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Original Article

Fungi Spectrum Evaluation, Clinical Features and Risk factors of Egyptian patients with onychomycosis in a tertiary care hospital

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ABSTRACT

Background: An effective treatment for onychomycosis can be achieved by identifying the etiological organism and antifungal susceptibility testing. This study aimed to isolate most prevalent fungi causing onychomycosis, detecting risk factors and clinical features of the disease.

Methods: This cross-sectional prospective clinical study has been performed on 80 patients clinically diagnosed to have onychomycosis. After a careful history taking (age, sex, underlying medical conditions and possible risk factors) a nail sample was obtained and subjected to mycological examination (10% KOH microscopic examination and fungal culture for identification of causative fungi.

Results: The most frequent isolated fungi were non dermatophytes which were isolated from 42.5% of the patients followed by dermatophytes in 37.5% then candida in 7.5% of the patients. The isolated non dermatophytes were A. flavus 10%A.niger10%,A.fumigatus7%,alternaria7%,scopulariopsis7% ,Cladosporium5% , Penicillium 1%. The isolated dermatophytes were T.Rubrum 20%, T.Mentagrophytes 14%, T.interdigitale 2%, E.floccosum 2%, T verrucosum 2%.

Conclusions: The incidence of non-dermatophytes onychomycosis has been increasing recently.
non-dermatophytes are emerging pathogens of onychomycosis among patients attending Zagazig University Hospitals.

Keywords: Fungi spectrum; Prevalence; Onychomycosis

INTRODUCTION

Nail fungus (onychomycosis) is a common infection affecting the nail, its underlying bed, or the area where the nail grows (the matrix) [1]. Its appearance varies considerably, and it can be caused by several different types of fungi, including dermatophytes, non-dermatophytes moulds (NDM), and yeasts. This condition affects a significant portion of the global population, with an estimated prevalence of 5.5%[2].

Distal and lateral subungual onychomycosis (DLSO) is the most frequently observed type of nail fungus, distinguished by its characteristic pattern of spread. Other presentations include white

superficial onychomycosis (WSO), endonyx infection, total dystrophic onychomycosis (TDO), as well as proximal subungual onychomycosis (PSO), with various combinations of these also possible[3].

Although onychomycosis does not pose a direct threat to human life, it is a significant public health concern due to its high prevalence rate and the associated morbidity, including psychological and occupational distress, long-term nail damage, infection transmission, and expensive treatment [3, 4].

Nondermatophyte molds, in particular, are responsible for a rise in the incidence of onychomycosis [5]. The mycoses have been found to be on the rise due to a number of factors, including an aging population, more people taking immunosuppressant drugs, more people suffering from underlying diseases like diabetes and HIV that lower immune systems, more people going to public pools and spas, more people wearing shoes that don't fit properly for fashion, and more people participating in long-distance running events [6, 7].

Dermatophytes, especially *Trichophyton rubrum* and *Trichophyton mentagrophytes*, are the most common culprits in temperate regions [4]. Twenty percent of fungal nail infections are caused by non-dermatophytes [5]. About 10% to 20% of onychomycosis among patients are caused by yeasts, such as *candida species* [6].

The use of molecular biology in the diagnosis of onychomycosis caused by several fungal organisms is on the rise [2,7]. Oral and topical therapy efficacy can vary by species, thus knowing the fungal viability and identifying the causal organism are important preparatory steps [8].

To ensure successful treatment and minimize risks, accurate diagnosis of onychomycosis is paramount[9]. This requires mycological testing to confirm the presence of fungus and identify the specific type. Diagnostic methods include microscopy, fungal culture, histopathology, and molecular techniques, often used in combination to provide the most comprehensive assessment. [2].

Successful treatment of onychomycosis and reduced recurrence rates depend on appropriate medication selection, guided by identification of the causative fungus and antifungal susceptibility testing[10].So, this study aimed to isolate fungi causing onychomycosis and to detect prevalence and risk factors of the disease.

METHODS

This prospective cross-sectional study was conducted at the Outpatient Dermatology, Venereology, and Andrology Clinic and the Medical Microbiology and Immunology Department of Zagazig University Hospitals and Faculty of Medicine, respectively. All participants provided written informed consent. The study protocol was approved by the Zagazig University Faculty of Medicine Research Ethics Committee (ZU-IRB# 9314-9-2-2022) and adhered to the Declaration of Helsinki guidelines.

Patients

This study included eighty patients with a clinical diagnosis of onychomycosis recruited from the Dermatology, Venereology, and Andrology outpatient clinic. Patients were excluded if they had received topical or systemic antifungal therapy within the previous month or had other nail disorders, such as psoriasis or lichen planus.

All patients were subjected to the following:complete history taking, present history (onset, course and duration of nail affection), past history of any systemic or other dermatological diseases, drug intake. Complete general examination was done to exclude any associated medical problems. Dermatological examination: Fingers and toenails were examined to diagnose onychomycosis and exclude other nail disorders as psoriasis and lichen planus. The description included specifics about the clinical subtype and the number of nails that were involved.

A- Collection of nail specimens

Wiping the affected nail and skin surrounding with 70% alcohol removing any debris. The next step was to gather the nail clippings and scrapings using

a sterile scalpel. Specimen in DLSO patients were cut close to the nail bed. Splitting the sample in halves allowed for separate microscopical analysis and culturing. The patient's information (name, age, sex, and time of collection) was labeled on sterile petri dishes before placing the sample between two sterile slides.

B- 10 % KOH examination of nail specimen

The portion of the sample for microscopy was screened with a 10% KOH mount. KOH makes the preparation more sensitive and dissolves keratin more easily to identify any fungal elements (arthrocondia,hyphea). A grease-free glass slide was used to hold two or three droplets of 10 % KOH. The sample was placed in it, then a clean cover slip was placed carefully to avoid formation of air bubbles . After soaking in KOH for 5–8 minutes, the material was examined by light microscope for presence of hyphae and arthroconidia using both low power (10×) and high power (40×) magnification.

C-Fungal culture

The other portion for culture was inoculated on Fungibioticagar medium (Himedia ,USA) at room temperature (25˚C), cultures were incubated aerobically for four weeks. In the first week, the culture was checked daily; thereafter, it was checked twice weekly. If there was no growth after four weeks, the culture was deemed negative.

D- Identification of fungal growth.

Suspected filamentous fungi were identified to the species or genus level using macroscopic and microscopic examination of cultures (including texture, growth rate, morphology, and pigmentation). Yeasts were identified using standard diagnostic methods such as colony morphology and Gram staining.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 23.0. Qualitative data are presented as frequencies and percentages, while quantitative data are presented as mean ± standard deviation and range. Chi-square and Fisher's exact tests were used to analyze associations between qualitative variables. Independent t-tests and Mann-Whitney U tests compared quantitative variables between groups. Kruskal-Wallis or ANOVA tests were used to analyze relationships among multiple quantitative variables. Statistical significance was set at $p < 0.05$.

RESULTS

The studied groups had ages ranged from 14 to 66 years with mean ± SD of 40.6 ± 12.7. (78.8%) of the cases were females and (21.3%) were males. 52 patients were housewives. With respect to the patients' family histories, 33.8% had a positive history of onychomycosis and 66.3% had a negative one (Table 1, Figure 1).

Table (1) shows that the most frequent clinical subtype detected was DLSO (53.8%) of the patients, followed by PSO (32.5%) of the patients, while the least frequent subtype detected was TDO (13.8%) of the patients. As regard previous antifungal treatment, before washout period, 40 patients received systemic antifungals, 2 patients received local antifungals, while 38 patients didn't receive any antifungal treatment. During patient examination, (20%) had eczema, (6.3%) had tinea pedis, while (73.7%) didn't have any skin disease. KOH examination showed 47 positive samples and 33 negatives. While culture results showed 70 positive samples; 34 of them were non dermatophytes, 30 dermatophytes and 6 were candida.

On comparing between clinical types as regard age, sex, KOH examination, culture results and type of isolated fungi, there was no statistically significant difference (P>0.05) (Table 2).

The most frequent isolated fungi were non dermatophytes which were isolated from 42.5% of the patients followed by dermatophytes in 37.5% then candida in 7.5% of the patients. The isolated non dermatophytes were *A. flavus 10% A.niger10%, A.fumigatus7%, alternaria7%, scopulariopsis7% ,Cladosporium5% , Penicillium 1%. The isolated dermatophytes were T.Rubrum*

20%, T.Mentagrophytes 14%, T.interdigitale 2%, E.floccosum 2%, T verrucosum 2% (Table 3).

Table(1): Demographic, clinicaldata,Past history and Association of onychomycosis with risk factors of thestudiedpatients:

Variabes	All patients(n=80)
Age*(years)* • Mean ± SD • Range	 40.6 ±12.7 (14–66)
Sex (N.%) • **Male** • **Female**	 17 (21.3%) 63 (78.8%)
Clinicalsubtype*(N.%)*	
• DLSO	43 (53.8%)
• PSO	26 (32.5%)
• TDO	11 (13.8%)
Previousantifungal treatment*(N. %)*	
• Absent	38 (47.5%)
• Systemicantifungals	40 (50%)
• Local antifungals	2 (2.5%)
Other dermatologicalconditions*(N. %)*	
• Negative	59 (73.7%)
• Eczema	16 (20%)
• Tinea pedis	5 (6.3%)
KOHexamination	
• **Negative**	33 (41.3%)
• **Positive**	47 (58.8%)
Culture results	
• **Negative**	10 (12.5%)
• **Positive**	70 (87.5%)
Typesof isolatedfungi	
• **Negative**	10 (12.5%)
• **Candida**	6 (7.5%)
• **Dermatophytes**	30 (37.5%)
• **Molds**	34 (42.5%)
Medical history	
– *Negative*	*57 (71.3%)*
– *DM*	*11 (13.8%)*
– *HTN*	*10 (12.5%)*
– *IBS*	*1 (1.3%)*
– *RA*	*1 (1.3%)*
– *Cardiac*	*1 (1.3%)*
– *Hepatic*	*1 (1.3%)*
Family history	
– *Negative*	*53 (66.2%)*

Variabes	All patients(n=80)
– *Positive*	*27 (33.8%)*
Association of onychomycosis with risk factors	
Water contact	*60(75%)*
Soil contact	*5(6%)*
Diabetes	*11(13%)*
Trauma	*10(12%)*
Aging	*26(32%)*

**The same patient may have more than one medical presentation*

Table(2): Comparison between clinical subtypes as regard demographic, KOH examination and culture results:

Variables(N.%)	**DLO(n=44)**	**PSO(n=24)**	**TDO(n=12)**	**P value**
Age(years)				
• Mean ± SD • Range	40 ± 12.7 (14– 65)	45.7±11.44 (25–66)	40.4±9.58 (30–62)	0.07
Sex (N.%)				
• Male • Female	7(16.3%) 36(83.7%)	6(23.1%) 20 (76.9%)	4(36.4%) 7(63.6%)	0.28
KOHexamination				
• Negative • Positive	18 (41.9%) 25 (58.1%)	11 (42.3%) 15 (57.7%)	4(36.4%) 7(63.6%)	0.94
Culture results				
• Negative • Positive	5 (9.3%) 39 (90.6%)	4(15.3%) 22 (84.6%)	2(18.2%) 9(81.8%)	0.61
Isolated fungi				
• Negative	5(11.6%)	3(19.2%)	2(18.2%)	
• Candida	3(7%)	2 (7.7%)	1 (9.1%)	0.83
• Dermatophytes	15 (34.9%)	9(34.3%)	6(54.5%)	
• Molds	21 (48.8%)	10 (34.6%)	3(27.3%)	

***[1]One way ANOVA test,[2]Fisher's exact test,[2]Chi-square test, Non-significant: P >0.05, Significant: P ≤0.05**

Table (3): Results of fungalculture amongthe clinicalsubtypes:

Variables(N.%)	**DLO(n=43)**	**PSO(n=26)**	**TDO(n=11)**
Aspergillus Flavus	4(9%)	3(11.5%)	0(0%)
AspergillusFumigatus	3(7%)	2 (7.7%)	0(0%)
Aspergillus Niger	3(7%)	2 (3.8%)	2(18.2%)
Alternaria	5(11.6%)	0(0%)	0(0%)
Candida	3(7%)	2 (7.7%)	1 (9.1%)
Penicillium	1 (2.3%)	0(0%)	0(0%)
Scopulariopsis	2 (4.7%)	3(11.5%)	0(0%)
E. floccusom	2 (4.7%)	0(0%)	0(0%)
T.mentagrophytes	4 (9.3%)	3(11.5%)	3(27.3%)

Variables(N.%)	DLO(n=43)	PSO(n=26)	TDO(n=11)
T. rubrum	6(14%)	7(26.9%)	1 (9.1%)
T.interdigitale	1 (2.3%)	0(0%)	1 (9.1%)
T.verrucosum	2 (4.7%)	0(0%)	0(0%)
Cladosporium	3(7%)	0(0%)	1 (9.1%)
Negative	5(11.6%)	3(11.5%)	2(18.2%)

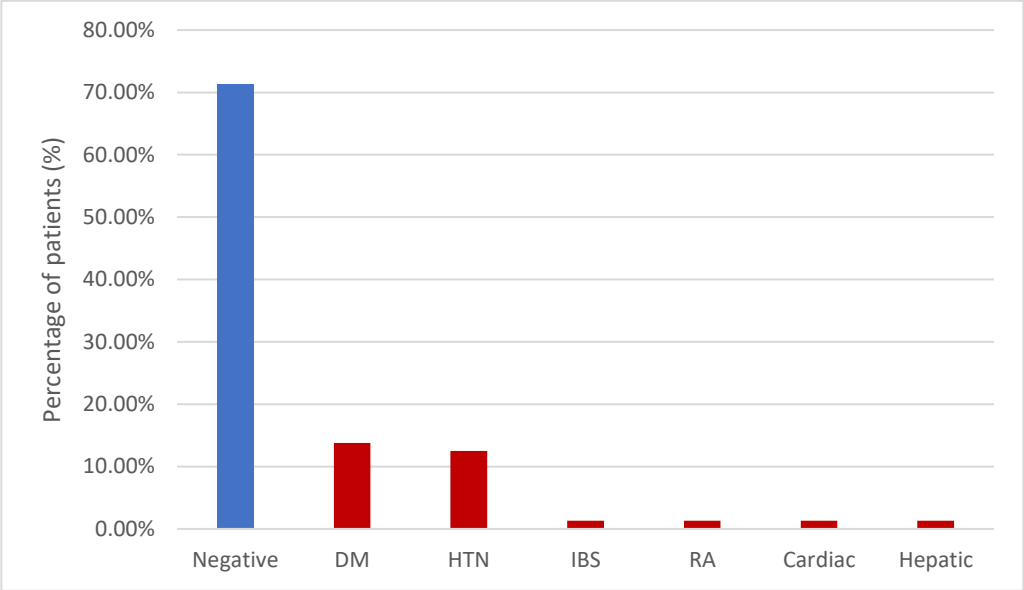

Figure 1: Medical history among the studied patients

DISCUSSION

Onychomycosis treatment shouldn't start without first conducting confirmatory testing. Confirmation can be achieved using histology, fungal culture, or direct microscopy. The infectious organism can be quickly identified using polymerase chain reaction [3]. In order to diagnose onychomycosis, the gold standard is fungal culture, which is currently the most common method for determining the viability of the causing organism [11].

This study aimed to isolate fungi causing onychomycosis and to detect prevalence and risk factors of the disease. Patienׅt's age ranged from 14 to 66 years with a mean age of 40.6 years. Factors such as occupational trauma, increased water exposure, cosmetic concerns among younger patients, and domestic duties may contribute to the higher occurrence of onychomycosis in this age range. This is in accordance with other studies on patients of onychomycosis of the same age group [12, 13, 14, 15, 16].

In the current study, 78.8% of patients were females. Female: male ratio was approximately 4:1. This finding was in line with Haghani et al. [13], Abu El hamd et al. [14] Nada et al. [15], who also reported a higher incidence in female than male. In contrast, Kaur et al. [17], Neupane et al. [18] and DAS et al. [19] reported that male outnumbered female. The higher incidence in female is more likely due to exposure to water and chemical detergents during daily domestic works and household responsibilities [20]. In our study 13% of patients were diabetics Because of its effects on microcirculation, diabetes increases the risk of onychomycosis [21].

This study found that onychomycosis developed in 10% of cases following trauma, despite trauma being a widely recognized risk factor for fungal nail infections.The precise mechanism remains unclear, but reduced blood flow to areas with traumatic neurovascular damage is a potential explanation for the observed impaired immune response to fungal infections in these regions[22].

Regarding clinical types of onychomycosis, the most common clinical pattern of onychomycosis is DLSO 43 (53.8%) followed by PSO 26 (32.5%),

followed by TDO 11 (13.8%). This is in accordance with Haghani et al. [13], Nada et al. [15], El Nagar et al. [23], and DAS et al. [19] all reported that DLSO was the most common clinical presentation followed by TDO**.** DLSO is the most common variety of onychomycosis, it is easily acquired as it is caused by distal invasion of nail bed and underside of nail plate [17].

For KOH examination & culture, there were 58.7% of patients with positive KOH and 87.5% of patients with positive culture results for fungi. Shenoy et al. [21] reported that 53% of cases were KOH positive and 35% were culture positive. Haghani et al. [13] reported that the positive rates of KOH preparation, fungal culture were 59.5%, 66.9%, respectively.Nada et al. [15] reported KOH positive in 52.5% of the patients and 75% were culture positive. Abu El Hamd. [14]. reported 77.9% were KOH positive and 80.9% were culture positive.

The variability between KOH & culture results in different studies can be attributed to multiple causes. KOH examination is a test of low sensitivity & high specificity, this can lead to some false negative results. Along with improper sample collection, scarce fungal spores in the sample or irregular fungal distribution in the lesion [23].

In the current study, as regard type of fungi isolated; the most frequent isolated fungi were non dermatophytes which were isolated from 42.5% of the patients followed by dermatophytes in 37.5% then candida sp. in 7.5% of the patients.

Several studies have reported the high prevalence of NDM onychomycosis compared to the dermatophytes [12,16]. In contrast**,** Nada et al. [15] reported 10% had dermatophytes, 3% had non-dermatophytes and 86 % had candida. Motamedi et al. [24] reported that dermatophytes accounted for 35.8% of cases, yeasts for 32.7%, NDMS for 29.3%, and mixed infections for 2.2%.

Potential risk factors for the rise in NDMS include the following: immunosuppression, chemotherapy, aging, occupational accidents, metabolic diseases, debilitating diseases, and the broad-spectrum antibiotics used by several individuals [25]. Not only does NDMS-induced nail infections create pain and anguish for patients, but antifungal medication often fails to alleviate their symptoms [26].

In the current study, the isolated non dermatophytes were *A. flavus* 10% *A. niger*10%, A. *fumigatus* 7%, *Alternari*a sp.7%, *Scopulariopsis sp.* 7%, *Cladosporium sp.* 5%, *Penicillium sp.* 1%.

El Nagar et al. [23] reported that The most prevalent fungal isolates among NDMS samples were

A. *fumigatus*, *A. niger*, and *A. flavus*. Haghani et al. [13] reported that *A. terreus*, *A. flavus*, *A niger*were the most frequently isolated non dermatophytes.

In the current study, the isolated dermatophytes were *T.Rubrum* 20%, *T.Mentagrophytes* 14%, *T.interdigitale*2%, *E.floccosum* 2%, *T verrucosum* 2%.Bueno et al. [26] reported that Two dermatophytes, *T. rubrum* and *T. mentagrophyte*s, were isolated more frequently than any other types. Halvaee et al. [16] reported that *T. mentagrophytes* was the most frequently isolated dermatophyte (48.9%) in their study, followed by *T. rubrum* (42.2%), and T. verrucosum (8.9%).

Namidi et al. [27] reported that the dermatophytes isolated were *T. rubrum 3*5%, *T.mentagrophyt*e 25%, *T.tonsurans*16% , *M .gypseum*10%,*T.verrucosum* 6%.

Because of regional differences in climate, the onychomycosis-causing agents vary with each area. The most common NDMS producing onychomycosis in Turkey were *fusarium spp.*, *acremonium spp.*, and*scopulariopsis spp*., but in Sri Lanka the most common fungal isolate was *Alternaria alternaea*, followed by *Cladosporium spp*. [28].

Possible explanations for the observed discrepancy in onychomycosis clinical patterns and mycological features between countries include variations in environmental factors, socioeconomic status, evaluation methods, and cutaneous fungal pathogen virulence [13].

Only by obtaining a culture-based diagnosis can we ensure the sensitivity and specificity of direct microscopy with simple KOH, identify the pathogen species, and conduct antifungal susceptibility testing to commence targeted antifungal therapy.

Limitations

Our study has a few limitations including that our results may not be applicable to a broader

population due to the small sample size (80 cases).Antifungal susceptibility pattern of dermatophytes isolated from clinically suspected cases of onychomycosis could be better understood with a bigger and more diversified cohort.

CONCLUSIONS

The incidence of non-dermatophytes onychomycosis has been increasing recently, non-dermatophytes are emerging pathogens of onychomycosis among patients attending Zagazig University Hospitals.

Conflict of Interest or financial disclosure: No potential conflict of interest to be reported by the authors.

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Citation

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