Frequency of Candida in onychomycosis
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Abstract
Objective: To assess the frequency of Candida in cases of onychomycosis at a tertiary care facility in an urban area.
Methods: The cross-sectional study was carried out from September 2009 to July 2010 at the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. It involved 124 patients, who were clinically diagnosed as cases of onychomycosis with the collaboration of the hospital’s Dermatology Department. The specimens were processed for the diagnosis by mycological culture using Sabouraud’s dextrose agar at 25ºC and at 37ºC. Observation for growth was done periodically for 4 weeks; if growth was present, the pathogen was identified by cultural characteristics and microscopy. Descriptive statistics (with frequency and percentage) of the type of fungus and organisms were evaluated.
Results: Of the 124 cases, 52 (42%) tested positive and represented the final study sample. Candida was observed in 16 (30.76%) patients.
Conclusion: There was high frequency of Candida involvement in clinically diagnosed cases of onychomycosis.
Keywords: Onychomycosis, KOH mount, Fungal culture, Candida. (JPMA 63: 350; 2013)

Introduction
Onychomycosis is defined as invasion of the nail plate by a fungus which may be due to dermatophyte and non-dermatophyte mold or yeast.1

It is a common problem and affects approximately 5% of the population worldwide2 and represents up to 50% of all nail diseases.3 The incidence is increasing worldwide and now it is no more considered merely a cosmetic problem.4

Among the dermatophytes, Trichophyton rubrum (T. rubrum) is the major cause of onychomycosis.5 Of the non-dermatophytes, Candida species is the most common, alongside saprophytes molds such as Acremonium species, Scopulariopsis species, Scytalidium species, Aspergillus species and Fusarium species. Many of these are found in soil or plant material.6

The leading non-dermatophyte mold cause of onychomycosis is Scopulariopsis brevicaulis and the leading yeast cause of onychomycosis is Candida albicans. More than one fungus species may be jointly involved in causing the infection.7,8

In 1998, a study presented a new classification of onychomycosis dividing the pattern of nail plate involvement by mode and site of invasion into five clinical types: distal lateral subungual onychomycosis (DLSO), white superficial onychomycosis (WSO), proximal subungual onychomycosis (PSO), endonyx onychomycosis (EO), and candidal onychomycosis. Patients may have a combination of these subtypes. Total dystrophic onychomycosis refers to the most advanced form of any subtype. Therefore, Candida onychomycosis is not a separate category in the new classification.9

Candida nail infections occur in patients with chronic mucocutaneous candidiasis, and are caused by C. albicans.10 Candida species (spp) may cause other syndromes, including onycholysis and paronychia.10 These forms occur more commonly in women than in men and often affect the middle finger, which may come into contact with Candida organisms that reside in the intestine or vagina.11 Candida onychomycosis can be divided into three general categories: Infection beginning as a paronychia, the most common type of Candida onychomycosis;12 patients with chronic mucocutaneous candidiasis are at risk for Candida granuloma and account for less than 1% of onychomycosis cases.11-13 It involves direct invasion of the nail plate;14 Candida onycholysis, which is more common on the hands than the feet.10

The general risk factors for any type of onychomycosis are increasing age, male gender, immunodeficiencies,
diabetes, nail trauma, hyperhydrosis, peripheral vascular diseases, poor hygiene and chronic exposure of nails to water in candidal onychomycosis.\textsuperscript{13}

**Materials and methods**

The cross-sectional study was carried out on 124 patients who were clinically diagnosed as cases of onychomycosis from September 2009 to July 2010.

The samples were taken from the Dermatology Department, Jinnah Postgraduate Medical Centre (JPMC), Karachi, and were processed for the diagnosis by mycological culture. Only culture-positive samples were included in the study.

The sample size calculation was done using the World Health Organization software for Sample Size Calculation edited by L. Lemeshow and S. K. Lwanga.\textsuperscript{14} The results of our study are valid where \( \alpha = 0.05 \), \( 1- \beta = 90 \), \( Po = 0.25 \), \( Pa = 0.37 \), and \( n \) (sample size) =124.

All patients suspected with clinical diagnosis of onychomycosis, irrespective of age or gender referred by the Dermatology Department were included in the study. Except for onychomycosis, all other lesions were excluded.

Specially-designed proforma was used for data collection. The 124 specimens were received at the Microbiology Department of the Basic Medical Sciences Institute (BMSI) of JPMC. They were examined and processed for fungal culture and only those testing positive for fungal culture were included.

Processing for mycological culture was done by using Sabouraud’s dextrose agar with (cycloheximide and chloramphenicol) and without antibiotics at 25ºC and at 37ºC. Specimens were placed into the Sabouraud’s dextrose agar accordingly. Observation for growth was done periodically for 4 weeks. If there was growth, the pathogen was identified by cultural characteristics and microscopy.\textsuperscript{15}

For observing the microscopic features of the isolates, lactophenol cotton blue stain (LPCB) was used. A piece of colony (fungal growth) was taken with the help of sterilised needle and put on a clean (flamed) slide containing one drop of LPCB. The slide was observed under the microscope using low X10 and high X40 power objectives.

The specimen were kept at room temperature and body temperature to know dimorphism. Sabouraud Dextrose Agar vials without antibiotic were used to observe the growth of non-dermatophytes. The growth on cycloheximide-free medium indicates that the infective agent may be a non-dermatophyte because cycloheximide inhibits the growth of non-dermatophytes.\textsuperscript{16}

Descriptive statistics (with frequency and percentage) of the type of fungus and organisms were evaluated. Data was collected and results tabulated.

**Result**

Of the 124 clinically diagnosed cases of onychomycosis, 52 (42\%) patients were included in the study who had tested positive. Among them 36 (69.23\%) were females, while 16 (30.77\%) were males. Of the 52, 35 (67\%) were between 14-30 years old; 6 (11.5\%) were 31-40 years old; 4 (7.7\%) were 41-50 years age; and 7 (13.46\%) patients were above 50 years of age. Age range was 14-80 years.

Maximum patients (n=19; 36.54\%) had attended dermatology clinic within six months of the disease; 6 (11.54\%) attended within 6-12 months; 13 (25\%) within 1-3 years; 6 (11.54\%) attended in 3-5 years; and 8 (15.38\%) consulted the clinic after 5 years of nail infection.

Thumb and fingernails were involved in 30 (57.77\%) patients, while in 22 (42.3\%) patients toenails were involved. More than one nail involvement was observed in 16 (30.76\%) patients.

The fungi, which were isolated from the culture,

<table>
<thead>
<tr>
<th>Type of fungus</th>
<th>No. of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatophytes</td>
<td>26</td>
<td>50%</td>
</tr>
<tr>
<td>Non-dermatophytes</td>
<td>26</td>
<td>50%</td>
</tr>
</tbody>
</table>

Table-2: Organisms identified.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Positive (n)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. rubrum</td>
<td>17</td>
<td>32.69%</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>8</td>
<td>15.39%</td>
</tr>
<tr>
<td>T. tonsurans</td>
<td>1</td>
<td>01.92%</td>
</tr>
<tr>
<td><strong>Candida Albicans</strong></td>
<td>14</td>
<td>26.92%</td>
</tr>
<tr>
<td>Other species</td>
<td>2 (16)</td>
<td>03.85%</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>4</td>
<td>07.69%</td>
</tr>
<tr>
<td>Chrysosporum spp.</td>
<td>2</td>
<td>03.85%</td>
</tr>
<tr>
<td>Aspergillus fumigates</td>
<td>2</td>
<td>03.85%</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>1</td>
<td>01.92%</td>
</tr>
<tr>
<td>Scopulariopsis spp.</td>
<td>1</td>
<td>01.92%</td>
</tr>
<tr>
<td>Total organisms</td>
<td>52</td>
<td>100%</td>
</tr>
</tbody>
</table>
reflected the presence of dermatophytes in 26 (50%) and non-dermatophytes in 26 (50%) isolates (Table-1).

Among the dermatophytes, T. rubrum (n=17; 32.69%), T. mantagrophytes (n=8; 15.39%), T. tonsurans (n=1; 1.92%) were isolated, while among the non-dermatophytes, Candida was the most common isolated species (n=16; 30.77%) (Table-2).

Discussion
Onychomycosis is the invasion of the nail plate by fungus which may be due to dermatophytes, non-dermatophyte molds or yeast. It occurs in all ages, but children have infection rates lower than the adults. Female preponderance was observed in our study with a male-to-female ratio of 1:2.25 while Elewski showed a male preponderance. Also, Cohen et al included the male gender as a general risk factor for onychomycosis. Bokhari et al observed 72% female patients in their study in Department of Dermatology, King Edward Medical College/Mayo Hospital, Lahore, Pakistan. This was a local study and the results were consistent with our findings.

Cohen et al described the general risk factors for any type of onychomycosis, and in particular pointed out chronic exposure of the nails to water as a significant risk factor which are consistent with our study as we found these factors present in most of the patients. In our study, Of the 16 patients in whom Candida was isolated, 14 were female, and majority of them were poor and working as housemaids which involves using water in the kitchen, in the cleaning of the house, washing clothes, using detergents etc.

Gianni et al found positive fungal culture in 90 (52.9%) cases, showing a dermatophyte in 45, a yeast in 23 and a mold in 22 samples. Of the 90 positive samples, 45 each were dermatophytes and non-dermatophytes. In the 45 non-dermatophytes, 23 (25.55%) were yeast, and 22 (24.44%) were molds. In our study dermatophytes were 26 (50%), while non-dermatophytes were 26 (50%) in number. In non-dermatophytes, 16 (30.77%) were yeast, while 10 (19.23%) were molds. In our study the percentage of the organisms isolated was consistent with the findings of Gianni et al.

In a study by Bokhari et al in Lahore, Candida was the most common pathogen (46%). Dermatophytes were found to be 43% which is comparable with our study in which the dermatophytes were 50%. Among these dermatophytes, Bokhari et al isolated Trichophyton rubrum in 31% cases, which is consistent with our study where it was 32.69%.

Shenoy et al isolated fungi in 35 (35%) patients, with dermatophytes in 11 (31%), Candida in 2 (6%), and non-dermatophyte molds in 22 (63%) patients. Similar results have been reported by others.

Elewski found onychomycosis by Candida in 8.7%, while Venugopal found exceptionally high incidence of Candida onychomycosis of the toenails and it was suggested that this phenomenon might be related to the Muslim religious practice of washing the feet five times a day.

Faergemann observed that Candida albicans was the most frequent species causing onychomycosis which is consistent with our study where Candida albicans was isolated in 14 patients in a total of 16 Candida spp. isolation.

In the USA, a survey of 1,038 outpatients in a dermatology clinic demonstrated culture-proven onychomycosis and that Candida was responsible for 50% of these cases. In this study Candida involvement was much higher than in our study.

Sarma et al in a study in north India observed Candida spp. in 40.4% of the patients in a study population of 302 clinically suspected cases of onychomycosis, which is comparable to our study.

Khosravi and Mansouri in a study conducted in Tehran, Iran, examined a total of 187 patients with suspected onychomycosis. Laboratory examination confirmed onychomycosis in 115 patients, of which 97 cases were presented with positive microscopic and cultural examinations. Among them 43.3% were infected with Candida spp, which is again very high compared to our study.

Conclusion
There was a high frequency of Candida involvement in clinically diagnosed cases of onychomycosis.

References