RESEARCH ARTICLE

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

Raziuddin Ahmed[1], Bahram Khan Khoso[2], Fakhur Uddin[3], Nida Rehman[4], Muhammad Sohail[5], Saleem Hafiz[6]

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.

Keywords: Candida, Dermatophytes, Fungal infections of nail, KOH mount, Non-dermatophyte molds, Onychomycosis, Tinea unguium. (JPMA 71: 51; 2021) **DOI: https://doi.org/10.47391/JPMA.390**

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 disease,[2] 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 health.[1]

Determining factors for prevalence of the disease include age, social status, nature of job and environmental conditions.[4] 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 maintained.[5]

Among the dermatophytes, the common organisms involved in onychomycosis are *Trichophyton (T.) rubrum, T. mentagrophytes and Epidermophyton (E.) floccosum*. Non-*dermatophytic molds (NDMs) include acremonium, alternaria*, aspergillus, botryodiplodia, fusarium, *onycochola* 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. glabarata, 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, Acremonium* spp., *scytalidium dimidiatum* and *Alternaria* spp. Yeast and dermatophytic infections were not significantly different. Both yeasts and dermatophytes were significantly more prevalent than NDMs.[7]

[1]Department of Pathology, SAHARA Medical College, Narowal, Karachi, Pakistan; [2]Department of Dermatology, Jinnah Postgraduate Medical Centre, Jinnah Sind Medical University, Karachi, Pakistan; [3]Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan; [4,5]Department of Microbiology, University of Karachi, Pakistan; [6]Department of Microbiology, Sind Institute of Urology and Transplant, Dow Medical College and Civil Hospital Karachi, Pakistan.

Correspondence: Muhammad Sohail e-mail: msohail@uok.edu.pk

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. verrucossum* 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 species.[8]

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 cells.[6]

Apparently onychomycosis seems like a cosmetic problem, but in old patients it may get complicated with cellulitis, while in diabetics there may be foot ulcers.[9] 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 interactions.[10] Commonly, topical or oral antifungal medications are used. Different topical medicines, such as ciclopirox 8% nail lacquer, tavaborole, amorolfine or efinaconazole, are prescribed for onychomycosis.[11] Oral medications include terbinafine, itraconazole and fluconazole. These antifungals penetrate the nail bed promptly and persist there for months after discontinuation of therapy.[12] Comparatively, terbinafine has fewer side effects than itraconazole. Ketoconazole is not recommended orally due to its side effects.[13]

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 JMPC 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 calculator[14] 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 2012-13.[16]

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 he 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,

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

Sex	Total (n=230) [n (%)]	Culture positive cases (n=111) [n (%)]	Culture negative cases (n=119) [n (%)]
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 (n=230) [n (%)]	Culture positive cases (n=111) [n (%)]	Culture negative cases (n=119) [n (%)]
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	n (%)	Fungal Culture	n (%)
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.

	TOTAL	Total Culture Positive (n=111) [n (%)]
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)

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. glabarata* 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 adults.[17] Female preponderance was observed in our study. In contrast, other studies[1,18] showed a male preponderance. One study[19] 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 afford the treatment. Most of the male patients attended the dermatology clinic after a long duration of the disease when they had some secondary infection.

In the current study the age ranged 11-75 years with a mean of 36.08 years and a median of 35 years. One study[1] observed that among 200 patients suffering from the disease, none was <20 years and almost 24% were aged >70years. Age-related incidence of onychomycosis has also been reported[5] that could be related to various physiological problems faced by the elderly.

The current study had most of the patients belonging to the poor community. In old age, patients are usually dependent on children/siblings. Treatment in such cases is sought if there is any other complaint along with onychomycosis. Therefore, the data of old patients attending dermatology clinics may not be a true reflection of the number of cases in the community.

In the current study, five patients aged up to 12 years were found to be very poor and were not attending any school, roaming around most of the time bare-footed. A study[17] has also reported prevalence of molds, particularly T. rubrum, in such cases.

Dermatophytes were less in number than NDMs in the current study, while yeasts were more frequently isolated amongst non-dermatophytes than molds. One study[20] reported dermatophytes in 50%, yeast in 25.55% and mold in 24.45% of its sample.

Since onychomycosis requires long-term antifungal therapy, therefore, it is imperative to have precise and rapid diagnostic methods to avoid side effects of the drugs. While comparing the results of KOH mount with culture positive results, it inferred that more samples could be diagnosed by KOH method (58.26%) than obtaining fungal cultures (48.26%), which was in line with earlier findings.[20] Indeed several studies[2,15,18] also reported variation in culture positivity with a consistent sensitivity of KOH mount.[21] In our study, laboratory evidence of fungal infection was obtained in 143 samples by at least one of 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 study[24] 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-albican *Candida* species. Their treatment regime is different from the traditional treatment of *C. albicans*.

Disclaimer: None.

Conflict of interest: None.

Source of Funding: None.

References

1. Elewski BE. Onychomycosis: Pathogenesis, Diagnosis, and Management. Clin Microbiol Rev. 1998; 11:415-29.
2. Kaur R, Panda PS, Khan S. Non dermatophytic molds causing onychomycosis: a rising trend in North India. Int J Community Med Public Health. 2017; 4:4532-7.
3. Emam SM, Abd El-salam OH. Real-time PCR: A rapid and sensitive method for diagnosis of dermatophyte induced onychomycosis, a comparative study. Alexandria J Med. 2016; 52: 83-90.
4. Williams HC. The epidemiology of onychomycosis in Britain. Br J Dermatol. 1993; 129:101-9.
5. Elewski BE, Charif MA. Prevalence of onychomycosis in patients attending a dermatology clinic in north-eastern Ohio for other conditions. Arch Dermatol. 1997; 133:1172-3.
6. Kaur R, Kashyap B, Bhalla P. Onychomycosis-epidemiology, diagnosis and management. Indian J Med Microbiol. 2008; 26:108-16.
7. Bokhari MA, Hussain I, Jahangir M, Tahir, Haroon S, Aman S, et al. Onychomycosis in Lahore, Pakistan. Int J Dermatol; 1999; 38:591-5.
8. Kaur R, Panda PS, Khan S. Non dermatophytic molds causing onychomycosis: a rising trend in North India. Int J Community Med Public Health. 2017; 4:4532-7.
9. Boyko EJ, Ahroni JH, Cohen V, Nelson KM, Heagerty PJ. Prediction of diabetic foot ulcer occurrence using commonly available clinical information: the Seattle Diabetic Foot Study. Diabetes Care. 2006; 29:1202-7.
10. Dyanne P. Westerberg, Michael J. Voyack, Onychomycosis: Current Trends in Diagnosis and Treatment. Am Fam Physician. 2013; 88:762-70.
11. James Q. Del Rosso. The Role of Topical Antifungal Therapy for Onychomycosis and the Emergence of Newer Agents. J Clin Aesthet Dermatol. 2014; 7:10-8.
12. Elewski BE, Hay RJ. "Update on the management of onychomycosis: highlights of the Third Annual International Summit on Cutaneous Antifungal Therapy." Clin Infect Dis. 1996; 23: 305-13.
13. "Nizoral (ketoconazole) Oral Tablets: Drug Safety Communication - Prescribing for Unapproved Uses including Skin and Nail Infections Continues; Linked to Patient Death". FDA. 19 May 2016.
14. Dean AG, Sullivan KM, Soe MM. Open Epi: Open Source Epidemiologic Statistics for Public Health, Version. [Online] [Cited 2017 April 04]. Available from: URL: www.OpenEpi.com.
15. Aggarwal P, Kashyap B, Kalita JM. A study to compare and classify etiological agents of onychomycosis. Indian J Med Special. 2017; 8:150-4.
16. Demographic and Health Survey 2012-13 by National Institute of Population Studies (2013) with Measure DHS, ICF International and Claverton, Maryland, USA. [Online] [Cited 2020 April 30]. Available from: URL: https://dhsprogram.com/pubs/pdf/fr290/fr290.pdf
17. Gupta AK, RG Sibbald, Lynde CW, Hull PR, Prussick R, Shear NH, et al. Onychomycosis in children: prevalence and treatment strategies. J Am Acad Dermatol. 1997; 36: 395-402.
18. Cohen JL, Scher RK, Pappert AS. The nail and fungus infections In: Cohen JL, Scher RK, Pappert AS, Elewski B, eds. Cutaneous fungal infections. New York: Lgaku-Shoin; 1992, pp-106-22.
19. Gelotar P, Vachhani S, Patel B, Makwana N. The Prevalence of Fungi in Fingernail onychomycosis. J Clin Diagn Res. 2013; 7:250-2.
20. Gianni C, Valeria Morelli, Amilcare Cerri, Consuelo Greco, Patrizia Rossini, Antonio Guiducci, et al. Usefulness of Histological Examination for the Diagnosis of Onychomycosis. Dermatology. 2001; 202:283-8.
21. Nargis T, Shenoy MM, Bejai V, Gopal V. A simple but yet novel stain in the diagnosis of onychomycosis. Indian J Dermatopathol Diagn Dermatol. 2018; 5:39-41.
22. Weinberg JM, Koestenblatt EK, Tutrone WD, Tishler HR, Najarian L. Comparison of diagnostic methods in the evaluation of onychomycosis. J Am Acad Dermatol. 2003; 49:193-7.
23. Haghani I, Shokohi T, Hajheidari Z, Khalilian A, Aghili SR. Comparison of diagnostic methods in the evaluation of onychomycosis. Mycopathologia. 2013; 175:315-21.
24. Chadeganipour M, Mohammadi R. Causative Agents of Onychomycosis: A 7-Year Study. J Clin Lab Anal. 2016; 30:1013-20.