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Research Article

Onychomycosis: role of non dermatophytes

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ABSTRACT

Background: Onychomycosis, a fungal infection of nails, is ordinarily be caused by dermatophytes, non dermatophytes and yeast. Therefore the aim of this study was to determine the role and pattern of non dermatophyte moulds as causative agents of onychomycosis.
Methods: The retrospective study was carried out from Jan, 2000 to March, 2014 at Allahabad. In more than a period of 14 years 3321 clinically suspected cases of onychomycosis are included in the study. KOH examination and culture have been carried out in all the patients. All the finger and toe nails were examined along with whole body skin for fungal infection.
Results: Positive cultures were obtained in 2906 cases. Dermatophytes were isolated in 1307 (44.97%), non dermatophytes in 1209 (41.60%), yeast in 284 (9.77%), mixed infections in 106 (3.64%) cases, and the remaining 415 cultures were sterile. Among ND the predominant isolates obtained were *Scopulariopsis bervicaulis* (20.51%), *Aspergillus terreus* (14.06%), *Aspergillus niger* (7.44%), *Aspergillus flavus* (8.10%), *Absidia* (7.22%), *Rhizopus* (7.60%), *Acremonium* (11.99%), *Fusarium* (7.60%), *Penicillium* (8.22%), *Mucor* (5.78%), and *Scytalidium dimidiatum* (1.48%).
Conclusions: It can be inferred from the study that NDMs play a significant role in the causation of onychomycosis. Hence it is necessary that fungus culture should be carried out in all cases of onychomycosis for proper therapeutic management.

Keywords: Onychomycosis, Non dermatophytes, True pathogens

INTRODUCTION

Onychomycosis accounts for upto 50% of all nail disorders.[1-3] Most cases are caused by dermatophytes but yeasts and non dermatophytes (ND) are being increasingly isolated from such cases. A number of species of ND have been implicated as the causative organisms, however their role is considered to be controversial.[3-7] They have generally been considered as either contaminants or commensals and thus ignored in the etiology of nail disorders. Ajello pointed out that nail invasion by ND is considered uncommon with prevalence rates ranging from 1.4% to 17.6% and almost all these reports are from the western countries.[8] Therefore the aim of this study was to determine the role and pattern of non dermatophyte moulds as causative agents of onychomycosis.

METHODS

The study included 3321 clinically diagnosed cases of onychomycosis received from the skin clinics of Allahabad, India. In all the cases data related to the age, sex, duration of the lesions, occupation, personal, habits etc were noted. After a detailed clinical examination, the physical features of the nails were recorded. Care was particularly taken to record the presence of superficial mycotic infections on other parts of the body.

Before obtaining a specimen, nails were cleansed by swabbing them liberally with alcohol to eliminate as many bacteria as possible, because they can overgrow and inhibit the growth of dermatophytes. Scrapings and clippings were collected from the deepest part of the nail (junction of the healthy and diseased portion of the nail).

When both toe and finger nails were affected, specimens were collected from both the sites. In patients with presumptive diagnosis of distal subungual onychomycosis (DSO) and Candida onychomycosis (CO), a nail clipper was used to cut away the nail plate; then a curette was used to scrape the debris from the nail bed at a site as proximal to the cuticle as possible. A No 15 blade scalpel was used to scrape debris from the nail surface in the cases of White superficial onychomycosis (WSO). In the cases of proximal subungual onychomycosis (PSO) healthy nail was pared back with a no 15 blade scalpel and a curette was used to remove the material from the proximal nail bed.[9]

Each specimen was divided into two parts, one was taken for direct microscopic examination after 10% KOH solution treatment and second was inoculated on Sabouraud Dextrose agar (M286) and Sabouraud Cycloheximide Chloramphenicol agar (M664). Two successive nail cultures were performed to establish the colonization of the pathogen because successive sampling rarely demonstrates the same contaminant. Cultures were routinely incubated at 25^0–30^0 c and examined daily for up to 4 weeks. The identification of individual fungi was based on standard methods such as microscopy, morphology, colonial characterization and pigment production, rate of growth and biochemical test (Table 1). Isolation criteria laid down by English were followed in the present study.[10]

Table 1: Identification criteria for non dermatophytes.

Fungus	Growth rate days	Pigment	Hyphae	Conidiophores/ Sporingophores	Micro conidia	Macro conidia
S.bravecaulis	2-6	Initially white, later become light brown	-	Hyaline, septate, branched, conidia, large, rough walled, flat base	-	-
Acremonium	2-6	Reddish orange	Septate, produce single unbranched tube like phialides	-	Elliptical single celled	-
Fusarium	2-6	Purple Yellow	Septate give rise to phialides	-	Single celled	Sickle shaped and contain numerous septa
A.fumigatus	2-6	Blue green	Septate, short conidiophores	-	Long chains of conidia	-
A.niger	2-6	Initially yellow turns into black	Septate, long conidiophores	-	Chains of brown conidia	-
A.terreus	2-6	Tan and resemble cinnamon	-	-	Globosed to elliptical	-
Penicillium	2-6	Green/purple green	Hyaline septate and produce brush like conidiophores	Exhibit branching metulae from which phialides producing conidia	Chains of conidia	-
Rhizopus	1-3	Black	Nonseptate, ribbon like with rhizoids	-	Yellow/ brown spores	-
Absidia	1-3	Grey	Septum is formed, rhizoides present between sporangiophores	Pyriform sporangia, swollen portion at the junction of the sporangium	-	-

RESULTS

Total 3321 clinically suspected cases of onychomycosis were undertaken for mycological studies. Out of 2906 culture proved cases, dermatophytes were obtained in 1307; non dermatophytes in 1209, yeast in 284, mixed infections 106 cases, and the remaining 415 cultures were sterile. Culture isolates in relation to the site of involvement are depicted (Table 2).

Table 2: Culture isolates in relation to the site of involvement.

Fungus isolated	Finger nail	Toe nail	Both
S.berviacaulis (248)	200	32	16
Penicillium (100)	73	20	7
A.flavus (98)	35	40	13
A.terreus (170)	140	26	4
A.niger (90)	54	33	13
Acremonium (145)	121	9	15
Rhizopus (92)	62	24	6
Fusarium (92)	76	14	2
Absidia (86)	60	21	5
Mucor (70)	55	22	3
Scytalidium dimidiatum (18)	11	6	1

Table 3: Clinical types and culture isolates.

Clinical types	Dermatophytes	Non dermatophytes	Yeast	Mixed
DSO	840	904	68	40
PSO	315	293	-	14
WSO	52	12	-	-
CO	-	-	216	52

Females outnumbered the males. The commonest age group affected was 35-50 years. The duration of the lesions varied from one month to 8 years but majority of cases were of less than one-year duration.

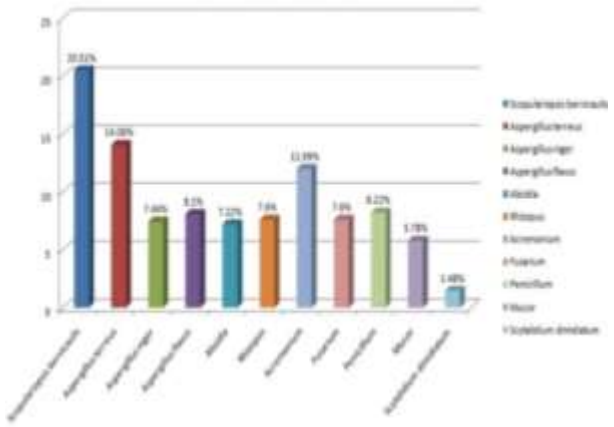

Figure 1: Percentage of non dermatophytes infections in 14 years (2000-March 2014).

The predominant clinical abnormalities observed were discoloration of the nail plate, subungual hyperkeratosis, leukonychia, total nail dystrophy, paronychia and pitting.

The common clinical types of nail involvement recorded were DSO in 904 cases, PSO in 293 cases and WSO in 12 cases (Table 3). Complete spectrums of non dermatophytes are depicted (Figure 1).

It was observed that when only a few nails were involved (in the absence of mycotic skin lesions) it was reasonable to suspect a mould as the causative agent. *Scopulariopsis brevicaulis* was isolated in 248 cases of onychomycosis. Patients described a very rapid spread of the disease since the appearance of the first sign.

Aspergillus onychomycosis was diagnosed in 358 patients. *Aspergillus flavus* is responsible for 98 cases, *Aspergillus terreus* for 170, *Aspergillus niger* for 90 cases. Seventy out of 90 cases of *Aspergillus niger* showed a black discolouration of the lunula. Twenty two patients affected by *Aspergillus terreus* presented a PSO associated with paronychia.

Acremonium was isolated in 145 cases. Onychomycosis was asymptomatic in all cases. *Fusarium* onychomycosis was diagnosed in 92 cases, 10 patients presented a DSO type. *Absidia* was found in 86 cases, *Rhizopus* and *Mucor* were isolated from 92 and 70 cases respectively. *Penicillium* was isolated in 100 cases. All presented DSO

with hyperkeratosis. Duration ranged from 1 month to 5 years.

DISCUSSION

Onychomycosis caused by non dermatophytes occurs with varying frequency according to the geographic location.[11-13] Frequencies of mould onychomycosis in European countries like Austria, Estonia, Italy, and Spain were reported as about 5%, 7%, 8%, and 17.2%, respectively.[14-17] The prevalence in North America is 4.3% in Canada and 20% in the United States, whereas in South America 4.5 and 9.5% in two different centers in Colombia and 1% in Argentina.[18-21] Tosti performed a mycological study on 1548 patients affected by nail disorders and diagnosed 431 cases of onychomycosis out of which 59 were caused by moulds.[22]

These included 17 patients caused by *Scopulariopsis berviacaulis*, *Fusarium* in 26, *Acremonium* in 9 and 7 patients were caused by *Aspergillus* species. In Asia the frequencies are given as 12% in Singapore and 22% in India.[23-25] Differences in lab techniques may explain the variation in the figures, a discrepancy which contributes to the controversy. Those who studied the subungual debris alone may report the incidence of non dermatophytes to be around 5% where as others who studied subungual debris and clippings of nail plates put the figure at 15-20%.[13,14] In our study culture isolates revealed 1209 of non dermatophytes. This could be due to truly high incidence of non dermatophytes in this part of the world and may be also due to the fact that both nail plate and sub ungual debris were taken for culture.

CONCLUSION

In present study many NDMs have been isolated though the prevalence rates of these fungal species do vary to some extent which could be explained on the basis of geographic variation. Still another reason for high incidence could be due to the fact that we took two consecutive samples and also sub culture was carried out only once and not five times as suggested by English MP. Two successive nail cultures were performed to establish the colonization of the pathogen because successive sampling rarely demonstrates the same contaminant. Therefore our results suggest that non dermatophytes can no longer be considered as secondary colonizer or contaminants but seem to play a definite role in the etiology of onychomycosis.

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