

Spectrum of fungi causing onychomycosis in a tertiary care hospital in Bangalore

Anjana Gopi¹, Swati Jain^{2*}, Faiza Samreen³

¹Professor & HOD, ^{2,3}Junior Resident, Dept. of Microbiology, KIMS, Bangalore

***Corresponding Author:**

Email: drswati_jain@gmail.com

Abstract

Introduction: Onychomycosis is a fungal infection of nails caused by dermatophytes, yeasts or non-dermatophyte moulds and represents about 30% of mycotic cutaneous infections. Onychomycosis manifests itself in various forms, notably onychodystrophy, onycholysis, subungual hyperkeratosis, or nail-plate discoloration. The objective of this study was isolation and speciation of various fungi by culture and to determine its prevalence.

Materials and Method: The study was conducted from Jan 2014 - June 2016 at a tertiary care hospital, Bangalore. Nail-clipping and subungual debris of clinically suspected patients were subjected to Potassium hydroxide (KOH) preparation. Culture was done on Sabouraud's dextrose agar (SDA) media. Species identification was done by colony character, pigment production, Lactophenol cotton blue (LPCB) staining, some special tests like germ tube test, inoculation into chrome agar in case of *Candida* and slide culture wherever necessary.

Result: Out of 400 cases, the KOH preparation was positive for fungal elements in 288 (72%) cases and culture grew fungi in 268 (67%) cases. Among those 268 cases, the infective fungal agents were predominantly dermatophytes (59.7%) and the rest were due to moulds (22.38%) and yeasts (17.91%). Among the different species, *Trichophyton rubrum* (T. rubrum-37.5%) accounted for the majority of dermatophytes, *Fusarium* (60%) was the commonest mould and *Candida albicans* (37.5%) the predominant yeast.

Conclusion: Direct microscopy and culture are extremely important in the diagnosis of onychomycosis and dermatophytes are the most common etiological agent in our region.

Keywords: Dermatophytes, KOH, LPCB, Onychomycosis

Introduction

Onychomycosis is defined as fungal infection of the nail units caused by dermatophytes, non-dermatophytic moulds or yeasts.⁽¹⁾ It may involve any component of the nail unit including the nail matrix, nail bed or nail plate.⁽²⁾ It accounts for almost 50% of all nail diseases and about 30% of mycotic cutaneous infections and is one of the most common causes of deformed nails.⁽³⁾

Dermatophytes are able to invade normal keratin while *Candida* species attack the soft tissues surrounding the nail and penetrate the keratin secondarily whereas moulds are opportunists and infect the keratin primarily. Moisture and warmth favours infection with dermatophytes.⁽⁴⁾

On the basis of types of lesions produced, classification of onychomycosis includes distal and lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), proximal subungual onychomycosis (PSO) and total dystrophic onychomycosis (TDO).⁽⁵⁾

Nail changes in onychomycosis can occur in various forms, like, onychodystrophy, onycholysis, subungual hyperkeratosis, discoloration or thickening of nail plate.⁽⁶⁾

Though not life threatening, it can cause pain, discomfort and disfigurement. It is worldwide in distribution. Prevalence rates range from 2%-3% in temperate climates to 12% in tropical climates. Prevalence rates in children are 30 times less than

adults, ranging from 0%-0.2%.⁽²⁾ This disease is more frequent among men than women.⁽⁷⁾

Several risk factors for onychomycosis have been identified, including older age, tinea pedis, cancer, psoriasis, immunodeficiency, swimming, diabetes and smoking.⁽⁸⁾ Predisposing factors include nail trauma, male gender, hyperhidrosis, peripheral vascular disease and poor hygiene.⁽²⁾

This study was undertaken to know the incidence of age, sex, distribution in finger/toenail or both and identification and speciation of fungus by KOH wet mount and culture.

Objectives

1. To correlate clinically suspected cases of onychomycosis with direct microscopy and culture.
2. To determine the age group, gender and finger/toe nail involvement.
3. Isolation and speciation of different fungal species based on culture.
4. To determine the incidence of dermatophytes, *Candida* and other non-dermatophytic moulds in the causation of onychomycosis.

Materials and Method

This study was conducted from January 2014 to June 2016 in South India, Bangalore. All the clinically suspected cases were subjected to mycological work-up.

The patients were off antifungal treatment for two to four weeks before collecting the sample.⁽⁹⁾

Nail clippings and undersurface scrapings were collected after thorough cleaning the affected nail with 70% ethanol. Each specimen was divided into two parts, one for KOH wet mount and the other for fungal culture.

Direct microscopic examination was done using 40% KOH and was looked for any fungal filaments and arthrospores.

Two different sets of SDA media were used for culture, one tube with chloramphenicol 50 mg/L and cycloheximide 500 mg/L and the second tube with only chloramphenicol. The culture tubes were incubated at 25°C (room temperature) and observed for growth for a period of 4-6 weeks.

Speciation was done by colony morphology, pigment production if any, LPCB mount and some special tests like germ tube test and inoculation into chrome agar in case of *Candida* & slide culture wherever morphological identification was in doubt.

Results

In the present study, totally 400 clinically suspected cases of onychomycosis were studied during the period of January 2014 to June 2016.

Of these 400 cases, 208 (52%) were male and 192 (48%) were female patients, male to female ratio being 1.08:1. (Fig. 1)

The commonest age group was 25-45 years. Infections were less common below the age group of 15 years. (Fig. 2)

The toenails were more frequently involved i.e., 280 (70%) cases followed by fingernails i.e., 100 (25%) cases and both toe and finger nails in 20 (5%) cases, toenail to fingernail ratio being 2.8:1. (Fig. 3)

Out of 400, 288 (72%) cases were positive for fungal filaments by KOH preparation and 268 (67%) cases were culture positive. (Table 1)

Among the culture positive cases, dermatophytes were the predominant fungal isolates (59.7%) and the rest were due to moulds (22.38%) and yeasts (17.91%). Among the different species, *T. rubrum* (37.5%) accounted for the majority of dermatophytes, *Fusarium* (60%) was the commonest mould and *Candida albicans* (37.5%) the predominant yeast. (Table 2)

Of the 160 dermatophytes isolated, 14 were overgrown by contamination, hence could not be speciated. Fig. 4 represents the distribution of various dermatophytes isolated.

Table 3 and 4 represent the distribution of various non-dermatophytic moulds (NDM) and yeasts isolated respectively and Table 5 represents organisms associated with onychomycosis according to gender.

Discussion

Our study has documented onychomycosis to be the disease of middle-aged (25-45 years) which is in

accordance with the study by Veer P et al.⁽⁷⁾ The increase in cases with age may be justified by repeated nail microtrauma and greater work activity, prolonged exposure to pathogenic fungi, venous insufficiency and diabetes.⁽¹⁰⁾ Higher incidence of onychomycosis was noted among males when compared with female patients in this study, the ratio being 1.08:1 which correlates well with most other studies.^(8,11) This may be because of more exposure of males to outdoors with greater physical activity and are more prone to trauma.

Many authors have reported high incidence of onychomycosis of the fingernail but in the present study we have reported more cases of toenail onychomycosis than fingernail, with a ratio of 2.8:1 which is in accordance with some other studies.^(4,12) The lesions commonly occur on feet as warmth and moisture promote the contamination, sweating, tight fitting footwears that prevent sweat evaporation and create the ideal pabulum.

In our study, dermatophytes were the primary pathogen involved and among the dermatophytes, *T. rubrum* (present in 37.5% cases) was the commonest fungus responsible for onychomycosis. Studies from across the globe^(13,14,15) as well as in India⁽¹⁶⁾ found the same predominant etiology. Among the non-dermatophytic moulds, *fusarium* was the commonest mould and *Candida albicans* accounted for the most common yeast. This result is comparable with Ramani et al study.⁽⁴⁾ Local factors such as manicure/pedicure which may cause repeated damage of cuticle, contact with substances containing sugars, hyperhidrosis promote the infection with *candida*.

Of the two conventional methods of fungi identification, i.e., wet mount with KOH and fungal culture on SDA, the direct microscopic method is more sensitive. In this study, 288 (72%) cases were positive for fungal filaments in KOH preparation and 268 (67%) cases showed growth of fungus in culture. This result is comparable with the study by Sujatha V et al.⁽¹⁷⁾

In a study by Dogra et al,⁽¹⁸⁾ prevalence of onychomycosis in diabetic patients was 17% while in our study diabetes was seen only in 6% of cases.

Conclusion

Onychomycosis is a frequent cause of nail infection and due to its increasing incidence, prompt and proper diagnosis is unavoidable. The present study highlights the need for microbiological confirmation in case of onychomycosis as the type of nail changes cannot be taken as a reliable marker for predicting the causative infection. The study has also proposed the prevalence of different fungi causing onychomycosis in an urban setting in the southern part of India, which can provide useful guidelines for the appropriate management of cases and further epidemiological study.

Acknowledgement

I sincerely thank Professor and Head, Department of Dermatology & venereology, KIMS, Dr. Sharath Kumar B.C for his invaluable support and constructive criticism.

References

1. Kaur R, Kashyap B, Bhalla P. A five year survey of onychomycosis in New Delhi, India: Epidemiological and laboratory aspects. *Indian J Dermatol* 2007;52(1):39-42.
2. Ngwogu AC, Mba IEK, Ngwogu KO. Onychomycosis: etiology, diagnosis and treatment. *Journal of medical Investigations and Practice* 2014;9(1):43-6.
3. Raghavendra KR, Yadav D, Kumar A, Sharma M, Bhuria J, Chand AE. The non-dermatophyte molds: Emerging as leading cause of onychomycosis in south-east Rajasthan. *Indian Dermatol Online J* 2015;6:92-7.
4. Ramani R, Kumari G.R, Shivanada PG. Onychomycosis in coastal Karnataka. *Indian J Med Microbiol* 1993;11(3):223-25.
5. Chander J. Textbook of medical mycology. Mehta publishers 2009;3Edn:132-4.
6. Gupta AK, Ricci MJ. Diagnosing onychomycosis. *Dermatol Clin* 2006;24:365-9.
7. Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: prevailing fungi and pattern of infection. *Indian J Med Microbiol* 2007;25(1):53-6.
8. Sharath Kumar BC, Dayananda TR, Gopal MG, Ramesh M, Nandini, Sreedevi Chandrika M. Clinico-mycological study of onychomycosis. *Journal of evolution of Medical and Dental sciences* 2007;2(40):7790-99.
9. Kaur R, Kashyap B, Bhalla P. Onychomycosis-epidemiology, diagnosis and treatment. *Indian J Med Microbiol* 2008;26(2):108-16.
10. Elewski BE. Onychomycosis: Pathogenesis, diagnosis and management. *Clin Microbiol Rev* 1998;11:415-29.
11. Ghannoum M, Isham N (2014) Fungal Nail Infections (Onychomycosis): A Never-Ending Story? *PLoS Pathog* 10(6):e1004105. doi:10.1371/journal.ppat.1004105.
12. Perea S, Ramos MJ, Garau M, Gonzalez A, Noriega AR, del Palacio A. Prevalence and risk factors of tinea unguium and tinea pedis in the general population in Spain. *J Clin Microbiol* 2000;38:3226-30.
13. Boukachabine K, Agoumi A. Onychomycosis in Morocco: experience of the parasitology and medical mycology laboratory from Rabat children hospital. *Ann Biol Clin (Paris)* 2005;63:639-42.
14. Wang SH, Chi CC. Onychomycosis in Taiwan. *Int J Clin Pract* 2005;59:906-11.
15. Araj GF, Racoubian ES, Daher NK. Etiologic agents of dermatophyte infection in Lebanon. *J Med Liban* 2004;52:59-63.
16. Das NK, Ghosh P, Das S, Bhattacharya S, Dutta RN, Sengupta SR. A study on the etiological agent and clinic-mycological correlation of fingernail onychomycosis in eastern India. *Indian J Dermatol* 2008;53(2):75-9.
17. Sujatha V, Grover S, Dash K, Singh G. A clinico - Mycological evaluation of onychomycosis. *Indian J Dermatol Venereol Leprol* 2000;66:238-40.
18. Dogra S., Kumar B., Bhansali A., Chakrabarty A. Epidemiology of onychomycosis in patients with diabetes mellitus in India. *International Journal of Dermatology* 2002;41:647-51.