INTRODUCTION

Onychomycosis is defined as a fungal infection of finger and toe nails. It occurs worldwide and is mainly caused by dermatophytes and yeasts. In recent years, non-dermatophytes have also been implicated as probable causative agents of onychomycosis. The incidence of nail infection has increased in parallel with an expanding number of elderly and immuno-compromised patients. Although fungal nail infections are not life-threatening, they are associated with secondary bacterial infection, chronicity of disease, therapeutic failures and disfigurement like hyperkeratosis, discoloration of nail plate, and brittle nails.

Non-dermatophytes are filamentous fungi which are commonly found in nature as soil saprophytes and plant pathogens. It is not known whether non-dermatophyte infections occur as a primary ailment on healthy nails or exist as secondary invaders in already damaged nails by ischemia, trauma or other diseases. Non-dermatophyte moulds (NDMs) which are regularly identified in onychomycosis include Alternaria species, Scytalidium species, Fusarium species, Acremonium species, Scopulariopsis species, Cladosporium species and Aspergillus species. Incidence of NDMs has risen dramatically due to wide spread use of broad spectrum antibiotics, immuno-suppression, chemotherapy, diabetes and invasive procedures. Treatment of onychomycosis caused by NDM is difficult as both topical and systemic antifungals are ineffective. Terbinafine has the highest in vitro anti-fungal activity against NDMs when compared to fluconazole, itraconazole and griseofulvin.

The causative agents of the disease may vary depending upon geographic or temporal distribution. Even in developed countries, the importance of nail infections has been highlighted only in the last decade. In developing countries like Pakistan, socioeconomic constraints and other common prevalent health issues have led to a low awareness of onychomycosis by physicians and general population. Thus, even in the presence of good personal hygiene, it has continued to persist and spread.

This study was aimed to find out the pattern of non-dermatophyte moulds (NDMs) as causative agents of onychomycosis.

METHODOLOGY

The study was carried out at Microbiology Department, Armed Forces Institute of Pathology, from November 2009 to July 2010. A total of 100 nail specimens were included in this study with different nail abnormalities like subungual hyperkeratosis, nail thickening, longitudinal and transverse grooves, brittle nails, pitting of nails, cracking of nails, any visible change in colour of nail plate such as blackening, white or yellow.
discoloration. Patients ending up with treatment failure for dermatophytes and direct microscopy negative samples were included. Patients receiving anti-fungals, those suffering from other skin diseases like Psoriasis and Lichen planus, and repeat samples of the same patient were excluded from the study.

After taking a thorough history and demographic details on specifically designed proforma, nail was scrubbed with 70% ethyl alcohol and subungual keratinous debris were collected along with nail clippings. Subungual keratinous debris was immersed in a drop of 40% KOH over a slide and kept in humidified boxes overnight at room temperature to be seen on direct microscopy to detect any hyphae. The nail samples were simultaneously inoculated on 3 different points on Sabouraud’s dextrose agar (SDA) (Oxoid, UK), SDA containing chloramphenicol (0.05 gm/l), with and without actidione (100 mg/l). The inoculated specimen were incubated in cold incubator (22°C) for up to 3 weeks after placing them in polythene zipper bags to prevent contamination.7,8 The culture plates were examined twice weekly for any growth. Colony morphology of any growth appearing was noted. Species identification was done through colony morphology and microscopic examination of lactophenol blue preparation. Indentification of Candida species was done by using Candida chromagar (DIFCO, USA) and API 20c Aux (Biomerieux, France). On chromagar, greenish growth was taken as Candida albicans, blue as C. tropicalis, purple as C. glabrata and pink as C. krusei.9

Statistical analysis was done using the SPSS (version 17.0) statistical package. Descriptive analysis were used to calculate mean and standard deviation for age. Frequencies and percentages were obtained for gender and pattern of species (spp.).

All ethical considerations and obligations were duly addressed and the study was conducted after approval of ethical committee.

RESULTS

Out of 100 nail specimens, 64 were from females and 36 from males. Male to female ratio turned out to be 1:1.8. Age of the patients ranged from 04-70 years. The age group most affected was 30-40 years with mean of 36.575±15.7 years.

Out of 100 nail specimens, 47 revealed positive fungal growth. Non-dermatophytes moulds (NDM) accounted for 32 (68%) of total culture positive cases, yeasts 8 (17%) and dermatophytes 7 (15%).

Among the NDMs Alternaria alternata was most common isolate followed by Trichophyton rubrum, Trichophyton mentagrophytes and Trichophyton tonsurans (Table II). Among yeasts, Candida glabrata was the most common followed by Candida albicans and Candida krusei (Table III).

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>15</td>
<td>46.8%</td>
</tr>
<tr>
<td>Fusarium dimerum</td>
<td>5</td>
<td>16%</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>3</td>
<td>9.2%</td>
</tr>
<tr>
<td>Cladosporium sphaerospermum</td>
<td>5</td>
<td>16%</td>
</tr>
<tr>
<td>Scopulariopsis brevicaulis</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Scytalidium dimidiatum</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Cladophialophora carrionii</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Ulocladium chartarum</td>
<td>1</td>
<td>3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton interdigitale</td>
<td>3</td>
<td>43.5%</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>2</td>
<td>28.5%</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>1</td>
<td>14.0%</td>
</tr>
<tr>
<td>Trichophyton tonsurans</td>
<td>1</td>
<td>14.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida glabrata</td>
<td>5</td>
<td>62.5%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>25.0%</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>1</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

DISCUSSION

Timely diagnosis of fungal infections is mandatory for administering appropriate anti-fungal therapy. Inaccurate clinical diagnosis not only prolongs symptoms and discomfort of the patient but it also results in economic drain due to expensive anti-fungal therapy.10 With growing interest in pathogenic fungi and better awareness of the importance of fungal infections in differential diagnosis, the results of this study would be very valuable for the dermatologists and physicians in the area.

The major finding of this study was that abnormal nails in patients who were treated for dermatophytes were frequently colonized by NDMs. Although it is difficult to ascertain the role of NDMs as primary pathogens of the nails but studies are required to evaluate their ability to invade intact healthy nails, which is the characteristic of dermatophytes. However, NDMs may colonize nails that were damaged by occupational related trauma especially in young age group, immunocompromised conditions, poor peripheral circulation and peripheral neuropathy.

In this study non-dermatophytes accounted for 68% of the total fungal isolates. Amongst NDMs Alternaria
*Alternaria alternata* was the most common isolate (46.8%). The prevalence of NDMs varies considerably in different studies reported in the literature. In a study conducted in 2006 in Egypt on 32 patients with different nail abnormalities, it was found that NDMs were isolated from 59% of the total culture positive cases. Most of these NDMs were recovered from specimen taken from housewives with *Aspergillus* species being the commonest.5

A study conducted in Iraq in 2008, revealed that *Candida* species were most common followed by *Trichophyton* spp., *Alternaria alternata* and *Cladosporium* spp.11 While in the present study *Alternaria alternata* was the commonest species followed by *Cladosporium* spp., *Candida* and *Trichophyton*. Comparable results were seen in a local study conducted at Rawalpindi in 2007 which showed that among non-dermatophytes *Alternaria alternata* was most commonly isolated species followed by *Scytalidium dimidiatum*, and *Penicillium marneffei*.12

A multi-centre study conducted on large scale in North America to find out the frequency of pathogens involved in onychomycosis revealed that NDMs and yeasts accounted for 20% each of the two varieties. *Fusarium* spp., *Acremonium* spp., and *Scopulariopsis* species, were the commonest non-dermatophyte species in that study accounting for 34%, 29% and 20% respectively of the culture positive NDMs.13 Comparable results to ours were found in a study conducted in Italy which revealed *Fusarium* species as the most common NDMs followed by *Scopulariopsis brevicaulis*, *Acremonium* and *Aspergillus* species.4 Similarly, *Fusarium* was the most common species accounting for 59% of total culture positive NDMs followed by *Acremonium* spp. *Scopulariopsis* spp., *Alternaria* spp. and *Geotrichum candidum* in a study carried out in Argentina.14 This study also yielded fairly large percentage of *Fusarium* spp. 25% of the total NDMs. Studies carried out in Sri Lanka,15 Colombia16 and Pakistan12 during the last couple of years also revealed large percentage of *Fusarium* spp. from patients of onychomycosis.

On the contrary, the most common NDM isolated in studies reported from Europe,17 North America4 and Mexico18 were *Scopulariopsis* followed by *Aspergillus*. In our case, the isolation of these two fungal pathogens were very low. *Scytalidium dimidiatum* has been reported previously at our centre12,19 in two different studies but in this study only one isolate of the species was recovered from patients of onychomycosis.

Looking back at some of the studies carried out in the past, it was evident that in our country *Candida* spp. was reported as commonest pathogen followed by *Trichophyton*, *Fusarium* and *Alternaria* species.20 Similarly, dermatophytes were reported as principal pathogen followed by non-dermatophytic moulds in studies from Mexico and Malaysia in patients of onychomycosis.21,22 The isolation of dermatophytes from cases of onychomycosis in this study was quite low accounting for only 15% of the total isolates. A probable explanation for this might be that the majority of patients in this study were immunocompromised in one way or the other and most of them have been previously treated for dermatophytes without improvement.

Contrary to the popular belief of NDMs esp. *Alternaria alternata* as a contaminant, in this study, a large percentage of *Alternaria alternata* were isolated. They were considered important because of point specific growth on multiple media used. They are being increasingly isolated and reported in different studies across the globe.5,11,12,14,20 Epidemiological investigations should be performed in every country in order to determine the fungal species associated with abnormal nails.

**CONCLUSION**

There was a large percentage of onychomycotic patients whose nails were colonized with non-dermatophytes. This group of fungi can assume the role of a pathogen in immunocompromised individuals, those using broad spectrum antibiotics or subjected to localized trauma. These non-dermatophytes should be considered as important pathogen with a high index of suspicion in evaluating the patients with culture negative for dermatophytes or those subjects ending up in treatment failure.

**REFERENCES**


22. Ng KP, Soo Hoo TS, Nik SL, Ang LS. Dermatophytes isolated from patients in University Hospital, Kuala Lumpur, Malaysia. *Mycopathologia* 2002; **155**:203-6.