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Original Research Article

Study of fungal pathogen in onycomycoses patients attending the teaching hospital

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ABSTRACT

Background: Onychomycosis is commonly caused by dermatophytic group of fungi. Non dermatophytic group of fungi are rarely isolated from nail Onychomycosis due to non dermatophytic molds *Cladosporium spp*. Aim of the study was to isolate and identify fungal agents from nail clipping of patient clinically having onycomycoses.
Methods: Clinical samples from 226 clinically suspected onychomycotic patients were processed in the microbiology laboratory. These nail samples were subjected to 40% KOH examination .Samples were cultured on SDA and SDA chloramphenicol and cycloheximide for isolation of causative agents. These were identified microscopically and macroscopically.
Results: Total 35.8% samples were positive for fungus and showed fungal elements in 40% KOH mount amongst which 53.08% showed culture positivity.
Conclusions: Dermatophytes are the predominant group of fungi causing onychomycoses. These studies signify the importance of mycological examination in the diagnosis of nail infections for further effective management.

Keywords: Dermatophytes, Nail pathogens, Onychomycoses, Superficial mycoses

INTRODUCTION

Onychomycosis this term was used to designate dermatophytic infection of nail but now it is used for any fungal infection of the nail. *Tinea unguinum* is dermatophytic infection of nail plate.[1] Fungal infection of nail causes cosmetic problem. The agents may be dermatophytes, yeast and non dermatophyte molds.[2] Trichophyton and Epidermophyton are predominantly infecting the nails amongst dermatophytes.[3] Several nail disorder like psoriasis, lichen planus, bacterial infection, contact dermatitis, traumatic dystrophies; paronychia that may mimic fungal nails infection need to be differentiated from one another to initiate the appropriate therapy.[3,4] Clinical diagnosis of onychomycosis is based on microscopy and culture of the sample of nail. There are sevsral geographical variations in the causative agents. Scanty data on the predominance of the species causing onychomycoses is available. This study was conducted to identify the fungal pathogens causing nail infection and the predominant fungi causing nail infection in patients attending teaching hospital. Aim and objectives of the study were to isolate and identify the fungal agents in onychomycotic patients attending in a teaching hospital.

METHODS

This observational study was carried out in Department of Microbiology from August 2008 to June 2014. Total

226 patients were included in this study. Inclusion criterias were: clinically diagnosed patients of onychomycosis of all ages and sex. Patients on treatment were excluded from study. The nails were cleaned with 70% alcohol and clippings from nail were collected. Scrapping of the undersurface of juncture of nail bed was also taken. Brittle, discolored; dystrophic portion of nail was clipped. Debris from under nails was removed with scalpel. The material was collected in a clean paper envelope. After labeling it with patient's details; it was processed in microbiology laboratory.

Direct microscopic examination using 40% KOH mount of nail was done. The samples were cultured on two sets of Sabouraud's dextrose agar (SDA) and SDA with actidione and chloramphenicol. Slants were incubated at 27°C and at 37°C separately. No growths slant after 6 weeks was declared as negative. Morphology of colony was studied by observing reverse and obverse of SDA slant. Dermatophytes were identified on the basis of presence of thin separate hyphe with arthoconida (Figure 1), after culture the colonies were observed on surface as well as reverse to see the pigmentation, topography, texture and rate of growth.

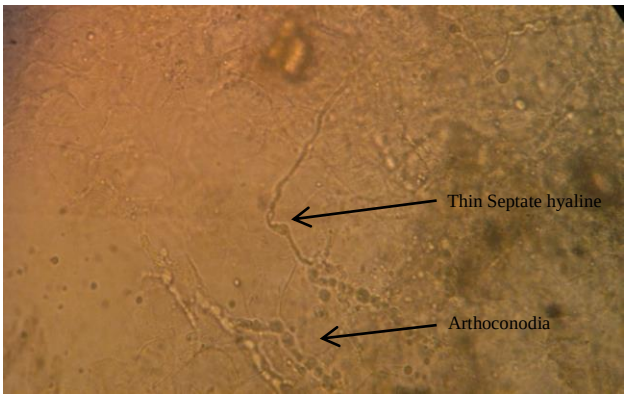


Figure1: KOH mount showing thin Septate hyaline hyphae with branched filaments and Arthoconodia.

Figure 2: Colony morphology of *Trichophyton verrucosum* on SDA (27OC) Yellowish white heaped, flat slightly downy with yellow reverse.

Lactophenol cotton blue (LPCB) stain of tease mounts of colony was done. Microscopic features of LPCB mount i.e. hyphae arrangement, presence of microconidia macroconida, shape and arrangement of it were observed and noted. Slide culture techniques were also performed to see the intact morphology of the fungi by using potato to dextrose agar. (Riddel Slide culture Method); Urease Test; and Hair perforation test were performed to identify species. The fungi were identified by following the standard guidelines and comparing them with the standard strains obtained from PGIMER, Chandigarh, India.

RESULTS

Total 226 samples from clinically suspected patients of onychomycosis were studied. Amongst which 81 (35.8%) were positive for fungus in the laboratory. Amongst the positive 43% patients were of 15-45 years age group. 30% patients were male and 14 % were female of this age group.

Table 1: Age and sex wise distribution of clinically suspected onychomycoses patients, positive for fungus in laboratory.

Age groups of patients (years) positive for fungus	Sex				Total	Percentage
	Male	(n %)	Female	(n %)		
0-14 years	7	8.64	4	4.94	11	13.58
15-45years	24	29.63	11	13.58	35	43.21
45-60years	15	18.52	8	9.88	23	28.40
>60	10	12.35	2	2.47	12	14.81
Total	56	69.14	25	30.86	81	100.00

KOH mount was positive of 81 (35.81%) specimens amongst which 43 (53%) samples fungi were positive for culture (Table 2). The distributions of fungi isolated from the specimens are mentioned in Table 3. The predominance of dermatophyte was observed in the study.

Amongst all isolates, 38 were identified as dermatophyte group. Four were of mold of non dermatophyte amongst which three were *Aspergillus niger*, one was *Cladosporium* species and one was *Candida albicans*.

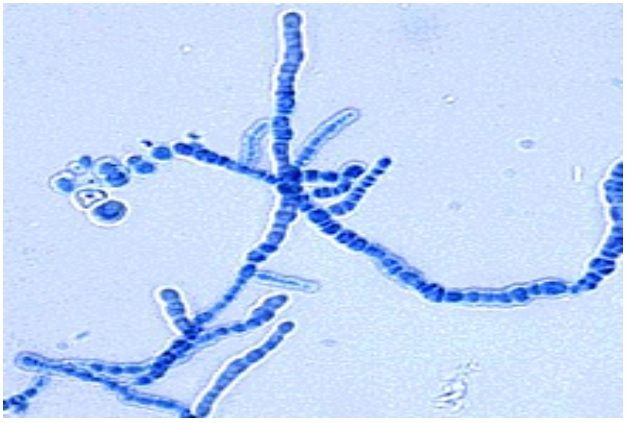
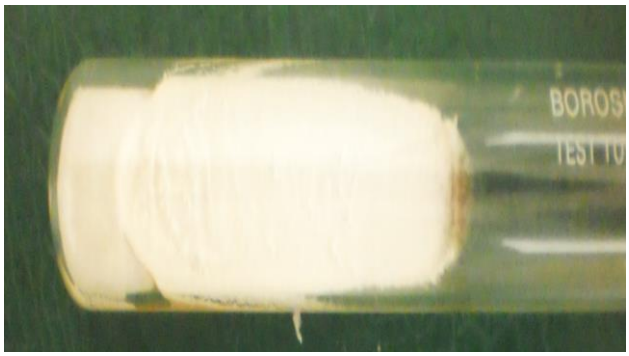


Figure 3: LPCB mount showing abundant chlamydospore in chains and some antler like branches.

As *Aspergillus* and *Cladosporium* are saprophytic confirmation was done by collecting the repeat samples. Amongst the dermatophytes, *Trichophyton rubrum* was the predominant dermatophyte isolate followed by *T. mentagrophyte* and *E. flocosum* species (Table 4).

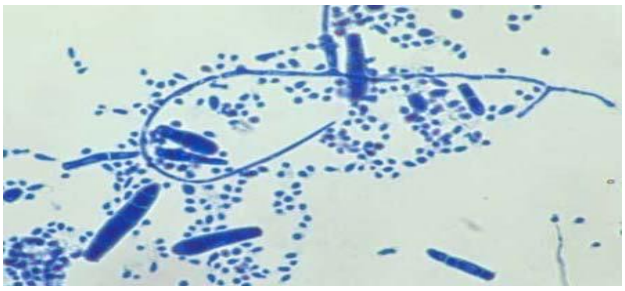


Colonies-white powdery, becomes yellowish forming radial folds. Reverse-colorless

Figure 4: Colony morphology of *Trichophyton mentagrophyte* on SDA (27°C)

Table 2: Positivity of specimen with microscopy and culture.

Number of specimen (n=226)	KOH positive	KOH positive culture positive	KOH negative culture negative
	81 (35.81%)	43 (19.2%)	145 (64.15)



Septate hyphae with abundant tear shaped round clustered microconidia & Cigar shaped Macroconidia.

Figure 5: LPCB mount of colony.

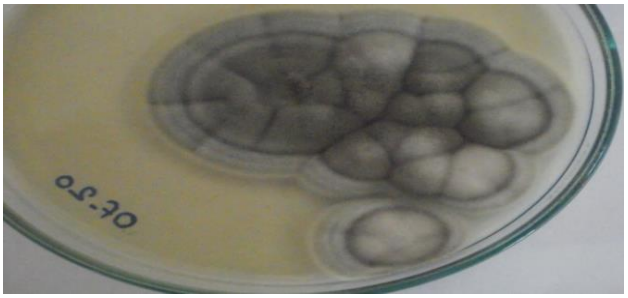


Figure 6: Colony morphology on SDA Greenish black with grayish velvety nap, becoming heaped and slightly folded, reverse was black.

Table 3: Fungi isolated on SDA showing onychomycosis due to different fungal groups.

Culture positive n=43(%)		
Dermatophyte	Non dermatophyte mold	Yeast
38 (88.3%)	4 (9.3%)	1 (2.3%)

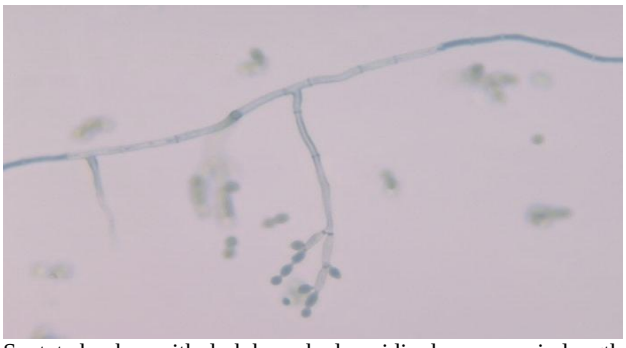
Table 4: Dermatophytes isolate from onychomycotic patients.

Dematophytes isolated	Number n=38 (%)
Trichophyton rubrum	16(42)
Trichophyton mentagrophyte	8(21)
Epidermatophyton flocossum	5(13.16)
T richophyton verrucosum	3(7.89)
Trichophyton schoenlenni	2(5.26)
Trichophyton terreste	2(5.26)
Trichophyton spp.	2(5.26)
Total	38

DISCUSSION

Onychomycosis is chronic fungal infections of the nail causing cosmetic problem. Commonly the dermatophytes have the capacity to invade keratinized tissue (skin, hair and nails) of humans and other animals to produce an

infection.[5] Diagnosis of onychomycosis is important because it helps to prevent nail dystrophy and the spread of infection. 5a Clinical and myocological features of onychomycosis show variation with time and place.[6]



Septate hyphae with dark branched conidiophores vary in length usually produce conidial chains

Figure 7: LPCB mount of colony.

Onychomycosis is most commonly found in age group of 15 to 45 years. Findings were similar observation in this study. The individuals of these age groups are comparatively more exposed to fungi due to more outdoor activities so the cases are more detected in them. Relatively males are more affected than female and children as there is less exposure to fungus as less time is spent in environments containing pathogens. Children have faster nail growth, smaller nail surface for invasion.[2] Male predominance of age group 25-45 years observed in this study. This could be the result of more traumas in nail, greater work activity which leads to more prolonged exposure to pathologic fungi.

In the present study, KOH positivity is 35.8% and culture positivity is 19%. In case of onychomycosis, it was always observed that microscopy has more sensitivity than culture. Most of the time the nail is thickened brittle or is difficult to dissolve. It may be due to non-viability of the fungal hyphae in the distal portion of the nail plate from where the scraping is done is well known.[2] In the present study commonest causative agent is dermatophyte which is observed in most of the studies on onychomycoses conducted in India and outside the India.[4,7] Amongst the dermatophyte *T. rubrum* was most common isolate (42%) followed by *T. mentagrophyte* and *E. flocossum*. Jha et al find the same observation in study on superficial mycoses and *T. mentagrophyte* followed by *T. rubrum* and *E. flocossum* Scher et al found that rate of isolation of *T. mentagrophytes* and *T. rubrum* was very high which can be explained on the basis of its capacity to infect the hard keratin of the nail.[8,9] Milos et al reported prevalence of nail infection by non-dermatophyte moulds 1.45% to 17.6%.[10]

Onychomycosis due to *Alternaria spp*, *Fusarium spp*., *Scytalidium spp*. and *Acremonium spp*. are also reported in the various studies.[11-14] In the present study 9.3% non dermatophytic fungi were isolated they were identified as *Aspergillus niger* and *Cladosporium species*. Common species of *aspergillus* like *A.niger, A. flavus, A. terruus, A. nidulans* as a cause of onychomycoses are also repoted.[15-19] Zotti et al reported a case of onychomycoses due to Aspergillus nomius Aspergillus persii, rare isolates.[20,21]

One of present fungal isolate is identified as Cladosporium which is rarely reported. Most of time it is considered as laboratory contaminant but after repeating samples and inoculating it on duplicate culture slants it was considered as etiological agent. The traditional microscopy and culture methods are slow and non-specific diagnostic approaches but to know the extact prevalence or predominant species, it will require studies by using molecular tools for fungal identification of dermatophytes. Only one isolate was *Candida albicans* which was also reported as a cause of onychomycoses. *Candida albicans* previously regarded as contaminants, yeasts are now increasingly recognized as pathogens in fingernail infections.[2,22] *Candida* species have emerged as second-line pathogens.[23] Onychomycosis is a chronic disease having therapeutic difficulties. In the present study, we have focused the microbiological aspect of onychomycosis, necessary for definitive identification of etiological agents.

CONCLUSION

Dermatophytes *T. rubrum* and *T. mentagrophytes* were predominant isolate responsible for onychomycosis. Awareness of microbiological examination of nail scrapping will help in identification of fungal pathogens and in management of onychomycosis.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Chander J. Textbook of Medical Mycology. Mehta publisher. 3rd edition. 2009.
2. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev. 1995;8:240-59.
3. Kaur R, Kashyap B, Bhalla P. A five-year survey of onychomycosis in New Delhi, India: Epidemiological and laboratory aspects. Indian J Dermatol. 2007;52:39-42.
4. Kaur R, Kashyap B, Bhalla P. Onychomycosis - epidemiology, diagnosis and management. Indian J Med Microbiol. 2008;26:108-16.
5. Sobhanadri C, Rao DT, Babu KS. Clinical and mycological study of superficial fungal infections at government hospital: Guntur and their response to treatment with Hamycin, Dermostatin and dermamycin. Indian J Dermatol Venereol Leprol. 1970;36:209-14.

6. Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: Prevailing fungi and pattern of infection. Indian J Med Microbiol. 2007;25:53-6.
7. Maraki S, Nioti E, Mantadakis E, Tselentis Y. A 7-year survey of dermatophytoses in Crete Greece. Mycoses. 2007;50(6):481-4.
8. Jha BK, Mahadevamurthy S, Sudisha J, Bora A. Isolation, Identification and Antifungal Susceptibility Test of Dermatophytes from the Patients with Onychomycosis in Central Nepal American Journal of Dermatology and Venereology. 2015;4(3):30-6.
9. Scher RK. Onychomycosis is more common than a cosmetic problem. Br J Dermatol. 1994;130;159.
10. Milos D, Pavlovic MD, Bulajic N. Great toenail onychomycosis caused by Syncephallastrum racemosum. Dermatol Online J. 2006;12:7.
11. Agarwalla A, Agrawal S, Khanal B. Onychomycosis in eastern Nepal. Nepal Med Coll J. 2006;8:1-7.
12. Farwa U, Abbasi SA, Mirza IA, Amjad A, Ikram A, Malik N, et al. Non-Dermatophyte Moulds as Pathogens of Onychomycosis. J Colle Phys Surg Pak. 2011; 21(10):597-600.
13. Malik NA, Raza N, Nasiruddin. Non-dermatophyte moulds and yeasts ascausative agents in onychomycosis. J Pak Asso Derm. 2009;19:74-8.
14. Tisti A, Piraccini BM, Lorenzi S. onychomycosis caused by non-dermatophytes: Clinical features and response to treatment of 59 cases. J Am Acad Dermatol. 2000;42:217-24.
15. Doddamani PV, Harshan KH, Kanta RC, Gangane R, Sunil KB. Isolation, Identification and Prevelance of Dermatophytes in Tertairy Care Hospital in Gulbarga District. People's J Scientific Research. 2013;6(2).
16. Garcia-Martos P, Guarro J, Gené J, Linares M, Ortoneda M. Onychomycosis caused by Aspergillus sclerotiorum. J Mycol Med. 2001;11:222-4.
17. Kristensen L, Stenderup J, Otkjaer A. Onychomycosis due to Aspergillus tamarii in a 3-year-old boy. Acta Derm Venereol. 2005;85:261-2.
18. Takahata Y, Hiruma M, Sugita T, Muto M. A case of onychomycosis due to Aspergillus sydowii diagnosed using DNA sequence analysis. Mycoses 2008;51:170-3.
19. Escobar ML, Carmona-Fonseca J. Onychomycosis by common non-dermatophyte moulds. Rev Iberoam Micol. 2003;20:6-10.
20. Zotti M, Machetti M, Perotti M, Barabino G, Persi A. A new species, Aspergillus persii, as an agent of onychomycosis. Med Mycol. 2010;48:656-60.
21. Zotti M, Machetti M, Persi A, Barabino G, Parodi, A. Onychomycosis: First case due to aspergillus nomius. Acta Dermato-Venereologica. 2011;91(5):591-2.
22. Pontes ZB, Lima Ede O, Oliveira NM, Das Santosh JP, Ramos AL, Carvalho MF. Onychomycosis in Pessoa city, Brazil. Rev Argent Microbiol. 2012;34:95.
23. Jayatilake, J.A.M.S., Tilakaratne, W.M. & Panagoda, G.J. Mycopathologia. 2009;168:165.

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