provinces of Iran such as Khuzestan (35.6%) (3), Tehran (39.6%) (27) and Qazvin (40.2%) (1).

In the present survey, the most frequency of onychomycosis in both genders has belonged to those over 45 years of age. The finding was similar to the results of studies conducted by Halvaei (40-60-year age group) (2), Rafat (>50 years in both genders) (4), and Aghamirian (40-49 years in both genders) (1). Different researches show onychomycosis is much more common in adults than in children, and the prevalence increases with age (2, 4). A prevalence of 0.4% in children of North America was reported for onychomycosis (28), whereas the prevalence may be as high as 33% in the elderly (> 60 years of age) (2). Also, in the present study, Fingernail onychomycosis was more common in females than men significantly. It was compatible with the results of a meta-analysis conducted by Rafat in Iran (4). Also, in the present study, 24.8% of specimens did not grow that may be due to unknown factors like washing the lesions, insufficient samples, or the use of antifungal drugs.

Furthermore, the results showed that females were more affected by onychomycosis than males that were compatible with the results of previous studies (2, 6). A higher ratio of women may be due to more hand eczema in the industrial cities in women (29), whom more refer to dermatology hospitals.

Regarding the fact that in the present study, the most frequent rate of onychomycosis was seen in 40-60-year-old people, the higher frequency of ony-
onychomycosis caused by NDMs may be due to the presence of predisposing factors such as occlusive and tight shoes footwear and trauma in this age group. This finding is similar to the results of studies conducted by Halvae (2), Hilmioglu (7), Moreno (8) and Gianni (9). More toenail involvement may be due to more trauma exposure in toenails, making them vulnerable to saprophytic fungi. Also, low hygiene in toenails than fingernails, the bareness of hands, high humidity, and more soil exposure are other reasons for this finding.

Also, in the present study, a 2.5-year patient with fingernail onychomycosis due to Candida species was found, which may be due to finger sucking or anal scratching. In the present study, the most common site of onychomycosis was the toenail.

CONCLUSION

This study is an advanced cause of the toenail problem that is more common in the middle-aged population. This finding is essential for health care that might be ignored besides more critical health problems like hypertension, diabetes, senile arthritis in this age group. Because toenails are an exceptional part of our beauty, the development of preventive and educational strategies about onychomycosis can help prevent nail dystrophy, the spread of infection and more flexibility in walking, running and other fast movements that depend on the precise balance of the weight tolerance. The present study started in the fall of 2018 and ended in May of 2019, and therefore the SARS- COVID-19 restrictions in the two last months of sampling led to a decreased number of patients referred to mycology laboratories.

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