DERMATOPHYTE AND NON-DERMATOPHYTE ONYCHOMYCOSIS IN SINGAPORE

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SUMMARY

Onychomycosis is caused by dermatophytes, moulds and yeasts. It is important to identify the non-dermatophyte moulds as they are resistant to the usual anti-fungals. A prospective study was undertaken in the National Skin Centre, Singapore to study the pattern of dermatophyte and non-dermatophyte onychomycosis. 53 male and 47 female patients seen between June 1990 and June 1991 were entered into the study. Direct microscopy was done and the nail clippings were cultured. Toe and finger nails were equally infected. Dermatophytes were isolated from 21 patients namely, T. rubrum (12/21), T. interdigitale (5/21), T. mentagrophytes (3/21) and T. violaceum (1/21). Candida onychomycosis occurred in 39 patients and was caused by C. albicans (38/39) and C. parapsilosis (1/39). 37/39 patients had associated paronychia. 5 types of moulds were isolated from 12 patients, namely Fusarium species (6/12), Aspergillus species (3/12), S. brevicaulis (1/12), Aureobasidium species (1/12) and Penicillium species (1/12). Although the clinical pattern and microscopy may predict the type of organisms, in practice this is difficult. Only cultures were confirmatory. 28% (28/100) had negative cultures despite a positive microscopy, and moulds (12/100) grown might be contaminants rather than pathogens.

Key words: Moulds, yeasts, fungi, tinea, onychomycosis, dermatophyte, non-dermatophyte

INTRODUCTION

Onychomycosis, a common nail disorder, is caused by dermatophytes, non-dermatophyte moulds, or yeasts. Mixed infections have been reported but are rare. The incidence of onychomycosis due to dermatophyte or non-dermatophyte varies from place to place. The routine incorporation of cycloheximide in mycological media which inhibits the growth of moulds may explain the low incidence in some reports. The incidence of these organisms causing onychomycosis in Singapore is unknown. We carried out a prospective study to determine the pattern of dermatophyte and non-dermatophyte onychomycosis in patients attending the National Skin Centre in Singapore.

METHODS AND MATERIALS

100 consecutive patients, seen in our centre between June 1990 and June 1991, with a new clinical diagnosis of onychomycosis were included in the study. Diagnosis was confirmed by the presence of fungal elements (mycelium, arthrospores and yeast) on direct microscopy of nail and subungal scrapings dissolved with 30% potassium hydroxide. Only patients with a positive microscopy were included in the study. Patients with skin disorders causing onychodystrophy or patients with chronic mucocutaneous candidiasis were excluded from the study. The patients’ demographic data were recorded. The duration, symptoms, characteristics and location of the onychomycosis were recorded.

Scrapings from nails and nail beds were put in 30% potassium hydroxide for microscopy. Nail clippings from all patients were dissolved in 30% potassium hydroxide overnight and inoculated into two types of media. Where more than one site was involved, the specimens were pooled. Mycobiotic agar (Difco Laboratories, Michigan, USA) was used for growing dermatophytes and it contained Bacto-Soytone (10g/l), Bacto-
Dextrose (10g/l), Bacto-Agar (15g/l), Actidione (0.5g/l) and Chloromycetin (0.05g/l). Modified Sabouraud Agar (Difco Laboratories, Michigan) containing Bacto-Neopeptone (10g/l), Bacto-Dextrose (20g/l), Bacto-Agar (20g/l) and Chloromycetin (0.05g/l) was used to culture non-dermatophytes. All cultures were incubated at room temperature (25°C to 30°C). The cultures were examined on the 3rd, 6th, 9th and 12th day. Morphology of fungi colonies and growth characteristics were recorded. Cultures were again examined at six weeks for slow-growers. No growth at six weeks was considered negative.

Positive cultures were confirmed and identified by wet mounts and/or slide cultures when necessary. Candida species isolated were identified using germ tube production, morphology on cornmeal media and mycotube identification system (“Mycotube” Roche). The χ² test was used for statistical analysis. P values <0.05 were considered significant.

RESULTS

100 patients (53 male and 47 female) were included in the study. Their ages ranged from 3 years to 79 years, with a mean age of 45.2 years. The majority were adults from the third to the fifth decade. Figure 1 shows the age distribution of the patients and the type of organisms cultured. There were 68% Chinese, 21% Indians, 8% Malays and 3% comprising the other minority racial group. The racial composition of the general dermatological patients attending the Centre during this time was 76.4% Chinese, 9.5% Indians, 8.6% Malays and 5.6% others. There were no significant differences in the incidence of infection in the different races, when compared to the general dermatological population. Figure 2 shows the racial distribution and the organisms cultured. Onychomycosis was seen in all the occupation groups. However, 66% of the female patients were housewives doing wet work.

50/100 patients had onychomycosis for more than two years, 5/100 patients for less than a month and the remaining (45/100) had the infection from between 1 to 24 months. 66/100 patients presented because of deformity or dyschromia of their nails. The others had pain (16/100), itch (13/100) or both (5/100). The total number of nails involved varied from less than 5 nails (57/100 patients) to more than 15 nails (10/100 patients). Onychomycosis involved the finger-nails in 43/100 patients, the toe-nails in 35/100 patients and both finger and toe nails in 22/100 patients.

28% (28/100) had a negative culture despite a positive microscopy. Table 1 shows the type of
organisms cultured and the site. Dermatophytes caused infection in 21/100 of the patients (Figure 3), and only four species were cultured. These were *T. rubrum* (12/21), *T. interdigitale* (5/21), *T. mentagrophytes* (3/21) and *T. violaceum* (1/21). Those patients who had *T. mentagrophytes* infection had no animal contact and no zoophilic infection on another part of the body. As the zoophilic variety is unusual in toe nails and these patients had no sign of zoophilic infection elsewhere, we assumed that they might be an anthropophilic variety, the variety not identified. 12/21 of these patients had associated dermatophyte infection elsewhere and the same species of dermatophyte were cultured from the skin sites.

39/100 had Candida onychomycosis and these were caused by 2 species namely, *C. albicans* (38/39) and *C. parapsilosis* (1/39). All but 2 patients with Candida onychomycosis had associated paronychia. 12/100 had a positive culture for non-dermatophyte mould. The moulds isolated were namely, Fusarium species (6/12), Aspergillus species (3/12), *S. brevicaulis* (1/12), Aureobasidium species (1/12) and Penicillium species (1/12).

Dermatophyte onychomycosis affected more male patients whereas Candida onychomycosis were common in female patients (Figure 3). Mould onychomycosis affected both male and female patients equally. However, moulds were not recovered from the two extremes of ages (Figure 1). There was also no difference in the type of organisms recovered from the different races (Figure 2).

### TABLE 1

<table>
<thead>
<tr>
<th>Type of Organism Cultured by Site</th>
<th>Finger Nails</th>
<th>Toe Nails</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dermatophytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. interdigitale</em></td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>T. violaceum</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Moulds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. brevicaulis</em></td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Aureobasidium</em></td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td><em>Aspergillus</em></td>
<td>—</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Candida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>34</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Onychomycosis in children is uncommon, with a prevalence rate of from 0.2% to 0.6% in the general population. When it does occur the parents may act as the source of infection. The usual organism isolated is *T. rubrum*. However, one of our children had Candida onychomycosis. This is uncommon in children unless it occurs secondary to an underlying nail or systemic pathology. Our patient had no such predisposing factors but her mother had a similar condition suggesting that she could have been the source of infection. 95% of our patients were adults concurring with reports that onychomycosis is an adult disorder.

Onychomycosis is a chronic disorder and is often resistant to treatment. Our study concurred with this. Patients are usually asymptomatic and do not seek treatment, another reason for the chronicity of the infection. The presenting complaint in our study was cosmetic, noted in a previous report.

Dermatophyte onychomycosis usually starts in the toe-nails, especially the big toes. Candida onychomycosis, in contrast, affects the finger-nails early. In our findings, the initial site of infection was not predictive of the causative organisms, as Candida had been isolated from toe-nails alone and dermatophytes from finger-nails alone.

Zaias divided onychomycosis into four clinical types namely, distal subungual onychomycosis (DSO), proximal subungal onychomycosis (PSO), superficial white onychomycosis (SWO) and Candida onychomycosis. The majority of our patients (30/100) had total subungal onychomycosis and it was difficult to determine the clinical type. One patient had SWO of the toenail caused by *T. interdigitale*, which was also reported to be the commonest dermatophyte causing this infection. PSO, the rarest clinical type, was present in 2 of our patients. Both affected the toe-nails and was caused by *T. rubrum*. However, morphology was not a predictive feature to differentiate dermatophyte from non-dermatophyte onychomycosis in our patients, except for the presence of paronychia which tended to suggest a diagnosis of Candida onychomycosis.

The positive culture rate in onychomycosis, even in the presence of positive direct microscopy, varies from 30 to 50%. Therefore, methods like suction drill sampling have been suggested to increase the yield. Other authors...
have used scanning electron microscopy\textsuperscript{10} to help make the diagnosis. Dermatophyte onychomycosis is commonly due to \textit{T. rubrum, T. mentagrophytes} and \textit{E. floccosum}.\textsuperscript{5,11,12} Four types namely \textit{T. rubrum, T. interdigitale, T. mentagrophytes} and \textit{T. violaceum} were isolated in our study. There was no infection by \textit{E. floccosum} as this was not a common cause of tinea pedis in Singapore. The commonest dermatophyte recovered was \textit{T. rubrum}, which is also the commonest dermatophyte reported previously.\textsuperscript{4,12,13} Dermatophyte onychomycosis occurred in all age groups and was more common in male than female patients, as is reported elsewhere.\textsuperscript{4,11}

Candida onychomycosis occurs in three patterns;\textsuperscript{4} namely (a) associated with chronic mucocutaneous candidiasis, (b) associated with paronychia and lateral onycholysis and (c) associated with primary distal and lateral onycholysis. The majority of our patients (37/39) had associated paronychia suggesting that the nail infection was secondary to Candida paronychia. However, in two patients paronychia was absent and there was also subungual hyperkeratosis suggesting that the onychomycosis was a primary event. It is important to differentiate between these two types as it has been reported that the latter pattern responds poorly to topical agents.\textsuperscript{14,15} The organisms responsible for the onychomycosis were \textit{C. albicans} and \textit{C. parapsilosis}. Our findings concurred with previous reports that Candida onychomycosis is more common in females and affects finger nails more.\textsuperscript{4,11} Those with toe-nail infections had concomitant finger-nail infections, which probably explained the relatively high numbers of toenail Candida onychomycosis in our study. The infection was uncommon in the extremes of age where wet-work and hence paronychia was less of a problem.

Onychomycosis due to moulds has been reported to occur on dystrophic nails and hence to have a higher incidence in older patients.\textsuperscript{12,13} Our study showed that it was common in the second to the fifth decade, with no cases in the extremes of age. The toe nails were affected in all cases, concurring with previous reports.\textsuperscript{4,11,13} In two patients their finger nails were infected in addition to their toe-nails. Moulds reported to cause onychomycosis include \textit{Aspergillus} species,\textsuperscript{13,16} \textit{Fusarium} species,\textsuperscript{17,18} \textit{Hendersonula toruloidea},\textsuperscript{19} \textit{S. brevicaulis}\textsuperscript{20} and others.\textsuperscript{4,11,13} \textit{S. brevicaulis}, Aureobasidium, Fusarium spp., Aspergillus spp. and Penicillium were isolated in our study. It was felt that \textit{Aureobasidium} and \textit{Penicillium} were contaminants as the cultures were not reproducible. The other moulds were considered to be significant using the criteria suggested by English,\textsuperscript{13} that is, positive microscopy and repeated cultures without isolation of dermatophytes.

Six of our patients had \textit{Fusarium} onychomycosis. Although normally known as a skin contaminant, \textit{Fusarium} had been reported to cause fungal keratitis,\textsuperscript{22} superficial onychomycosis\textsuperscript{4} and even osteomyelitis.\textsuperscript{23} The commonest species reported to cause disease was \textit{F. oxysporum}. We were unfortunately unable to identify the species.

This study showed that onychomycosis in Singapore is caused by either dermatophytes (21%) or yeasts (39%). Moulds (12%) are an uncommon cause. Moulds are resistant to the commonly used anti-fungal agents and hence their identification is important in preventing such medications from being prescribed. Methods used to distinguish them include the clinical pattern and site of infection. For example PSO has not been documented to be caused by mould and yeasts. Microscopy may help as some moulds have a fronded appearance. Their hyphae are hyaline, have a more variable width, swollen or distorted\textsuperscript{24} and in some may be pigmented or appear double-contoured.\textsuperscript{25} Although available, these measures are usually not helpful in the clinical situation. Therefore, culture media without cycloheximide is used if non-dermatophytes are suspected. The significance of any non-dermatophyte isolated must be confirmed by isolating the organisms in 5 out of 20 inocula without concomitant isolation of any dermatophytes (as suggested by English\textsuperscript{13}).

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