

“Microbiological Study of Onychomycosis at A Tertiary Care Hospital Kanpur”

R. Sujatha¹, Deepak Sameer*, Arunagiri D.²

Abstract:

Background: Onychomycosis is a common fungal infection of nail poses to be an important public health problem. Fungal nail infection accounts for almost 50% of all nail diseases it may be caused by dermatophytes, non-dermatophytic moulds or yeasts involving nail bed, nail plate or matrix of nail Onychomycosis. Further, management of fungal infections of nails usually requires a prolonged course with a tendency for relapse.

Aim: The present study was undertaken to isolate and identify the etiological agents of onychomycosis.

Material & Methods: A cross-sectional study was done over a 1 year period, in clinically suspected cases of onychomycosis, a thorough clinical evaluation was performed, followed by microscopically examining the infected nail specimen under 40% potassium hydroxide, which was succeeded by cultivating the organisms on fungal culture media and identifying the pathogen both by closely studying colony morphology in culture and also microscopically utilizing lacto phenol cotton blue staining.

Results: Females (60%) were more commonly affected than males (40%). The most common age group affected was 30-40 years (30%). Females showed more finger nail involvement (60%) and males toe nail involvement (40%)... Onychomycosis was most commonly seen in house wives (50%), followed by farmers (30%), Out of the 100 patients in our study, 10 demonstrated onychomycosis positive fungal growth on culture. Non dermatophyte moulds (NDM) (80%) were the most common fungal isolates followed by Candida species (10%) and dermatophytes (10%).

Conclusion: Unlike most studies, NDM constituted the major pathogen among our patients. Onychomycosis can affect a wide age group involving both males and females though fingernail involvement was mostly found in females, especially housewives doing greater household work. Despite low fungal isolation rate, fungal culture and antifungal susceptibility testing is utmost importance to know the major fungal a etiology and drug resistance pattern in the locality for better management of onychomycosis cases

Key Words: Candida, dermatophytes, non dermatophyte moulds, onychomycosis

Introduction

Onychomycosis is a common fungal infection of nail .Fungal nail infection accounts for almost 50% of all nail diseases [1]. It may be caused by dermatophytes, non-dermatophytic moulds or yeasts involving nail bed nail plate or matrix of nail. Dermatophytes are the predominant fungal isolates, of which *T.rubrum* is the main species and the rest are due to moulds (mainly *Fusarium* spp.) and yeasts (*Candida albicans* predominant one). Age, climate, occupation, travel and hygiene are the various factors contributing for the variation of prevalence rate of onychomycosis from region to region [2]. Onychomycosis can be classified into several clinical types: Distal and lateral subungual onychomycosis, proximal subungual onychomycosis, white superficial onychomycosis and Total dystrophic onychomycosis[3].

The clinical presentation may often be confused with other conditions like psoriasis, lichenplanus, onychodystrophy and nail trauma, making laboratory diagnosis and confirmation necessary. Fungal cultures are essential for accurate identification of the causative organism. This is of paramount importance because the clinical outcome of antifungal agents varies as to whether the aetiological agent is a dermatophyte, yeast or a non-dermatophytic mould (NDM). [4] The present study was undertaken to isolate and identify the aetiological agents of onychomycosis.

Material and Methods

This prospective study, over a period of one year (Dec2019-Nov 2020) was conducted on a total of 100 nail samples from patients with nail infection were collected after taking proper consent and was processed in the Department of Microbiology at RMCH & RC, Mandhana, Kanpur, Patients using topical or oral antifungal drugs at the time of sample collection or up to 15 days before the day of collection and patients with insufficient specimen were excluded from the study. Nail material was taken from clinically abnormal nails

¹Prof and Head, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)

^{*}Tutor, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)

²Professor & HOD, Dept of Endodontic, MDC,Kanpur(India)

or from the first right toenail if all nails appeared normal. Nails were cleaned with alcohol and nail clippings were collected on a sterile black filter paper or cardboard folder. The sample was divided in two portions: one part for fungal culture and another part for microscopy. The wet mount for microscopic examination was prepared using 20% KOH and examined after overnight incubation. Study Procedure

Culture was done in duplicate in SDA tubes; one with actidione (cycloheximide) and another without it and both were incubated at 25°C for four weeks in BOD incubator. Samples were considered negative if no growth was seen after four weeks of incubation. Positive cultures growing dermatophytes and moulds were processed further for identification and speciation by tests like Lacto phenol Cotton Blue (LPCB) staining, slide culture and unase production. Gupta AK et al., inoculums counting (Walsh and English criteria) method was followed for diagnosis of non-dermatophytic filamentous fungi [5]. Isolation of dermatophyte was considered as a pathogen irrespective of direct microscopy result. Culture tubes growing yeasts were further subjected to speciation by germ tube test, culture on CHROM agar (HiChrome)

Candida differential agar- Himedia and on cornmeal agar (Dalmau plate culture).In-vitro antifungal susceptibility testing was performed against Candida species using disc diffusion method on Muller Hinton agar with 2% glucose and methylene blue for fluconazole (10 µg), itraconazole (10 µg) and amphotericin B (20 µg) as per CLSI guidelines [6]

Results

Females (60%) were more commonly affected than males (40%). The most common age group affected was 30-40 years (30%). Females showed more finger nail involvement (60%) and males toe nail involvement (40%)... Onychomycosis was most commonly seen in house wives (50%), followed by farmers (30%), Out of the 100 patients in our study, 10 demonstrated onychomycosis positive fungal growth on culture. Non dermatophyte moulds (NDM) (80%) were the most common fungal isolates followed by Candida species (10%) and dermatophytes (10%)...

Table1: Shows nail involvement according to gender.

Gender	Type of nail Toe	Type of nail Finger	N=10	Percentage
Male	4 (40%)	0	4	40%
Female	0	6 (60%)	6	60%
Total	4	6	10	100%

Table 2:- Shows age wise distribution of the study group

Age	N=10	Percentage
<=20	0	0%
21-30	4	40%
31-40	3	30%
41-50	1	10%
51-60	0	0%
>60	2	20%

Table 3:- Shows occupational status of the study group

Occupation	N=10	Percentage
Student	0	0
House wife	5	50%
Business	2	20%
Farmer	3	30%
Others	0	0

Table 4:- shows KOH mount of the study group

KOH	N=10	Percentage
Positive	2	20%
Negative	8	80%

Table 5: Spectrum of fungi isolated

Fungal isolates	N=10	Percentage
Dermatophytes-(1)		
T.mentagrophytes	1	10%
Yeast (1)		
Candida albicans	1	10%
Non- dermatophytes (5)		
Cladosporium	1	10%
Fusarium	2	10%
Aspergillus niger	4	40%
Aspergillus flavus	1	10%



Figure 1: Onychomycosis of the finger nails

Discussion

Onychomycosis, the fungal infection of nail plate, though not a fatal disease is still clinically relevant due to its interference with patient’s quality of life, and

compromising the functional capacity. In the present study, Females(60%) were more commonly affected than males(40%), which is comparable with the studies of Madhuri JT et al(51.96%) and Bokhari et al(72%) Adhikari L et al, Neupane S et al, have reported higher prevalence in males[7-10]. Toe nail infection (40%) was commoner in males, while finger nail infection (60%) was common in females. This may be due to increased exposure to wet work in females, as most of them were house wives. In this study, age group most affected was 31-40 years (30%) which was in accordance with Gopi A et al., and Veer P et al., [1, 11]. Onychomycosis was most commonly seen in house wives (50%), followed by farmers (30%), similar to study by Veer P et al[10], High prevalence in farmers and house wives may be due to increased outdoor physical activity and increased exposure to wet work respectively. This variation in direct microscopy and culture may be due to non viability of fungal elements in some cases. Out of the 10 fungal isolates, non dermatophytic moulds (80%) were the commonest followed by yeasts 1 (10%) and 1 dermatophyte (10%). Among the dermatophytes, *T. mentagrophyte* was the predominate isolate 1 (10%) followed by the yeasts, *C.albicans* 1 (10%) was the most. Among the non dermatophytic moulds isolated, *A.niger* 4 (40%) was the most common followed by *Fusarium* spp 2 (20%). *A. niger* and *Cladospora* were isolated one each(10%) and is comparable with the studies of Niranjana et al Das NK et al, Veer P et al and Malik NA et al, 7, and Adhikari L et al [9,11,12,13,14]. Treatment of onychomycosis has been attempted throughout the ages, but success has been limited until the current decade. In this study, *Candida* isolates showed maximum susceptibility towards amphotericin B (100%); 85.7% and 75% towards fluconazole and itraconazole, respectively.

Conclusion

Onychomycosis is a common fungal infection of nails It can affect a wide age group involving both males and females though fingernail involvement was mostly found in females, especially housewives doing greater household work. It is caused by yeast, moulds and dermatophytes. In this study non-dermatophytes have outnumbered the dermatophytes, Despite low fungal isolation rate, fungal culture and antifungal susceptibility testing is utmost importance to know the major fungal aetiology and drug resistance pattern in the locality for better management of onychomycosis cases.

References

- Gopi A, Jain S, Samreen F. Spectrum of fungi causing onychomycosis in a tertiary care hospital in Bangalore. *Indian J Microbiol Res.* 2017; 4(2):207-09.
- Shenoy MS, Shenoy MM. Fungal nail disease (Onychomycosis); Challenges and solutions. *Arch Med Health Sci.* 2014; 2:48-53.

- Chander J(2009).Text book of Medical mycology.3rded.Newdelhi:Mehta publisher;pg:132-4, 56-63.
- RamaniR,Srinivas CR, et al.Moulds in onychomycosis.*Int J Dermatol* 1993;32:877-8.
- Gupta AK, Cooper EA, MacDonald P, Summerbell RC. Utility of inoculum counting (Walshe and English criteria) in clinical diagnosis of onychomycosis caused by nondermatophytic filamentous fungi. *J Clin Microbiol.* 2001; 39(6):2115-21.
- CLSI. Performance Standards for Antifungal Susceptibility Testing of Yeasts. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
- MadhuriJT,Rama RGR, et al.Onychomycosis:A significant medical problem.*Indian J DermatolVenerol Leprol*2002;68(6):326-9.
- Bokhari MA et al. onychomycosis in Lahore.*PakistanInt J Dermatol* 1999; 38:5915.
- Adhikari L, Gupta AD et al.Clinico-aetiologic correlates of onychomycosis in Sikkim.*Indian J PatholMicrobiol* 2009; 52:194-7.
- Neupane S, Pokhrel DB and Pokhrel BM. onychomycosis: A clinic-epidemiological study.*Nepal Med Coll J* 2009;11(2):92-95.
- Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: Prevailing fungi and pattern of infection. *Indian J Med Microbiol.* 2007;25:53-56
- Niranjana H. P.et al study of onychomycosis at a tertiary care hospital in south india journal of Evolution of Medical and Dental Sciences/Volume1/Issue5/November-2012Page-823-890.
- DasNK,Ghosh P, et al.A study on the aetiological agent and clinico-mycological correlation of finger nail onychomycosis in eastern india. *Indian J Dermatol* 2008; 53:75-9.
- Malik NA, Raza N, et al. Non dermatophyte moulds and yeasts as causative agents in onychomycosis. *Journal of Pakistan Association of Dermatologists* 2009; 19:74-78.