

Onychomycosis due to Dermatophytes Species in the University Hospital Hassan II of FEZ: Epidemiological and Mycological Profile

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Background and study aims: Dermatophytes are filamentous fungi characterized by their increased affinity to the keratin of the skin and phanera, and represent the main etiological agent of onychomycosis. The objective of this work is to identify the epidemiological and mycological profile of dermatophyte onychomycoses diagnosed at the laboratory of parasitology and mycology of the Hassan II university hospital of Fez.

Patients and Methods: This is a retrospective descriptive study conducted in the laboratory of parasitology mycology of the Hassan II University Hospital of Fez including patients referred for mycological nail sampling during a period of 6 years from November 2015 to October 2021. The identification of the different species of dermatophytes responsible for onychomycosis was based on the observation of macroscopic and microscopic aspects of the colonies.

Results: Of these 1673 patients, 460 cases of dermatophyte onychomycosis were confirmed with a prevalence of 27.5%. The age ranged from 9 to 87 years with an average age of 47.94 years. The disease was more common in women than in men, with a sex ratio of 0.63. Localization on the feet was predominant with a percentage of 93.7%. *Trichophyton rubrum* was the commonest dermatophyte isolated (95.7%), followed by *Trichophyton mentagrophytes var interdigitalis* (3.3%), *Trichophyton verrucosum* (0.7%), *Trichophyton soudanense* (0.2%), and *Epidermophyton floccosum* (0.2%).

Conclusion: *Trichophyton rubrum* is the genus most involved in dermatophyte onychomycosis in accordance with the data in the literature. This work emphasizes the contribution of mycological examination in the confirmation of the fungal origin of nail diseases, thus orienting the treatment.

INTRODUCTION

Onychomycosis is a fungal infection of the nails caused by dermatophytes, yeasts or molds. It accounts for nearly 50% of all nail diseases [1].

The incidence of onychomycosis is estimated to be over 10% in the healthy population and 40% in the elderly. This difference in incidence can be explained in the elderly by a lack of maintenance of good foot hygiene, a weakened immune system, and a regression of nail plate growth with age [1,2].

Dermatophytes are microscopic filamentous fungi characterized by a strong affinity for keratin. They

belong to the class Ascomycetes and are responsible for superficial lesions affecting mainly nails, but also skin and hair [3].

According to the latest classification of De Hoog et al, dermatophytes can be classified into seven genera: *Arthroderma*, *Epidermophyton*, *Lophophyton*, *Microsporum*, *Nannizzia*, *Paraphyton* and *Trichophyton* [4].

Almost 90% of onychomycosis of the feet and 75% of hand infections are caused by dermatophytes, notably *Trichophyton rubrum* (*T. rubrum*) and *Trichophyton mentagrophytes var interdigitalis* (*T. mentagrophytes var interdigitalis*) [2]. Onychomycosis

could also be caused by *Trichophyton tonsurans* (*T. tonsurans*), *Epidermophyton floccosum*, *Trichophyton verrucosum* (*T. verrucosum*), *Trichophyton soudanense* (*T. soudanense*) and *Trichophyton violaceum* (*T. violaceum*) [5].

PATIENTS AND METHODS

Our work is a retrospective study conducted in the laboratory of parasitology-mycology of Hassan II university hospital of Fez, over a period of six years from November 2015 to October 2021. The study involved nail samples from referred patients, as well as patients hospitalized in the different departments of the Hassan II University Hospital of Fez.

The samples were collected before any antifungal treatment or after a therapeutic window (15 days window for the local treatment and 3 months window for systemic treatment) in order to eliminate false negative results. After deleting any environmental mold contamination by cleaning the nails with alcohol at 70%, the samples were obtained from the area where the fungus is active and which corresponds to the junction between the healthy and the pathological area. 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. In case of perionychia, the pus was collected with a sterile swab.

The samples were then processed in two steps: first, direct examination, the objective of which was to analyze the product with observation after clearing with potassium hydroxide (potassium hydroxide 30%), and which was considered positive in case of evidence of regular segmented hyphae (Figure 1). The sample was then systematically cultured in three media: Sabouraud, Sabouraud-Chloramphenicol (SC) and Sabouraud- Actidione-Chloramphenicol (SCA) with incubation of the samples in an oven at 27 °C and 37 °C for at least 3 weeks. The tubes were checked after 48h and then 2 to 3 times a week during 4 to 6 weeks to follow the evolution of the growth.

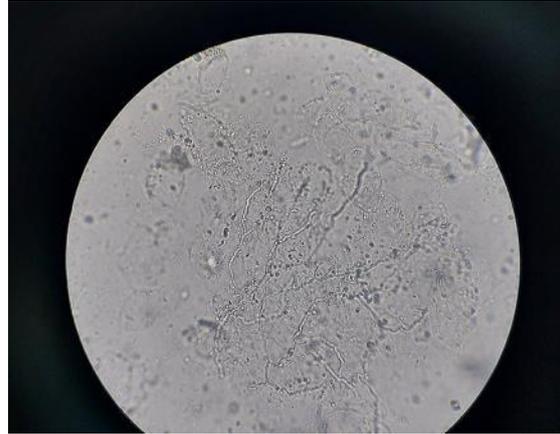


Figure (1): Direct examination of the nail mounted between slide and coverslip in 30% potassium hydroxide, showing numerous mycelial hyphae (magnification X400).

The identification of the dermatophyte species was based on the speed of growth, the macroscopic aspect of the surface and reverse of the colonies (Figure 2), the elaboration of pigments as well as their microscopic aspects after spreading the colonies between slide and coverslip in lactophenol blue solution (Figure 3), with observation of the fungal fruiting bodies.

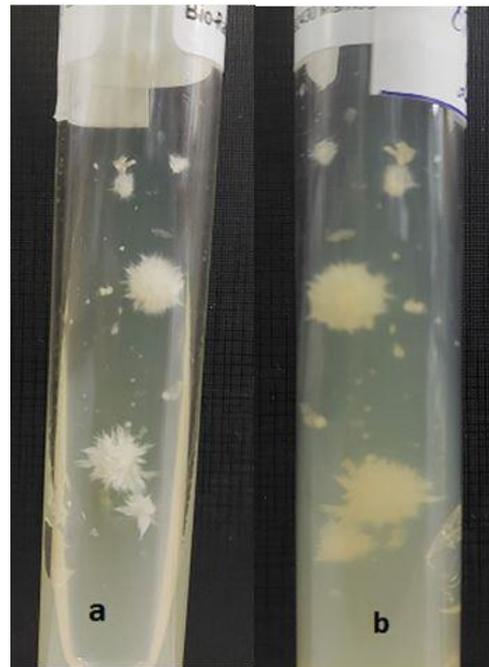


Figure (2): Culture showing white fluffy colonies on the front (a) and yellowish on the back (b) in favor of *Trichophyton rubrum*.

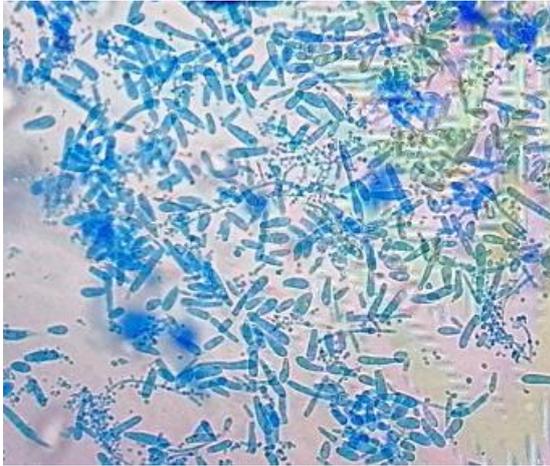


Figure (3): Examination between slide and coverslip after mounting in lactophenol blue solution of a sample of *T. mentagrophytes* colonies showing filaments with the presence of numerous round microconidia arranged in caladium form and club-shaped macroconidia. (Magnification X400).

RESULTS:

During the study period, we received 1673 nail specimens. 460 samples were confirmed as dermatophyte onychomycosis. Thus, the prevalence of dermatophyte onychomycosis was 27.49%.

The sex ratio M/F was 0.63, with women being more affected with a percentage of 61.1% (n=281).

The mean age of our patients was 47.94 years (SD=14.68) with extremes ranging from 9 to 87 years. Subjects between 25 and 45 years of age were the most presented with an estimated rate of 49.78%.

Foot involvement was predominant, representing 93.7%, followed by hand involvement at 6.3%. Only 8 cases of dual involvement were found.

The majority of samples processed in the parasitology-mycology laboratory came from referred patients (95.5%); the remaining specimens were referred mainly from the departments of dermatology (13 cases), rheumatology (3 cases), endocrinology, cardiology and intensive care, with 1 case for each department.

Direct microscopy was positive in 87.2% of cases, showing fungal hyphae in 77.4% of cases. It was negative in 12.8% of cases.

Among the different species of dermatophytes isolated in culture, *T. rubrum* ranked first with 95.7%, followed by *T. mentagrophytes* var. *interdigitalis* with 3.3% and *T. verrucosum* with 0.7% (3/640). *E. floccosum* and *T. soudanense* were isolated only once each (0.2%).

DISCUSSION

Onychomycosis is one of the most common fungal infections and is becoming increasingly prevalent. It represents about 39.6% of superficial mycoses [6] and its prevalence is estimated between 6 and 9% in the general population, and between 14 and 18% in patients seen in dermatology consultations [7].

Onychomycosis rarely occurs on a healthy nail. Older age, gender, diabetes, repeated nail trauma, poor peripheral circulation, prolonged contact with pathogenic fungi, hot and humid climate, excessive sweating, poor hygiene and immunosuppression are predisposing factors for the development of the infection [8].

Dermatophytes are the main agents of onychomycosis, causing more than 90% of toenail infections and 50% of nail infections [8]. The prevalence found in our study (27.49%) is close to the results described in the literature [6, 9, 10].

We found in our study that 61.1% of the patients were female. This predominance of women, also reported in other studies, can be explained by the aesthetic concern and functional discomfort more often expressed by women, thus motivating their consultation [11]. Microtrauma and prolonged contact with water during household chores, as well as the wearing of artificial nails and nail polish, are also among the contributing factors.

The average age of our population was 47 years, with a more pronounced attack in the 25-45 age group. Our results are consistent with those found in the literature, which reports a higher incidence in young adults [11-15, 18]. This could be attributed to the higher exposure related to occupation, sports-related trauma and the use of occlusive footwear [11-15, 18]. In addition, the younger population is generally more concerned about their appearance and therefore seeks early and frequent dermatologic check-up [13].

The involvement of the toenails was the most frequent (93.7%) concurring with the results revealed by other studies [8, 12, 16, 17]. The

frequency of foot infection from soils contaminated by anthropophilic dermatophytes, maceration favored by the wearing of closed shoes and microtrauma may explain the predominance of foot involvement. In addition, the slow growth of toenails slows down the elimination of the fungus [16].

In our series, *Trichophyton rubrum* was the most incriminated species (95.7%). This is in complete agreement with the data reported in various studies. Indeed, *Trichophyton rubrum* is the first fungus responsible for dermatophytic onychomycosis. Its prevalence is 97% in Casablanca [11], 80.8% in Marrakech [18], 40.6% in Singapore [5], 96.9% in Tunisia [19], 85.1% in France [20], 53.6% in Senegal [21]. *T. rubrum* is an anthropophilic species that is responsible for chronic skin and nail infections [5]; it is transmitted through the moist floors of showers, swimming pools and ablution areas in mosques. Therefore, the high frequency of use of Moorish baths could explain in part the frequency of these mycoses in the Moroccan population [22].

In second place, *Trichophyton mentagrophytes* var *interdigitalis* was found with a percentage of 3.3%, which is in line with the results reported in the literature [5, 11, 18].

Trichophyton verrucosum was found in 3 of our patients (0.7%). It is a zoophilic dermatophyte responsible mainly for inflammatory lesions of the skin and scalp. Nail involvement is very rarely reported in the literature. In a study conducted in Singapore on a population of 229 subjects, *T. verrucosum* was isolated from two patients; one of them was a kidney transplant patient whose immunocompromised state predisposed her to infection [5].

A few uncommon species were identified in our series. *T. soudanense*, an anthropophilic dermatophyte that mainly causes ringworm of the scalp (tinea capitis), was isolated from one patient, in line with the results of the study by Shiu Ming Pang et al. [5]. *E. floccosum*, often responsible for intertrigo, can also cause onychomycosis but its prevalence remains low. Only one case of *E. floccosum* onychomycosis was recorded in our series, in line with the results found in several studies [5, 8, 23].

CONCLUSION

Onychomycosis is the first cause of nail diseases with a predominance of dermatophytic origin. Women are mostly affected with preferential involvement of the toenails. The dermatophytes most involved are *T. rubrum* and *T. mentagrophytes* var *interdigitalis*. Since an onychopathy is not always related to onychomycosis, we should shed the light on the importance of practicing a quality sample, in order to confirm the diagnosis through direct examination and culture on an appropriate medium, and help the clinician through the therapeutic strategy.

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ETHICAL CONSIDERATION: all the informations gathered from the patients were handled confidentially, and they were used only for research purpose.

HIGHLIGHTS

- Onychomycosis represent the first cause of consultations in dermatology and the first etiology of nail diseases with a predominance of dermatophytic origin.
- Dermatophytes are microscopic filamentous fungi characterized by a strong affinity for keratin. They are responsible for superficial lesions affecting mainly nails, but also skin and hair
- An onychopathy is not always a synonym to onychomycosis. Therefore, it is important to practice a quality sample, in order to confirm the diagnosis through direct examination and culture on an appropriate medium, and help the clinician through the therapeutic strategy that can be very expensive for the patient and can expose him to side effects.

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