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A cross sectional study to observe the diversity of fungal species in Onychomycosis isolated from a tertiary care hospital in Karachi.

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Abstract

Objective: To study the frequency and diversity of fungi involved in onychomycosis.

Method: The cross sectional study was conducted from February 2018 to February 2019 at the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, and the University of Karachi. Specimens were taken from patients suffering with onychomycosis attending the Dermatology Department of Jinnah Postgraduate Medical Centre and were processed for the isolation of fungi. Specimens were processed for the diagnosis by potassium hydroxide mount for microscopic evaluation and mycological culture to determine the type of fungus involved in onychomycosis. Fungi were isolated using Sabouraud Dextrose Agar with antibiotics cycloheximide and chloramphenicol, and without antibiotics. Plates were observed for the growth

periodically until 4th week. Appearance of any colony was studied further for identification on the basis of cultural characteristics and microscopy. Precise identification of *Candida* species was done.

Results: Of the 230 samples, 85(36.96%) were from males and 145(63.04%) from females. Potassium hydroxide mount showed fungal element in 134(58.26%) specimens, while in 111(48.26%) patients the fungal culture was positive. Nine (3.9%) cases were culture-positive but potassium hydroxide-negative, while among the 134(58.26%) potassium hydroxide-positive cases, 32(23.9%) were culture-negative.

Key Words: *Candida*, Dermatophytes, Fungal infections of nail, KOH mount, Non-dermatophyte molds, Onychomycosis, *Tinea unguium*.

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41 Introduction

Onychomycosis is a fungal disease of nail plate caused by either dermatophytes or non-dermatophytes. When the onychomycosis is due to invasion by dermatophytes, the condition is termed "tinea unguium"¹. Among all the nail diseases, proportion of onychomycosis is 50%, and while just 5% of the population suffers from this disease², in old age the occurrence is as high as up to 48%³. It affects the quality of life, as the nail is disfigured and the patient feels discomfort which ultimately influences the patient's physical and psychological health¹.

Determining factors for prevalence of the disease include age, social status, nature of job and environmental conditions⁴. With increasing age, the peripheral circulation becomes poor, and chance of diabetes increases. In addition, growth rate of the nail slows down and consequently the exposure of nail to pathogenic fungi is increased. Low immunity at old age is also a factor and there is increased chance of onychomycosis in the elderly if sufficient hygiene is not maintained⁵.

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Among the dermatophytes, the common organisms involved in onychomycosis are *Trichophyton* (T.) *rubrum*, *T. mentagrophytes* and *Epidermophyton* (E.) *floccosum*. Non-dermatophytic molds (NDMs) include *acromonium*, *alternaria*, *aspergillus*, *botryodiplodia*, *fusarium*, *onychochola canadensis*, *scytalidium dimidiatum*, *scytalidium hyalinum*, *geotrichum candidum*, *cladosporium carrionii*, and *scopulariopsis brevicaulis*. Among yeasts, *Candida* (C.) *albicans* is the main pathogen responsible for onychomycosis, while less frequent are *C. krusei*, *C. parapsilosis*, *C. glabrata*, *C. guilliermondii*, *C. tropicalis* and *blastoschizomyces capitatus*⁶.

In the local setting, the most common pathogen isolated in Lahore, Pakistan, was *Candida* spp. (46%), followed by dermatophytes (43%) which include *T. rubrum*, *T. violaceum*, *T. mentagrophytes*, *T. tonsurans* and *E. floccosum*. NDMs were isolated in 11% of cases, including *Fusarium* spp., *Aspergillus* spp., *scopulariopsis brevicaulis*, *Acromonium* spp., *scytalidium dimidiatum* and *Alternaria* spp. Yeast and dermatophytic infections were not significantly different. Both yeasts and dermatophytes were significantly more prevalent than NDMs⁷.

In India, NDMs predominated (55%) and included *Aspergillus* (A.) *niger*, *A. flavus*, *Mucor* spp., *alternaria alternata*, *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Bipolaris* spp., *A. fumigatus* and *Curvularia* spp. Amongst the dermatophytes, *T. verrucosum* and *T. rubrum* were commonly isolated followed by *T. schoenleinii*, *T. violaceum*, *Microsporum* spp and *T. mentagrophytes*. Amongst the yeasts, *C. albicans* was the most commonly isolated species⁸.

Dermatophytes get involved in superficial keratinised structures (nails, hair and skin) and do not penetrate deeper tissues. Hyphae of the organism penetrate the stratum corneum, secrete keratinolytic enzyme and thereby enter into the living cells⁶.

Apparently onychomycosis seems like a cosmetic problem, but in old patients it may get complicated with cellulitis, while in diabetics there may be foot ulcers⁹. The aim of treatment is to improve the shape of the nail and to avoid complications. Many drugs cannot penetrate the layer of keratin, and therefore it is very difficult to achieve the target. The choice of treatment depends on the severity of disease, type of organism involved, adverse effects of the drugs and their interactions¹⁰. Commonly, topical or oral antifungal medications are used. Different topical medicines, such as ciclopirox 8% nail lacquer, tavaborole, amorolfine or efinaconazole, are prescribed for onychomycosis¹¹. Oral medications include terbinafine, itraconazole and fluconazole. These antifungals penetrate the nail bed promptly and persist there for months after discontinuation of therapy¹². Comparatively, terbinafine has fewer side effects than itraconazole. Ketoconazole is not recommended orally due to its side effects¹³. The current study was planned to observe the diversity of fungi involved in onychomycosis.

Materials and Methods

The cross-sectional study was conducted at the Department of Microbiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, and the University of Karachi from February 2018 to February 2019. Specimens were taken from patients suffering with a clinical diagnosis of onychomycosis attending the Dermatology Department of JPMC and were processed for the isolation of fungi.

After approval from the Pakistan Medical Association (PMA) ethics review board, the sample size was calculated using OpenEpi calculator¹⁴ at confidence level 95% and bound of error 5% and taking relevant references from literature.¹⁵

All patients with a clinical diagnosis of onychomycosis, irrespective of age and gender, were included, while all lesions other than onychomycosis were excluded.

The patients were also categorised into socioeconomic status (SES) as poor, average or fair, according to the Demographic and Health Survey 2012-13¹⁶.

Specimens were processed for diagnosis by potassium hydroxide (KOH) mount for microscopic evaluation and mycological culture to determine the type of fungus involved in onychomycosis. Fungi were isolated by spreading the specimen on Sabouraud Dextrose Agar (SDA) plated with cycloheximide and chloramphenicol antibiotics, and without antibiotics. The plates were observed for the growth periodically for 4 weeks. Appearance of any colony was studied further for identification on the basis of cultural characteristics and microscopy. For observing the microscopic features of the isolates, lactophenol cotton blue stain (LPCB) was used. Precise identification of candida species was done using API 20C AUX system (Bio Merieux, USA).

Results

Of the 230 samples, 85(36.96%) came from males and 145(63.04%) from females (Table 1).

The age of the subjects ranged from 11 to 75 years; and the highest number of patients 77(33.5%) were aged 31-40 years (Table 2).

KOH mount showed fungal element in 134(58.26%) specimens, while in 111(48.26%) patients the fungal culture was positive (Table 3). Nine (3.9%) cases were culture-positive but KOH-negative, while among the 134(58.26%) KOH-positive cases, 32(23.9%) were culture-negative (Table 4).

Of the 111 fungi isolated, 44(39.64%) were dermatophytes, 26(23.42%) NDMs and 41(36.94%) were yeast belonging to *Candida* species. Among dermatophytes, *T. rubrum* 26(59%), *T. mentagrophytes* 7(16%), *T. tonsurans* 4(9%) *T. soudanense* 1(2.27%), *T. schoenleinii* 1(2.27%), *T. verrucosum*

1(2.27%) and epidermophyton 4(9%) were isolated. Among the NDMs, there were *Fusarium* spp. 7(27%), *aspergillus* 6(23%), *Cephalosporium* spp. 4(15.38%), *Penicillium* spp. 4(15.38%), *Alternaria* spp. 2(7.69%), *Cladosporium* spp. 2(7.69%) and *Chrysosporium* spp. 1(3.84%). Among the *Candida* species, there were 27(65.85%) *C. albicans* and 14(34.14%) non-*albicans*, including *C. tropicalis* 10(71.42%), *C. parapsilosis* 3(21.42%) and *C. glabrata* 1(7.14%).

Overall, 81(35.21%) samples came from the dermatology clinic within 6 months of the disease; 70(30.43%) patients consulted dermatologist in 6-12 months of the symptoms 43(18.69%) waited 1-3 years; 17(7.39%) took 3-5 years; and 19(8.26%) took >5 years to consult a dermatologist.

In terms of SES, 181(78.69%) belonged to the poor class, 45(19.56%) to average class, and 4(1.74%) belonged to the fair class. Of the 111 culture-positive specimens, 95(85.58%) came from patients of poor class and 16(14.41%) from individuals of the average class. No samples belonged to a patient of the fair SES.

Overall, 25(10.86%) patients were diabetics. The occurrence of culture-positive specimen among the diabetics was 23(92%). Among the diabetics, 11(47.82%) showed growth of *Candida* spp., 7(30.43%) dermatophytes and 5(21.73%) NDMs.

Discussion

Onychomycosis occurs in all ages but children have infection rates lower than the adults¹⁷. Female preponderance was observed in our study. In contrast, other studies^{1,18} showed a male preponderance. One study¹⁹ reported female preponderance of 80% which was higher than the current study.

The reason for the female preponderance in our study can be linked to multiple factors, such as majority of the patients belonging to low SES and unable to

170 afford the treatment. Most of the male patients attended the dermatology clinic
171 after a long duration of the disease when they had some secondary infection.
172 In the current study the age ranged 11-75 years with a mean of 36.08 years and
173 a median of 35 years. One study¹ observed that among 200 patients suffering
174 from the disease, none was <20 years and almost 24% were aged ≥ 70 years.
175 Age-related incidence of onychomycosis has also been reported⁵ that could be
176 related to various physiological problems faced by the elderly.
177 The current study had most of the patients belonging to the poor community. In
178 old age, patients are usually dependent on children/siblings. Treatment in such
179 cases is sought if there is any other complaint along with onychomycosis.
180 Therefore, the data of old patients attending dermatology clinics may not be a
181 true reflection of the number of cases in the community.
182 In the current study, five patients aged up to 12 years were found to be very
183 poor and were not attending any school, roaming around most of the time bare-
184 footed. A study¹⁷ has also reported prevalence of molds, particularly *T. rubrum*,
185 in such cases.
186 Dermatophytes were less in number than NDMs in the current study, while
187 yeasts were more frequently isolated amongst non-dermatophytes than molds.
188 One study²⁰ reported dermatophytes in 50%, yeast in 25.55% and mold in
189 24.45% of its sample.
190 Since onychomycosis requires long-term antifungal therapy, therefore, it is
191 imperative to have precise and rapid diagnostic methods to avoid side effects of
192 the drugs. While comparing the results of KOH mount with culture positive
193 results, it inferred that more samples could be diagnosed by KOH method
194 (58.26%) than obtaining fungal cultures (48.26%), which was in line with
195 earlier findings²⁰. Indeed several studies^{2, 15, 18} also reported variation in culture
196 positivity with a consistent sensitivity of KOH mount.²¹ In our study, laboratory
197 evidence of fungal infection was obtained in 143 samples by at least one of
198 these methods. Using this as the denominator, KOH mount had a sensitivity of

93% while mycological culture had 78% sensitivity. A slight variation to this data has been reported in literature^{22, 23}.

In the current study, 25(10.86%) patients were diabetics, and 23(92%) of them were culture-positive which accounted for 10% of the total cases. This reflects that although diabetes is not a causative factor for the disease, there is association between the two. *Candida* species were most frequent organisms (47.83%) isolated from these 23 culture-positive cases, amongst which *C. albicans* was the most predominant. A study²⁴ found a higher coincidence (22.9%) of diabetes in cases of onychomycosis, and reported a higher occurrence of *C. albicans* which is in accordance with the current study.

Conclusion

There was a high prevalence of dermatophytes, followed by *Candida* species whose involvement was much higher in diabetics. Antifungal drugs should be started, but it is necessary to diagnose the infection with some laboratory evidence due to a significant proportion of non-*albicans* *Candida* species. Their treatment regime is different from the traditional treatment of *C. albicans*.

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Table 1: Gender-wise distribution of patients and the proportion of culture-positive sample with that of culture-negative samples

Sex	Total number (n=230)	Percentage %	Culture positive cases (n=111)	Percentage %	Culture negative cases (n=119)	Percentage %
Male	85	36.96 %	36	32.43 %	49	41.18%
Female	145	63.04 %	75	67.57 %	70	58.82%
Total	230	100 %	111	100 %	119	100%

Table 2: Distribution of patients of onychomycosis with respect to age

Age	Total number (n=230)	Percentage %	Culture positive cases (n=111)	Percentage %	Culture negative cases (n=119)	Percentage %
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11-20 years	26	11.30 %	15	13.51 %	11	9.24%
21-30 years	56	24.35 %	21	18.92 %	35	29.41%
31-40 years	77	33.48 %	32	28.83 %	45	37.82%
41-50 years	41	17.83 %	28	25.23 %	13	10.92%
51-60 years	21	9.13 %	9	8.11 %	12	10.09%
> 60 years	9	3.91 %	6	5.40 %	3	2.52%
Total	230	100 %	111	100 %	119	100 %

Table 3: Proportion of culture-positive and potassium hydroxide (KOH) positive specimen

KOH mount	Number	Percentage %	Fungal Culture	Number	Percentage %
Positive	134	58.26 %	Positive	111	48.26%
Negative	96	41.74 %	Negative	119	51.74 %
Total	230	100 %	Total	230	100 %

Table: 4: Association between potassium hydroxide (KOH) mount and fungal culture: (n=111)

	TOTAL	Total Culture Positive (111)	% Positive (n=111)
KOH Positive Culture –(ve)	32	102	91.89 %
KOH Positive Culture +(ve)	102		
KOH Negative Culture –(ve)	87	9	8.11 %
KOH Negative Culture +(ve)	9		
Total	230	111	100 %