Incidence of Dermatophyts Infection in Patients Visiting a Tertiary Care Centre

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\textbf{Abstract}

Introduction: India is a large subcontinent with remarkably varied topography, situated within the tropical and subtropical belts of the world. Its climate is conducive to the acquisition and maintenance of mycotic infection. Hence, dermatophytosis is an important disease affecting the dermal structures in people of India. It is most frequently during the monsoon. Aim & Objective: To study the incidence of Dermatophytosis. The isolation and identification of various Dermatophytes causing skin, hair and nail infection in the patients attending a tertiary care center in Navi Mumbai. Materials & Methods: A total of 66 patients were selected from MGM Hospital in Navi Mumbai. The patients were examined under normal lights for removal of infected hair, skin and nails in the form of scales, crust and hair stumps. The infected hair, scalp and nail particles were inoculated on the slanted surface of Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide (SDA+cc) and Dermatophyte Test Medium (DTM) and incubated at 27–30 °C for up to six weeks. Species of the dermatophytes isolated during this study were identified on the basis of their growth characteristics and microscope morphology. Results: Sixty-six clinically diagnosed cases of dermatophytosis were studied. Tinea corporis 35 (53%) was the commonest clinical type followed by Tinea unguium 16 (24%), Tinea cruris 5 (7.5%), Tinea pedis 3 (4.5%), Tinea barbae 3 (4.5%), Tinea capitis 3 (4.5%) and Tinea manuum 1 (2%). Commonest age group affected was 21-30 years. The ratio of Male and female was found to be 3:4:1. Fungi were demonstrated in 78.89% cases, either by direct microscopy and/or culture. Of the dermatophytes isolated, Tricophyton rubrum 15 (38.46%) was the commonest followed by Tricophyton mentagrophytes 13 (33.33%) and Tricophyton tonsurans 7 (17.95%). In the 4 cases (10.26%) of tinea unguium fungi other than dermatophytes were isolated. Conclusion: Dermatophyte infections are very common in our country where hot and humid climate in association with poor hygienic conditions play an important role in the growth of these fungi. Trichophyton species forms commonest a etiological agent of dermatophytosis. Tricophyton rubrum was the commonest isolate Tricophyton mentagrophytes was second and Tricophyton tonsurans was third commonest isolates in different species of dermatophytes.

1. Introduction

Dermatophytosis is an important disease affecting the dermal structures in people of India. Its climate is conducive to the acquisition and maintenance of mycotic infection. Hence, it is most frequently
during the monsoon (Singh Suman., et al., 2003). Dermatophytes are keratinophilic fungi, all of which produce keratinases, which cause infections of the skin, hair and nails called “dermatophytosis” or “ring worm”. These infections generally remain limited to non-living keratinized layers, but the infection may proceed more deeply than for the superficial mycoses, and a variety of pathologic changes can occur depending on the fungus, the site of infection, and the immune status of the host (Woods JP., 2002).

Dermatophyte infections can affect the skin on almost any area of the body. Redness, scaling, crack or crack of the skin, or a ring with outer edge is more inflamed and scaly than the plane centre. So, it often looks like a ring that becomes gradually larger. These infections are usually pruritus. An area of hair loss may result due to infection involvement of the scalp, an abscess or cellulitis may lead to more aggressive infections. Areas infected by dermatophytes may become secondarily infected through bacteria. Symptoms usually appear between 4 and 14 days following exposure.

The dermatophytes having three genera (http://www.cdc.gov/nczved/divisions/dfbmd/diseases/dermatophytes/)

Epidermophyton- Its produces only macroconidia, no microconidia and consists of 2 species, one of which is a pathogen.

Microsporum- Both microconidia and rough-walled macroconidia characterize Microsporum species. The 19 species are described but only 9 species are involved in human or animal infections.

Trichophyton- characterized by the development of both smooth-walled macro- and microconidia. 22 species are known and most of the species causing infections in humans or animals (www.provlab.ab.ca/mycol/tutorialderm/dermwhat).

The species of dermatophytes are differentiated by Microconidia & Macroconidia. Clinically, ringworm can be classified depending on the site involved. These include *Tinea capitis* (scalp), *Tinea corporis* (non-hairy skin of the body), *Tinea cruris* (groin), *Tinea pedis* (foot) or athlete’s foot and *Tinea barbae* or barber’s itch (bearded areas of the face and neck). Favus is a chronic type of ringworm involving the hair follicles (Ananthanarayan R., et al., 2009). The diseases caused by non-dermatophytic fungi infecting skin are called as dermatomycoses whereas hair and nail are known as piedra and onychomycoses (http://www.cdc.gov/nczved/divisions/dfbmd/diseases/dermatophytes/).

In the recent times few cases of subcutaneous and deep fungal infections have been reported to be caused by dermatophytes. Dermatophyte infections are more common in adolescents and adults.

For a definitive diagnosis and proper treatment of such cases, a proper laboratory back up is important. There have been no previous studies regarding the incidence of Dermatophytosis in Navi Mumbai. Hence the present study was undertaken.

2. Aims & Objective

To study the incidence of dermatophytosis in various patients attending a tertiary care centre in Navi Mumbai.

To isolate and identify various dermatophytes causing skin, hair and nail infection.

3. Materials and Methods

Dermatophytosis is an important disease affecting the dermal structures in people of India. Dermatophytes can survive solely on outer cornified layers of the skin. The ability of certain fungi to adhere to particular host arises from numerous mechanism and host factors including the ability to adapt to the human body. Natural infection is acquired by the deposition of viable arthrospores or hyphae on the surface of the susceptible individual. After the inoculation in the host skin, suitable conditions favour the infection to progress through the stages of adherence and penetration. After overcoming obstacles (ultraviolet light, temperature and moisture variation) and competing with the normal flora and sphingosines produced by keratinocytes and the fatty acid produced by the sebaceous glands, the arthroconidia (infectious element) adhere to the keratinized tissue. The germination of arthroconidia and hyphal growth adherence proceed radialy in multiple directions it is most frequently during the monsoon sessions.

A total of 66 patients were selected from MGM Hospital in Navi Mumbai. The patients were examined under normal lights for removal of infected hair, skin and nails in the form of scales, crust and hair stumps. The infected hair, scalp and nail particles were inoculated on the slanted surface of Sabouraud dextrose agar with chloramphenicol and cycloheximide (SDA+cc) and Dermatophyte test medium (DTM) and incubated at 27–30 °C for up to six weeks. Species of the dermatophytes isolated during this study were identified on the basis of their growth characteristics and microscope morphology.
3.1 Selection of Cases:

Inclusion criteria – Both sexes of all age groups patients, attending outpatient department of Skin and Venereology, MGM hospital, Kamothe, Navi Mumbai were taken for the study.

Exclusion criteria – Patients already under antifungal treatment were excluded from the study group.

After taking detailed history and clinical examination of patient was made in good light which included site of lesion, number of lesions, types, presence of inflammatory margin, etc.

3.2 Specimen Collection:
The affected area was cleaned with 70% ethyl alcohol, skin scales, crusts and pieces of nail or hairs were collected in clean black paper and in the case of hair collected on clean white paper packets. Skin specimen was collected by scraping across the inflamed margin of lesion into the apparently healthy tissue.

Nail specimen – The specimens of nails was collected by taking small pieces of the infected part and scrapings beneath the nail.

Hair specimen – The specimens of infected hairs was collected by tweezing with epilating forceps along with the base of the hair shaft around the follicle.

3.3 Direct Microscopic Examination

KOH mount:– Emulsify the specimen in a drop of 10% or 40% KOH on a microscopic slide with help of a straight wire.
Apply gentle heat by passing the slide over a Bunsen flame for 3-4 times.
Cover the smear with cover slip.
Leave it for 10-15 minutes. But in the case of hair or nail wait for overnight.
Examine the slide under low power (10x) and high power (40x) magnification.
Examine the slide for 15-20 minutes for demonstration of shining fungal elements.

3.4 Culture of Sample:
After direct microscopic examination, the specimen was inoculated on Sabouraud’s dextrose agar containing 0.05% chloramphenicol and 0.5% cycloheximide and the other Dermatophyte test medium (DTM).

3.4a Sabouraud’s dextrose agar with chloramphenicol and cycloheximide:
The standard medium for growing dermatophyte is Sabouraud’s dextrose agar containing chloramphenicol and cycloheximide, which inhibit the growth of bacteria and saprophytic fungi respectively. The cycloheximide (Actidione) in a concentration of 0.1 to 0.4 mg per ml suppresses the growth of most saprophytic fungi without deterring the growth of dermatophytes.

Autoclave the above mentioned ingredients at 121 °C for 15 minutes and adjust final pH at 5.6. Dispense in tubes and allow cooling in slanted position.

3.4b Dermatophyte test medium:
Specimens from skin, hair or nail were inoculated directly onto DTM and incubated at room temperature with the cap. Dermatophytes change the medium turned yellow to red within two weeks. Care must be taken because as many contaminants and other fungi increase the number of false positive changes in colour. DTM does not interfere with macroscopic morphology and microscopic characteristics of the dermatophytes, but it cannot be used to study pigment production because of the intense red colour of the indicator. The phenol red solution is 0.5 gm in 15 ml of 1N NaOH made up to 100 ml with distilled water. The medium is incubated at 25 °C.

3.5 Macroscopic Examination of Culture:
The growth on Sabouraud’s dextrose agar was examined to study the colony morphology based on following characteristics.

a) Colony characters on obverse: The colour (white, pearl, ivory) and consistency (cottony, velvety, fluffy, suede).

b) Colony characters on the reverse: presence or absence of pigment production and diffusion.

3.6 Microscopic Examination of Culture:

Tease mount:– The tease mount was observed under low and high power objective of microscope, for the presence of hyphae, macroconidia, microconidia and other accessory structures of vegetative hyphae and the characters of each was noted.

Procedure:–
Place a drop of lactophenol cotton blue on a clean glass slide.
Remove a small portion of the colony and the supporting agar at a point between the centre and periphery and place it in the drop of LPCB.
With a needle, tease the fungal culture first and spread in the LPCB and cover with cover slip.
Examine microscopically after giving sufficient time for the structure to take up the stain, usually 30 minutes.
Examine the slide under low power (10x) and high power (40x) magnification.

3.7 Slide Culture of Fungal Isolates:
Procedure:–
From the petri dish containing Potato dextrose agar cut out 1 cm square block of agar for each slide culture to be inoculated. With a flat side of a sterile inoculating striate wire, or with a spatula, place an agar block in the centre of the slide in the slide culture set up. Around the periphery of the one square centimeter block of agar, inoculate the fungal strain under identification at four sides of agar block. Cover the inoculated block with sterile coverslip with help of sterile foresip and incubate at 25°C in BOD incubator. With a pipette, thoroughly moisten, but not to excess, the filter paper with sterile distilled water. Incubate the slide culture at room temperature. Remove the slide culture from the petri dish and dry the bottom of the slide with a tissue. When growth appears, take a slide place a drop of LPCB on it, and place the cover slip removed from the block on the LPCB. Place the slide on the microscope stage and examine. The aerial hyphae including the conidiophores will be seen to grow along the undersurface of the cover slip.

**Advantage**:- Advantage of slide culture over tease mount technique is that it allows the study of microscopic features of fungus preserving the continuity between the hyphae, microconidia and macroconidia. Dermatophyte species were further confirmed based on urease test.

**3.8 Urease Test**:-
This test is to differentiate between *T. mentagrophytes* and *T. rubrum*. Christensen’s urea agar slant was inoculated with the test fungus. *T. mentagrophytes* demonstrated the urease activity usually within seven days changing the colour of the medium to pink. *T. rubrum* isolates were negative for urease test.

**Procedure**:-
1) Pick up the growth of fungi from culture tube.
2) Inoculated Christensen’s urea agar slope with these fungal growth.
3) Incubate the tube at room temperature for 2-4 days.
4) Observe any change of colour in the inoculated medium

**Results**
A total of sixty six patients with dermatophytosis were studied over a period of one year. Following are demographic details of the study group.

Table no. 3 showed *Tinea corporis* was more common in the age group 21-30 years with 13 cases (37.14%) and in males with 9 cases (69.23%) than females with 4 cases (23.5%). *Tinea unguium* was more common in the age group of 31-40 years with 6 cases (37.5%) and in males with 10 cases (62.5%) than females with 6 cases (37.5%). *Tinea cruris* was more common in the age group 51-60 years with 2 cases (40%) and was more common in males with 5 cases (100%). In *Tinea pedis*, one case was seen in the age group of 11-20 years and the other in the age group of 41-50 and 51-60 years, and was more common in males with 3 cases (100%). *Tinea barbae* was more common in the age group 21-30 years with 2 cases (66.66%) and was more common in males with 3 cases (100%). *Tinea capitis* was more common in the age group of 31-40 years with 2 cases (66.66%) and was more common in females with 3 cases (100%). *Tinea manuum* was more common in the age group of 31-40 years and in males with 1 case (100%). In males, commonest infection was *T. corporis* followed by *T. unguium, T. cruris, T. pedis, T. barbae* and *T. manuum*. In females, commonest infection was *T. corporis* followed by *T. unguium* and *T. capitis*.

**Table 1**: Sex wise distribution in the study group

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>M:F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cases</td>
<td>51</td>
<td>15</td>
<td>66</td>
<td>3:4:1</td>
</tr>
<tr>
<td>Percentage</td>
<td>77%</td>
<td>23%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

Males were predominantly affected 51 cases (77%) as compared to females’ 15 cases 23%. Male to female ratio was 3:4:1.

**Table 2**: Age wise distribution in the study group

<table>
<thead>
<tr>
<th>Age in Year</th>
<th>Number of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>2</td>
<td>03%</td>
</tr>
<tr>
<td>11-20</td>
<td>6</td>
<td>09%</td>
</tr>
<tr>
<td>21-30</td>
<td>20</td>
<td>30%</td>
</tr>
<tr>
<td>31-40</td>
<td>15</td>
<td>23%</td>
</tr>
<tr>
<td>41-50</td>
<td>11</td>
<td>17%</td>
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<tr>
<td>51-60</td>
<td>9</td>
<td>14%</td>
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<tr>
<td>61-70</td>
<td>3</td>
<td>4%</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Discussion**
In the present study, 66 clinically diagnosed cases of dermatophytosis attending Dermatology and Venereology outpatient Department of M.G.M. Hospital Kamoth, Navi Mumbai were studied. A comparison of demographic correlation of dermatophytosis in our study along with other studies is given below.
### Table 3: Age and sex wise distribution in relation to clinical types

<table>
<thead>
<tr>
<th>Clinical Type</th>
<th>Age groups in Years</th>
<th>Sex Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;10 (2.8%)</td>
<td>1 (2.8%)</td>
<td>29 (82.8%)</td>
<td>6 (17.2%)</td>
</tr>
<tr>
<td></td>
<td>11-20 (5.7%)</td>
<td>2 (5.7%)</td>
<td>13 (37.1%)</td>
<td>5 (14.2%)</td>
</tr>
<tr>
<td></td>
<td>21-30 (12.5%)</td>
<td>13 (37.1%)</td>
<td>5 (14.2%)</td>
<td>1 (2.8%)</td>
</tr>
<tr>
<td></td>
<td>31-40 (22.8%)</td>
<td>2 (5.7%)</td>
<td>8 (22.8%)</td>
<td>5 (14.2%)</td>
</tr>
<tr>
<td></td>
<td>41-50 (14.2%)</td>
<td>1 (2.8%)</td>
<td>1 (2.8%)</td>
<td>2 (5.7%)</td>
</tr>
<tr>
<td></td>
<td>51-60 (22.8%)</td>
<td>5 (14.2%)</td>
<td>8 (22.8%)</td>
<td>2 (5.7%)</td>
</tr>
<tr>
<td></td>
<td>61-70 (14.2%)</td>
<td>2 (5.7%)</td>
<td>1 (2.8%)</td>
<td>3 (4.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (4.5%)</td>
<td>1 (2.8%)</td>
<td>1 (2.8%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35 (100%)</td>
<td>5 (100%)</td>
<td>13 (100%)</td>
</tr>
</tbody>
</table>

### Table 4: Shows correlation between findings of KOH mount and culture n=66

<table>
<thead>
<tr>
<th>Total KOH and/or Culture positive</th>
<th>KOH +ve Culture +ve</th>
<th>KOH +ve Culture -ve</th>
<th>KOH -ve Culture +ve</th>
<th>KOH -ve Culture -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cases</td>
<td>52</td>
<td>35</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Percentage</td>
<td>78.78%</td>
<td>53.03%</td>
<td>19.69%</td>
<td>6.06%</td>
</tr>
</tbody>
</table>

Out of 66 clinically suspected cases of dermatophytosis, fungi were demonstrated in 54 cases (78.79%) either by direct microscopy and/or culture. Thirty-five cases (53.03%) were positive by both microscopy and culture. 13 cases (19.70%) were positive by microscopy and negative by culture. 4 cases (6.06%) were negative by microscopy but culture positive. 14 cases (21.21%) were negative both by microscopy and culture.

### Table 5: Age distribution as found in various studies (in percent)

<table>
<thead>
<tr>
<th>Name of Author</th>
<th>Place of study</th>
<th>Year</th>
<th>Commonest Age Groups (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>Navi Mumbai</td>
<td>2012</td>
<td>21-30 years (28%)</td>
</tr>
<tr>
<td>Karmakar S et al</td>
<td>Rajasthan</td>
<td>1995</td>
<td>0-30 years (64%)</td>
</tr>
<tr>
<td>Mishra M et al</td>
<td>Sambalpur</td>
<td>1998</td>
<td>15-35 years (30%)</td>
</tr>
<tr>
<td>Bokhari MA et al</td>
<td>Lahore</td>
<td>1999</td>
<td>20-40 years (36%)</td>
</tr>
<tr>
<td>Singh S et al</td>
<td>Baroda</td>
<td>2003</td>
<td>16-45 years (31.36%)</td>
</tr>
<tr>
<td>Sen SS et al</td>
<td>Assam</td>
<td>2006</td>
<td>21-30 years (40%)</td>
</tr>
<tr>
<td>Veep P et al</td>
<td>Aurangabad</td>
<td>2007</td>
<td>31-40 years (39.4%)</td>
</tr>
</tbody>
</table>
**Figure 1:** Culture of *T. viride* in obverse (A) show fluffy white growth and reverse (B) show wine red pigmentation. Microconidia (C) arranged in birds-on-a-fence seen in Lactophenol cotton blue mount (40X).

**Figure 2:** Culture of *T. maritimum* in obverse (A) show buff and powdery white growth and reverse (B) show no pigmentation. In lactophenol cotton blue (C) spiral hyphae, microconidia and macroconidia seen (40X).

**Figure 3:** Culture of *T. koningii* in obverse show white powdery with flat and folded edges growth and reverse show no pigmentation. In lactophenol cotton blue tear drop microconidia and terminal chlamydoconidia seen (40X).
The present study shows that dermatophytosis was more common in the age group of 21-30 years (30%) followed by 31-40 years (23%), which is comparable with other studies done by (Mishra M et al., 1998; Sen SS et al., 2006). However Veer P has reported that the most common age group affected was 31-40 years followed by 41-50 years. The highest incidence in young adults aged 21-30 years may be due to increased physical activity and increased opportunity for exposure.

In the present study, Tinea corporis was the commonest clinical type encountered (53%) followed by Tinea unguium (24%) and the commonest age group affected was 21-30 years (30%). Males were predominantly affected with male to female ratio being 3.4:1, which is comparable with other studies done by (Grover S., 2003; Vijaya D et al., 2003; Sen SS et al., 2006; Venkatesan G et al., 2007). Onychomycosis was second commonest clinical type and more common in males. Male to female ratio was 1.6:1, which is comparable with other studies done by (Cordeiro et al., 2005; Nada H et al., 2005) in their study reported that females were commonly affected than males, with male to female ratio being 0.31:1 and 0.69:1 respectively. Tinea cruris was the more common clinical type encountered (7.5%) and commonest age group affected was 51-60 years (40%). Males (100%) were more commonly affected than females, which is comparable with other studies done by (Siddappa K et al., 1982; Mishra M et al., 1998; Sen SS et al., 2006).

Tinea pedis was seen in 4.5% cases, which is comparable with the study done by (Siddappa K et al., 1982) whereas (Chimelli PAV et al., 2003) in their study on dermatophytosis, reported Tinea pedis in 9.9% cases respectively. Tinea barbae was seen in 4.5% cases, which is comparable with the study done by (Singh S et al., 2006; Sen SS et al., 2006; Keyvan Pakshir et al., 2006).

out of 66 cases, Tinea capitis was seen in 4.5% cases, more common age group of 31-40 years (66.66%), which is comparable with other studies done by (Siddappa K et al., 1982; Kumar AG et al., 1990; Reddy BSN et al., 1991; Kalla G et al., 1995) Tinea manuum was 1 case (2%), which is comparable with other studies done 1.53% (Siddappa K et al., 1982) and 1.9% (Chimelli PAV et al., 2003).

Conclusion

Dermatophyte infections are very common in our country where hot and humid climate in association with poor hygienic conditions play an important role in the growth of these fungi. There is varying difference in isolation of different species from southern and northern part of India. By and large Trichophyton species forms the commonest aetiological agent of dermatophytosis. Trichophyton rubrum was the commonest isolate in Tinea corporis, Tinea unguium, Tinea cruris and Tinea barbae, T. mentagrophytes was second commonest isolate in Tinea corporis, Tinea unguium, Tinea pedis and Tinea capitis. T. tonsurans was third commonest isolated in Tinea corporis, Tinea barbae and Tinea capitis. In the 4 cases of Tinea unguium fungi other than dermatophytes were isolated.

Acknowledgement

At the completion of this research work, it is with immense pleasure that I express my deep sense of gratitude and indebtedness to my esteemed teacher and guide Dr Chitra Pai, Professor, Department of Microbiology and Dr. Harpriya Kar, lecture, Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India for their keen interest, valuable suggestions, encouragement, supervision and whole hearted help throughout this study. They have been a role model and a constant source of inspiration to me.

References


