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Onychomycoses at the Mycology Laboratory of Mohammed Vi University Hospital Center in Marrakech

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Abstract **Original Research Article**

The study conducted at the Laboratory of Parasitology-Mycology of the Mohammed VI University Hospital Center in Marrakech, Morocco, aimed to describe the epidemiology of onychomycoses and identify the predominant fungal agents. Over a two-year period, 156 samples were collected and analyzed. Results showed a prevalence of confirmed onychomycosis of 34%, with a male predominance and a peak incidence in individuals aged 51 to 70 years. Trichophyton rubrum was the most commonly isolated dermatophyte, particularly affecting toenails, while candidiasis was more frequent in fingernails. The study underscores the importance of mycological diagnosis in distinguishing onychomycosis from other nail disorders, advocating for improved awareness and timely management strategies, including the exploration of alternative diagnostic approaches like PCR, to enhance the specificity and sensitivity of identification.

Keywords: Onychomycosis, Epidemiology, Fungal Agents, Mycological Diagnosis, Dermatophytes.

INTRODUCTION

Onychomycosis accounts for the majority of nail diseases and superficial fungal infections, caused by dermatophytes, yeasts, or molds commonly observed in clinical practice [1]. This condition is chronic and characterized by thickening, fragility, discoloration, and separation of the nail plate [1].

It is one of the most common reasons for consultation in mycological dermatology in Morocco [2]. The existence of numerous differential diagnoses (such as nail psoriasis, mechanical nail dystrophy, etc.) underscores the importance of mycological diagnosis to avoid inappropriate management leading to unnecessary, risky, and costly treatments [2].

The objective of this study is to describe the current epidemiology of onychomycoses and determine the most frequently isolated fungal agents at the Laboratory of Parasitology-Mycology of the Mohammed VI University Hospital Center in Marrakech, Morocco.

PATIENTS AND METHODS

This is a retrospective study conducted over a period of two years (January 2022-December 2023) at the Laboratory of Parasitology-Mycology of the Mohammed VI University Hospital Center in Marrakech, Morocco.

During the study period, 156 samples were collected at the laboratory by a medical biologist. This is a retrospective study conducted over a period of two years (January 2022-December 2023) at the Laboratory of Parasitology-Mycology of the Mohammed VI University Hospital Center in Marrakech, Morocco. During the study period, 156 samples were collected at the laboratory by a medical biologist.

For the sampling technique, damaged nails were first disinfected with alcohol and then cut with sterile nail clippers up to the junction with the healthy nail. Samples were collected by scraping the subungual scales or powder at the junction of the diseased nail and healthy nail, or at the area of leukonychia. The scales were collected in a sterile Petri dish. In case of paronychia, pus was collected using a sterile swab. Each sample was identified, and a clinical and epidemiological data sheet was completed (including age, sex, previous treatments especially phytotherapy, medical history, profession, and appearance of the nail at the time of sampling). A systematic examination of all nails of the feet and hands, as well as interdigital and intertoe folds, palm of the hands, and soles of the feet, was performed

to search for associated lesions.

The mycological examination is conducted in two stages: Firstly, a direct examination involves microscopic study of the samples in fresh state after the addition of 10% potassium hydroxide (KOH), aiming to identify septate and/or arthrospore-bearing mycelial filaments, as well as yeast cells and/or pseudohyphae. Subsequently, the samples are cultured on Sabouraud agar supplemented with chloramphenicol and chloramphenicol with cycloheximide. The samples are inoculated into slanted agar tubes under aseptic conditions. The tubes are then incubated in an oven at 25-37 °C for at least 30 days for dermatophytes and 72 hours for yeast and/or molds.

The cultures are monitored twice a week until the fungal agent is identified. Haut du formulaire. In case of growth of yeast-like colonies, species identification is performed using YST VITEK 2® identification cards [3].

The identification of dermatophyte colonies is based on the growth delay, the macroscopic appearance of colonies on both the front and back sides. These macroscopic morphological criteria are coupled with microscopic morphological criteria (mode of fruiting and spores), observed after conducting the flag test and staining with Lactophenol Blue.

The diagnosis of onychomycosis is confirmed when the direct examination is positive and the culture is positive with identification of dermatophytes or pseudodermatophytes, Candida albicans or non-albicans, or molds in pure culture after control on a second sample.

When the direct examination is negative, the diagnosis of onychomycosis is only confirmed if the culture is positive and dermatophytes or pseudodermatophytes or Candida albicans are identified.

RESULTS

This study reported a male predominance with a sex ratio (M: F) of 1.2.

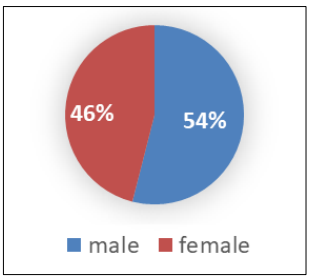

Figure 1: Distribution of patients with onychomycosis by gender

The average age of the patients was 56 years with a range from 13 to 86 years. The majority of patients were aged between 51 and 70 years (47.4%).

The prevalence of confirmed onychomycosis in this study was 34% (53 cases).

Hand onychomycosis affected 22 patients (41.5%), of whom 13 (59%) were women. These lesions were unilateral in 7 cases (46.6%) and bilateral in 15 cases (53.3%).

Foot onychomycosis affected 45 patients (84.9%), of whom 23 (51%) were women. Unilateral lesions were observed in 14 cases (31.1%) and bilateral lesions in 31 cases (68.9%).

The observed nail dystrophy aspects at the time of sampling, in decreasing order of frequency, were: pachyonychia (39.6% (21/53)), xanthonychia (28.3% (15/53)), subungual hyperkeratosis (15.1% (8/53)), paronychia (7.5% (4/53)), leukonychia (7.5% (4/53)), and onycholysis in (1.9% (1/53)).

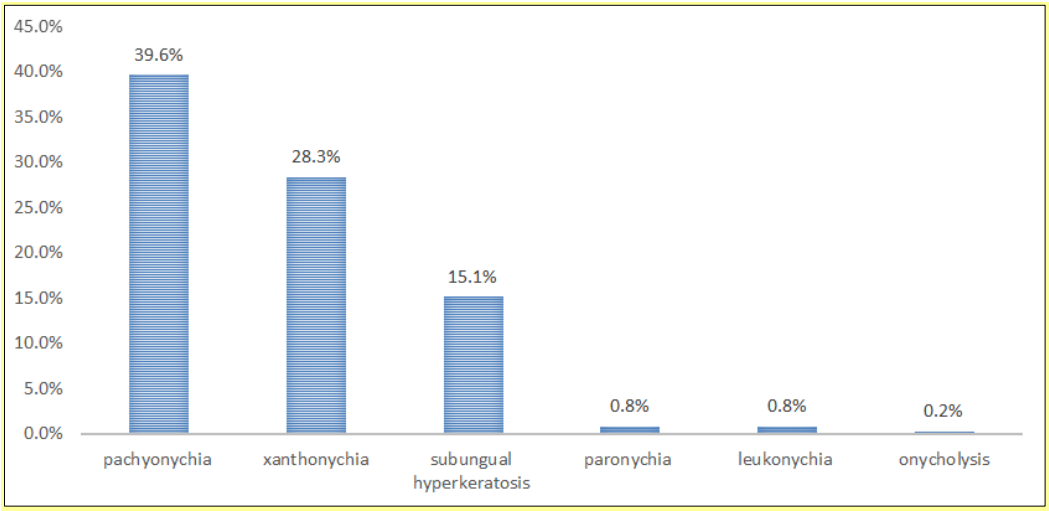

Figure 2: Nail dystrophy aspects in cases of onychomycosis.

The positivity of direct examination and culture is detailed in Table 1. Direct examination was positive

for 74 samples (47.4%). Culture was positive for 67 samples (42.9%). Both direct examination and culture were positive for 53 samples (33.9%).

Table 1: Results of direct examination and culture

	Culture (+)	Culture (-)	Total
Direct examination (+)	53 (34%)	21 (13%)	74 (47%)
Direct examination (-)	14 (9%)	68 (44%)	82 (53%)
Total	67 (43%)	89 (57%)	156 (100%)

Regarding toenails, direct examination was positive in 80% of the samples (32/39: 80%).

Yeasts were observed on direct examination in 71.4% of the fingernail samples (10/14: 71.4%).

In culture, two groups of fungal agents were isolated, among which dermatophytes were predominant with 23 positive cultures for Trichophyton rubrum (43.4%) and 16 for Trichophyton interdigitale (30%).

Yeasts were responsible for 26.4% of diagnosed onychomycoses (14 cases), and non-albicans Candida species were the most commonly isolated and identified (8/14).

Table 2: Distribution of fungi responsible for onychomycoses (n=53)

Fungus		(%)
Dermatophytes (n=39)	*Trichophyton rubrum*	*23 (43%)*
	Trichophyton interdigitale	*16 (30%)*
yeasts (n=14)	*Candida albicans*	*6 (11%)*
	Candida non albicans	*8 (16%)*
TOTAL		*53 (100%)*

DISCUSSION

Onychomycosis is a chronic nail infection, and its prevalence in the general population varies from 2 to 18%. It accounts for 18 to 50% of nail pathologies and constitutes 1.5 to 18% of dermatological consultations [4].

In Morocco, the true prevalence of onychomycosis is certainly underestimated due to the high cost of medical care and the preference for traditional phytotherapy methods, leading to low rates of mycological examinations being prescribed. Consequently, there is a lack of confirmation of mycological etiologies in cases of nail lesions.

Additionally, many patients with onychomycosis are unaware of their condition and its potential complications, leading them to not seek medical attention systematically for painless nail dystrophy.

According to this study, the most represented age group was 51 to 70 years old, with an average age of 56 years. This result is similar to that reported by Ab Youssef in Tunisia, who found a predominance in the same age group (over 60 years old) [5], but differs from the findings of Halim *et al*., [6], (46 years) in Morocco and Kouotou in Cameroon (40.7 years) [7].

Indeed, onychomycosis is more common in the elderly population due to physiological decline in immunity and slowing of circulation. Additionally, difficulty in nail care contributes to fungal colonization [8].

In this study, men were affected in over half of the cases (54.4%), contrary to several studies that have reported a female predominance [5-9]. This difference may be explained by higher exposure of men to factors favoring onychomycosis, such as wearing shoes and prolonged foot maceration.

In this study, as well as in the literature [5-9], onychomycosis predominates in the feet. This is explained by toe contamination from floors contaminated with anthropophilic fungi, moisture, and maceration facilitated by wearing closed shoes. Additionally, the slower nail growth rate on the toes promotes colonization and reduces the elimination of the fungal agent [4].

Out of the 156 patients included in our study, direct examination was positive for 74 specimens (47.4%). Direct microscopy examination is necessary, reliable, and can provide a quick answer to the clinician; indeed, it can refer to the specific fungus by showing fungal hyphae in cases of dermatophytosis or directly visualizing yeasts [10, 11]. However, culture was positive in only 42.9% of cases (67/156 cultures). Culture should systematically be associated with direct examination. Indeed, it is important to isolate and identify fungi in culture, as treatment may differ depending on the species isolated [10].

The diagnosis of dermatophytes has its limitations due to cultures, which can sometimes take 2 to 4 weeks and require subculturing, time, and well-

trained personnel. Studies report the use of PCR for rapid identification of the main species involved in onychopathies, including T. rubrum, M. canis, M. audouinii, and E. floccosum. Other tests are used for direct screening of dermatophytes in collected samples. A specific kit commercialized for the detection of dermatophytes in nails (OnychoDiag® kit) is available, which allows the detection of the dermatophyte but without species identification. The presence of non-pathogenic telluric dermatophytes or those in a simple "colonizer" situation limits the use of this method. These techniques are still in the experimental phase, not validated, expensive, and used only in reference laboratories [10].

Dermatophytes represent the most commonly implicated pathogens in diagnosed onychomycoses, with Trichophyton rubrum as the primary fungal agent responsible for dermatophytic onychomycoses (43.4% of cases). This predominance is similar to that found in Casablanca (75%), [6], Tunisia (96.4%), [5], Senegal (53.6%), [11], and France (85.1%), [4].

The second isolated dermatophyte is T. interdigitale, implicated in 30% of patients. This figure differs from that reported in Casablanca (2%), but is similar to that in Senegal (26.4%) [6-11].

The transmission of these anthropophilic species is ensured by humid floors (showers, swimming pools, and spaces for ablutions in mosques) and the high attendance at public baths, which justifies the frequency of these mycoses among the Moroccan population [6].

Onychomycoses caused by Candida (27%), mainly isolated in the fingers, could be explained by prolonged and repeated contact of the hands with water in certain professions and during household tasks [12].

The non-albicans Candida species were more prevalent (57%) than Candida albicans (43%) in our series. These figures differ from those found in Casablanca (62%), Tunis (57%), and France (71%), where Candida albicans predominated [4-6]. The low number of onychomycoses caused by Candida albicans may be explained by the low number of patients consulting for hand onychomycosis.

Regarding onychomycoses caused by molds and pseudodermatophytes, no cases were recorded in our study.

This could be explained by the difficulty in confirming the pathogenicity of a mold, which relies on the positivity of direct examination and isolation of the same mold in pure culture from two samples taken from the same site.

CONCLUSION

Prevalence of confirmed onychomycosis in our study is 34%, with 47% of patients aged over 50 years, and 54% of patients being male. Trichophyton rubrum is the most isolated dermatophyte, primarily affecting the toes, while candidiasis was more frequent in the fingers.

The role of the Medical Biology Laboratory is crucial in diagnosing the fungal origin of onychodystrophies by isolating and identifying the responsible agents. Faced with various challenges encountered during the precise identification of pathogens, it is necessary to explore alternative approaches such as PCR, which allows for faster and more accurate identification of fungal agents involved in onychomycoses.

In conclusion, this study emphasizes the importance of raising awareness among patients and healthcare professionals about the necessity of mycological diagnosis of onychomycosis at an early stage, before initiating any treatment. There is a need to improve the timeliness, sensitivity, and specificity of mycological diagnosis for the widespread diagnosis and treatment of onychomycoses in the general population, particularly in the elderly, where this fungal infection is common and often neglected.

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