## **ARTICLE IN PRESS**

Journal de Mycologie Médicale (2017) xxx, xxx-xxx



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ORIGINAL ARTICLE/ARTICLE ORIGINAL

# Onychomycosis due to dermatophytes species in Iran: Prevalence rates, causative agents, predisposing factors and diagnosis based on microscopic morphometric findings

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Received 7 August 2017; received in revised form 18 November 2017; accepted 15 December 2017

KEYWORDS Onychomycosis; Dermatophytes spp.; Causative agents; Predisposing factor; Iran

#### Summary

*Objective.* — Onychomycosis (OM) or fungal nail infection is one of the most common fungal infections, which is increasingly prevalent. OM is caused by *dermatophytes* spp, yeasts and non-dermatophyte moulds (NDMs). The purpose of this study was to identify and determine the prevalence rates, predisposing factors and causative agents of OM using clinical symptoms and microscopic morphometric findings.

*Materials and methods.* — In the present study, 180 patients suspected of OM were evaluated by direct microscopy using KOH 20%, culturing in Mycosel and Sabouraud dextrose agar media and Olysia software for identifying the causative fungi of OM.

*Results.* — From 180 referred patients, 118 (65.56%) had OM, of whom 79 (66.94%) were positive for infection with *dermatophytes* spp. Of the 79 cases, the commonest age group was 61-70 years (21%) with males being 46 (58.23%) and females being 33 (41.77%). Both the fingernail and toenail infections were most prevalent in male patients. Sex, diabetes and age above 60 years were

https://doi.org/10.1016/j.mycmed.2017.12.009

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Please cite this article in press as: Babayani M, et al. Onychomycosis due to dermatophytes species in Iran: Prevalence rates, causative agents, predisposing factors and diagnosis based on microscopic morphometric findings. Journal De Mycologie Médicale (2018), https://doi.org/10.1016/j.mycmed.2017.12.009

Abbreviations: OM, onychomycosis; NDMs, nondermatophytic molds; SDA, Sabouraud dextrose agar; DLSO, distal and lateral subungual onychomycosis; LSO, lateral subungual onychomycosis; DSO, distal subungual onychomycosis; PSO, proximal subungual onychomycosis; WSO, white superficial onychomycosis; CI, confidence interval; OR, odds ratio

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significant predisposing factors for OM development. DLSO was observed as the only clinical pattern of OM and *T. rubrum* was the commonest dermatophyte isolate (49.34%). *Conclusion.* — This study showed that *T. rubrum* was the most common dermatophyte agent of OM in Iran.

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### Introduction

Onychomycosis (OM) or fungal nail infection is one of the most common fungal infections, which is increasingly prevalent. This infection occurs as a result of fungus penetration to the nail plate. It involves toenails or fingernails, and shows about 39.6% of superficial mycoses [25]. Major etiological agents of OM comprise dermatophytes spp., yeasts (Candida spp, especially C. albicans) [31,34], and NDMs (Fusarium spp., Aspergillus spp., and S. brevicaulis) [26,28]. Some fungi such as Malassezia spp. especially M. Furfur [11,46], Rhodotorula spp.[43], Exophiala dermatitidis [13], Rhizomucor [19], Neoscytalidium dimidiatum [29], Cunninghamella bertholletiae [41] and Fonsecaea pedrosoi [37] are known as the uncommon agents of OM.

Dermatophyte infection of the nail is called tinea ungium [7]. *Dermatophytes* are the main agent of OM, which cause more than 90% of toenail infections and 50% of fingernail infections [28]. OM rarely occurs in a healthy nail. Older age, sex, diabetes, repeated nail trauma, poor peripheral circulation, longer contact with pathogenic fungi, warm and humid climate, excessive sweating, poor hygiene, and immunocompromised condition are predisposing factors for the development of the infection [18]. OM is divided into five main clinical patterns, based on the fungal invasion of the nail: proximal subungual onychomycosis (PSO), distal and/or lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), Candida onychomycosis (CO), and total dystrophic onychomycosis (TDO) [9].

Prevalence rates of OM, due to dermatophyte agents, vary in different regions of Iran, being, for instance, 35.8% in Tehran [25], 23% in Sari [3], 28.18% in Kerman [30], 18.6% in Kermanshah [23], 13.9% in Esfahan [10] and 50.2% in Qazvin [4]. The purpose of this study was to identify and determine the prevalence rates, predisposing factors and causative agents of OM using clinical symptoms and microscopic morphometric findings in Tehran, Iran.

### Materials and methods

#### Subjects

The population of this study included one hundred and eighty subjects suspected to have been infected with OM with different ages and both sexes who referred to the School of Public Health, the Tehran University of Medical Sciences, from September 2014 to September 2015. Each subject completed a structured questionnaire that was employed to determine demographic related factors (age, sex, personal health status), behavioral factors (occupation, socioeconomic status, level of education and hobbies), and clinical factors (underlying physiology or immune status, previous treatments, presence of foot ulcer, existence of other diseases, nail structural deformity, number of involved nails, family and personal history of OM, trauma and clinical manifestation of OM). The subjects with history of receiving topical and/or systemic antifungal or corticosteroid agents for at least 7 and 21 days, respectively, prior to collecting the samples were excluded. This study was approved by the ethics committee of the Kerman University of Medical Sciences (k/93/665).

#### Specimen collection

A total of 231 nail-clipping specimens were obtained from one hundred and eighty subjects suspected to be OM. From each subject, after cleaning the affected areas with 70% alcohol, nail specimens were obtained from the deepest part, underside of the nail plate and the hyponychium of the abnormal nails by a sterile nail clipper and scalpel blade. In some subjects, more than one nail was involved and specimen collection was done from all of the involved nails.

#### Identification of dermatophytes isolates

#### Direct microscopic examination

A portion of the collected nails specimens was digested by 20% potassium hydroxide (KOH) (Merck, Germany). Then, a glass slide containing the nails specimens and 20% KOH was examined carefully using light microscopy in 100 × and 400 × magnifications to identify the existence of fungal elements, including arthrospores, segmented hyphae, and or pseudohyphae and yeast cells.

#### Specimen cultivation on specific media

Another part of the nail chips obtained from each subject was cultured on Mycosel agar (Merck, Darmstadt, Germany), Sabouraud dextrose agar (SDA) (Merck, Germany), and also SDA supplemented with chloramphenicol (50 mg/ml). The inoculated plates were incubated at 28°C for four weeks. The cultures were examined every two to three days for fungal growth. The plates without fungal growth were considered negative after four weeks of incubation. Identification of the dermatophytes species was performed based on colony morphology, color of colonies (surface and reverse), macro- and microscopic characteristics by slide culture procedure, urease test, and hair perforation test [8,24].

#### Measurement with Olysia Software

In cases where direct microscopy examination was positive for the presence of *dermotophyte* mycelium, suitable

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