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RESEARCH ARTICLE

TREND OF ONYCHOMYCOSIS IN RIMS HOSPITAL IMPHAL

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ABSTRACT

Background: Onychomycosis is a commonly encountered superficial fungal infection. Beside causative dermatophytes and yeasts, present data shows that non-dermatophytic filamentous fungi can also be the potential cause of this ungual disease. The aim of this study was to analyse the causative agents of onychomycosis in patients attending dermatology OPD of a tertiary care hospital. **Methods:** A mycological study of onychomycosis was undertaken in 881 patients suspected to have fungal nail infection by their clinical appearance referred from the Dermatology OPD, January 2016 to July 2018 and processed in the Department of Microbiology RIMS. Direct microscopy of the nail clippings in 10% KOH followed by culture in SDA and DTM was performed to identify the causative agent. **Results:** Direct microscopy of the nail clippings in 10% KOH was positive in 318 (35%) and culture was positive in 592 (67.1%) cases. Out of the samples cultured, dermatophytes were grown in 166 (28.04%), non – dermatophytes moulds grown in 331 (55.9%), yeast and yeast like grown in 73 (10.8%) and mixed isolates grown in 22 (3.7%) Among the dermatophytes Trichophyton spp. was found to be commonest etiological agent followed by Microsporum spp. Among the non – dermatophyte moulds Aspergillus spp. was the most prevalent species followed by Penicillium spp. **Conclusions:** In the previous study done during the period from January 2013 to December 2015 in RIMS Imphal, among the dermatophytes Trichophyton spp. was the commonest followed by Epidermophyton species. Among non-dermatophytes Aspergillus species was the commonest followed by Fusarium species that caused onychomycosis. The prevalence of non-dermatophytic onychomycosis was more than the dermatophytic onychomycosis. Here in this comparative study the change in the trend of onychomycosis is not very significant. Hence the trend of onychomycosis by dermatophytes and Non-dermatophytes remains the same in RIMS Hospital.

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INTRODUCTION

Onychomycosis is the most common nail infection, with an incidence rate of more than 10% in the general population worldwide (Hilmioğlu-Polat, 2005). Onychomycosis can be caused by dermatophytes (tinea unguium), non-dermatophytic molds, or yeast. While dermatophytes account for the majority of onychomycosis cases in temperate Western countries, non-dermatophytic filamentous fungi and yeast are more commonly implicated in countries with a humid and hot climate (Ghannoum, 2018). Onychomycosis can be classified into distinct clinical categories based on the region of the nail unit that is affected. These categories include distal/lateral onychomycosis, proximal subungual onychomycosis, and superficial white onychomycosis (Tosti, 2000). The causative organisms have different entry sites, resulting in different clinical variants of onychomycosis.

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For instance, *T. rubrum* and *Epidermophyton floccosum* usually infect the distal and lateral parts of the nail, while *T. soudanense* usually manifests as endonyx subungual disease. *T. mentagrophytes* and non-dermatophyte molds normally invade the superficial layer of the nail plate causing superficial white onychomycosis (SWO). By contrast, *Candida* spp. invade the subcuticular space, eventually resulting in proximal nail dystrophy (Bongomin, 2018). Approximately 10 % of onychomycoses are caused by non-dermatophytic molds (Ghannoum et al., 2018). Non-dermatophytic molds (NDM) are filamentous fungi that are commonly found in nature as soil saprophytes and plant pathogens. Nail invasion by NDM is considered uncommon with prevalence rates ranging from 1.45% to 17.6% (Maddy, 2017). The literature about the pathogenic role of NDMs is controversial because they can act as contaminants, colonisers or pathogens (Lipner, 2019). It has not been fully established whether non-dermatophyte infections occur as a primary event on healthy nails or can only affect nails already damaged by ischemia, trauma or other diseases such as diabetes and psoriasis which predisposes the NDMs nail colonization (Bombace, 2016).

Some non-dermatophyte molds that cause infections of the nail include species of Scopulariopsis, Scytalidium, Fusarium, Aspergillus, and Onychocola canadensis. Candida species, especially C. albicans and C. parapsilosis, are the major yeasts that cause onychomycosis (Gupta, 2003). NDM onychomycosis presents with clinical features mimicking dermatophytic onychomycosis, making clinical diagnosis difficult and unreliable (Bongomin et al., 2018). Very little is known regarding the ability of NDM to invade an intact nail plate. Fungal infections of the fingernails and toenails, in contrast to those at other body sites, are particularly difficult to eradicate with drug treatment. This is the consequence of factors intrinsic to the nail such as the hard nail plate, sequestration of pathogens between the nail bed and plate and slow growth of the nail and also the pathophysiology of onychomycosis is complex (**S**hemer, 2019).

Since the nail unit lacks effective cell-mediated immunity, it is susceptible to infection by fungal organisms. The pathogen first adheres to the nail apparatus and then infects the sublayers, using its keratinolytic, proteolytic and lipolytic properties (Grover, 2012). Only 50% of nail dystrophy is because of fungal infection, with several other conditions, including inflammatory disorders such as psoriasis and lichen planus, presenting nail changes that clinically mimic onychomycosis (Allevato, 2010). Additionally, the clinical appearances of onychomycosis caused by different fungal species are often indistinguishable, thus indicating that laboratory testing is required for identification of the infecting organism (Singal, 2011). Onychomycosis if left untreated, will often worsen in severity, leading to marked dystrophic changes in affected nails (Elewski, 1996). Furthermore, onychomycosis has been reported to have a significant impact on patient quality of life due to a variety of physical changes (e.g., pain, discomfort, difficulty trimming thick nail plates, and difficulty walking) and psychosocial consequences (e.g., embarrassment and avoidance of intimacy) (Thomas, 2010). This study has been taken up to determine the trend of onychomycosis caused by dermatophytes and Non-dermatophytes in RIMS Hospital, Imphal, Manipur.

METHODS

During the period of January 2016 to July 2018, 881 patients, with clinically suspected onychomycosis, referred to Department of Microbiology, Regional Institute of Medical Sciences, Imphal, Manipur from Dermatology OPD to confirm the diagnosis. All patients signed an informed consent form. Patients who had undergone treatment with topical antifungals during the previous 4 months and those who had undergone treatment with systemic antifungals during the previous 9 months were excluded from the study. History were collected from all patients and specific data related to predisposing factors for onychomycosis were collected for each subject. Nails and the surrounding skin were cleaned with 70% ethyl alcohol before taking the specimens. The specimens were collected using surgical forceps and/or a sterile disposable scalpel at the site of potentially infected portion of the nail. Samples were collected depending on the variety of onychomycosis. In distal subungual onychomycosis (DLSO) nail bed underside of the nail plate from the advancing edge, most proximal to the cuticle was taken. In PSO, the specimen was taken from nail plate and proximal nail bed as close to lunula as possible. In WSO, the surface scrapings of the nail plate were taken. For candida infection, the material closest to the proximal, lateral nail edges and scrapings from under surface of the nail were obtained which were ideal specimens to demonstrate the fungi. Maximum care was taken to avoid penetration of the nail plate and bleeding. For total dystrophic onychomycosis, any abnormal area of the nail or bed were used as a specimen. A clean sheet of white paper or brown paper folded was used for the specimen transport. For each patient, direct microscopic examination and mycological culture method were performed. One portion of the sample from the patient was allowed to dissolve in 10% KOH overnight and then examined for the presence of fungal elements under the microscope. Another portion of the sample was again divided into two portions for culture on two sets of SDA and one is incorporated with chloramphenicol (0.5mg/ml) and cycloheximide (0.5mg/ml) and incubated at 25^0C for 3 weeks. The fungal species that grow in SDA were identified based on cultural characteristics, pigment production, rate of growth, microscopic examination in LCB and slide culture. Yeasts identification were done using standard mycological procedures. Non-dermatophytes were confirmed by repeated isolation of same fungus twice at intervals.

RESULTS

Out of 881 patients 318 were positive for fungal elements under direct microscopy with 10% KOH and a total of 592 patients were culture positive following the criteria and diagnosed with onychomycosis. Out of total 592 patients 356 (60.1%) were females and 236 (39.9%) were males -Table 1.

Age group	Total no. of people	Males	females
0-15	84	33	51
16-30	224	71	153
31-45	334	104	230
46-60	190	66	124
>61	49	18	31
Total	881	292	589

Table 2. Distribution of fungal agents in males and females

Fungal isolate	No. of Females	No. of Males	Total
Dermatophytes	94	72	166
Non- dermatophytes	201	130	331
Yeast and yeast like	48	25	73
Mixed growth	13	9	22
Total	356	236	592

Table 3. Different forms of fungal elements under 10% KOH

Age in Years	Hyphae		Hyphae with Yeast		Yeast cells	
	Male	Female	Male	Female	Male	Female
0-15	7	12	2	3	1	4
16-30	28	37	4	5	8	11
31-45	42	53	6	7	10	19
46-60	14	18	-	2		4
>61	6	4	1	1	-	2
Total	97	128	13	18	22	40

Table 4. Distribution of onychomycosis by fingernails/toenails involvement

Fungal Isolate	No. of isolates	Toe Nail	Finger Nail
Dermatophytes	166	75	91
Non- dermatophytes	331	177	154
Yeast and yeast like	73	16	57
Mixed growth	22	10	12
Total	592	278	314

Table 5. Different isolates of Dermatophytes

Dermatophytes	Number of isolates	Percentage
Trichophyton spp.	155	93.3%
Microsporum spp.	7	4.2%
Epidermophyton spp.	4	2.5%
Total	166	100%

Table 6. Different fungal isolates of Non-Dermatophytes

Non-Dermatophytes	No. of fungi	Percentage
Aspergillus spp.	118	35.6%
Penicillium spp.	57	17.2%
Mucor spp.	28	8.4%
Nigrospora spp.	24	7.25%
Fusarium spp.	23	6.9%
Rhizopus spp.	21	6.3%
Zygomycetes spp.	9	2.7%
Cladosporium spp.	8	2.4%
Acremonium spp	8	2.4%
Alternaria spp.	8	2.4%
Scopulariopsis spp.	6	1.8%
Bipolaris spp.	4	1.2%
Trichoderma spp.	3	0.9%
Scedosporium spp.	3	0.9%
Curvularia spp.	2	0.6%
Chaetomium spp.	2	0.6%
Scytalidium spp.	2	0.6%
Epicoccum spp.	1	0.3%
Paecilomyces spp.	1	0.3%
Sepedonium spp.	1	0.3%
Hormonema spp.	1	0.3%
Chrysosporium spp.	1	0.3%

Table 6. Different fungal isolates of Non-Dermatophytes

Yeast and yeast like	No. of isolates	percentage
Candida spp	54	73.9%
Aureobasidium spp	10	13.6%
Geotrichum spp	6	8.2%
Rhodotorula spp	3	4.1%

Table 8. Different fungal isolates of Mixed isolates

Mixed fungal isolate	Number of isolates	percentage
Trichophyton with Candida species	8	36.3%
Candida with Fusarium species	3	13.6%
Candida with Penicillium species	3	13.6%
Trichophyton with Fusarium species	3	13.6%
Trichophyton with Penicillium species	2	9.09%
Trichophyton with Acremonium species	1	4.5%
Candida with Aureobasidium species	1	4.5%
Scopulariopsis with Fusarium	1	4.5%

The highest prevalence was among the age group 16-30 years age group and second highest was among 31-45 years age group. The different fungi isolated were dermatophytes 166 (27.3%), non-dermatophytes 331 (55.9%), yeast and yeast like 73 (12.3%) and mixed fungal isolates 22 (3.7%) are shown in - Table 2. Different forms of fungal elements seen under direct microscopic examination is shown in Table 3. Finger nail involvement was more common 52.02% (308 patients) compared to toe nail involvement 47.9% (284 patients) (Table-4). Among the dermatophytes Trichophyton spp. 155 (93.3%) was the commonest isolate followed by microsporum spp. 7 (4.21%) – (Table – 5). Among non-dermatophytes, Aspergillus spp. 118 (35.6%) was the commonest followed by Penicillium spp. 57(17.2%), Mucor spp. 28(8.4%), Nigrospora spp. 24 (7.25%), Fusarium spp. 23 (6.9%), Rhizopus spp - 21 (6.3%), Zygomycetes spp - 9 (2.7%), Cladosporium spp- 8 (2.4%), Acremonium spp.- 8 (2.4%), Alternaria spp.- 8 (2.4%), Scopulariopsis spp. 6 (1.8%), Bipolaris spp - 4 (1.2%), Trichoderma spp - 3 (0.9%), Scedosporium spp - 3(0.9%), Curvularia spp - 2 (0.6%), Chaetomium spp - 2 (0.6%), Scytalidium spp - 2 (0.6%), Epicoccum spp - 1 (0.3%), Paecilomyces spp - 1(0.3%), Sepedonium spp - 1(0.3%), Hormonema spp -1(0.3%), Chrysosporium spp - 1(0.3%). Different fungal isolates of non-dermatophytes are shown in Table – 6. Among the yeast (Rhodotorula spp. 3) and yeast like (Candida spp 54, Geotrichum spp. 6, Aureobasidium spp -10) were 73 (12.3%). Different fungal isolates of yeast and yeast like is shown in Table – 7. And mixed fungal isolates were 22 (3.7%). Consisted of Trichophyton with Candida 8 (36.3%), Candida with Fusarium – 3 (13.6%), Candida with Penicillium – 3 (13.6), Trichophyton with Fusarium – 3 (13.6%), Trichophyton with Penicillium – 2 (9.09%), Candida with Aureobasidium – 1(4.5%), Trichophyton with Acremonium – 1(4.5%), and Scopulariopsis with Fusarium – 1(4.5%). Different fungal isolates of mixed growth are shown in Table – 8.

DISCUSSION

As onychomycosis is a chronic disorder with frequent relapses, it becomes imperative on the part of the clinician to identify the causative organism. Further, studying its various characteristics also gives an insight to the treating dermatologist with regard to management strategies. In the present study, 67.1% (592) samples were positive by direct examination and/or culture. In studies which were conducted by Kaur et al., Das et al., Jesudanam et al., and Aghamirian et al., 54.5 %, 51.76%, 45.53% and 40.2% samples respectively were found to be positive by direct examination and/or culture. (Kaur, 2007; Das, 2008; Jesudanam, 2002; Aghamirian, 2010). In our study, direct microscopy was positive in less cases than culture. The results were in accordance with the findings of study which was carried by Das et al, direct microscopy was positive in 32.94% cases, while culture was positive in 49.4% cases (Das, 2008). However, in the study which was conducted by Manjunath Shenoy et al., which showed positive results in 53% and 35% cases by direct microscopy and culture respectively (Devi, 2011). In contrast to most other studies that depicted a male preponderance for onychomycosis, our study demonstrated a female preponderance. Female susceptibility has also been demonstrated in our previous study Pukhrambam et al. (2011) and also reported by Jesudanam etal., (Jesudanam, 2002) Bokhari et al., (1999) Banerjee et al., (Velez et al., 1997) and Khosravi et al. (1994) Maximum patients in our study belonged to the 20-40 years age group. In contrast to Velez et al., and Mercantini et al. reported higher prevalence among adults who were over 50 years of age (Velez et al., 1997; Mercantini et al., 1996). Higher prevalence in this age group has also been substantiated by other studies. The occurrence of onychomycosis in this age group could be related to trauma following occupational and sporting activities, use of occlusive footwear, and cosmetic awareness. Moreover, in the elderly, even with the presence of this disease, many may not report to a clinician because of its asymptomatic presentation. In our study finger nails are mostly involved when compared to toe nails. In almost all studies, finger nail involvement predominated, except for studies by Gupta et al. (2007) and Ilkit (Ilkit, 2005), wherein toe nails were majorly involved. In our study, non-dermatophytes were the most common aetiological agents of onychomycosis; the roles of dermatophytes and yeasts in causing infections were also demonstrated.

In our study, the combined sensitivity of direct microscopy and culture was greater than those of direct microscopy and culture alone. This emphasizes the need of performing both tests. In the present study, females were more commonly infected and the age group of 31 - 45 years was more commonly involved. The changing trend of the causative organisms in causing onychomycosis should be given due importance. In this study we found that the prevalence of onychomycosis due to NDM and yeasts are taking an upper hand and this was one of the important findings of this study. In our previous study done during the period from January 2013 to December 2015 in RIMS hospital, Imphal the prevalence of non-dermatophytic onychomycosis was more than that of dermatophytic onychomycosis[21]. Here in this comparative study the change in the trend of onychomycosis is not very significant. Hence the trend of onychomycosis by dermatophytes and Non-dermatophytes remains the same in RIMS Hospital. Once known as soil contaminant, yeast and non - dermatophytic fungi has become the most common pathogen of onychomycosis which showed resistance with common available anti-fungal drugs as well. More studies like sensitivity of these organisms to suitable anti-fungal agents and the role of non - dermatophytic fungi in causing onychomycosis should be undertaken.

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