Changing trend in clinico-mycological profile of dermatophytosis of skin in Eastern India

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Abstract
Introduction: Dermatophytosis is one of the most prevalent cutaneous mycosis and a public health problem in West Bengal as well as in India. Its footprint spans far and wide across urban and rural Bengal, with a distribution that is determined by a multitude of factors like geography, environmental conditions, hygiene and sanitation. Despite its surging incidence, Dermatophytosis in Bengal remains a seldom studied topic.
Aim: The present study was undertaken to assess the burden of dermatophytosis by clinico-mycological evaluation using standard laboratory protocol and its correlation with clinical parameters.
Material and Methods: A cross sectional and observational study was conducted in 100 clinically diagnosed patients of dermatophytosis of skin attending the dermatology outpatient department of our tertiary care hospital. Skin scrapings, were collected from the active margins of the obvious new lesions and processed in the Mycology laboratory following standard protocol. All specimens were screened for presence of dermatophytes by direct microscopy using KOH DMSO preparation and were confirmed by fungal culture.
Results: Among all clinical types, Tinea corporis (58.0%) was the predominant clinical condition. Males were affected more (60.0%) than females. The predominantly affected age group was found to be 21-30 years (35.0%) followed by 31-40 years (24.0%). Fungi were demonstrated in 72.0% cases by direct microscopy in KOH mount while 51.0% cases were found as culture positive. However 02.0% cases were KOH negative but culture positive. Microsporum audouinii (20.0%) was the predominant species identified followed by Trichophyton rubrum (10%). Some rare species like Microsporum persicolor, Microsporum distortum, Microsporum fulvum were also isolated in our study which are rarely documented in the literature till date.
Conclusions: In our study, males in the age group 21-40 years were most commonly affected. Tinea corporis was found to be the most common clinical presentation likely due to the poor hygiene and lack of sanitation amongst the study population, most of whom were manual labourers. Microsporum audouinii was the most common isolate.

Keywords: Dermatophyte, Dermatophytosis, Tinea, Microsporum audouinii.

Introduction

Dermatophytes are a set of fungi with capability to invade and infect keratinized tissue such as hair, nail and skin. Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts. Reactions to dermatophyte infection may range from mild to severe as a consequence of the host’s reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors.

The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, Epidermophyton, Microsporum, and Trichophyton, of anamorphic class Hyphomycetes of the Deuteromycota (Fungi Imperfecti). The descriptions of the genera essentially follow the classification scheme of Emmons on the bases of conidial morphology and formation of conidia.

Traditionally infections caused by dermatophytes (ringworm) have been named according to the anatomical locations involved, by appending the Latin term designating the body site after the word Tinea. Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical lesions.

Dermatophytosis is a major public health problem in the world today. Dermatophytosis has higher prevalence in the tropics because of high humidity, higher population density and poor sanitation and may even reach epidemic proportions. The disease is more frequent among men than woman. Several factors have been implicated to increase in disease such as trauma, increased sweating and diabetes. Despite increasing reports of dermatophytoses in different tropical and subtropical countries, there is scanty data on this issue from India especially from West Bengal which is situated in the Eastern part of India. In the current study, we have undertaken a clinico-mycological approach, correlating various demographic data such as age, and sex with identification of the fungus using standard techniques. As the dermatophytic skin infections are more frequent when compared to those of hair and nails the study was confined to skin infections alone. The present study was undertaken with the objectives include to determine the incidence, contributing factors associated with dermatophytoses...
and occupational consequences related to disease. Also
to isolate and characterise the causative dermatophytes
and the species prevalent in this part of country.

Material and Methods
This is a cross-sectional and observational study
over a period of one year from October 2014 to
September 2015 conducted at R. G. Kar Medical
College & Hospital, Kolkata in West Bengal. The study
population comprised of 100 clinically suspected cases
of dermatophytoes attending Dermatology outpatients
department at R. G. Kar Medical College & Hospital,
Kolkata during a period of one year. Our hospital which
is a tertiary care hospital caters patients from densely
populated metro city of Kolkata along with a wide
suburban area surrounding it. Majority of the people are
from rural areas with low socioeconomic background
and poor literacy rate. Detailed history of onset of
disease, duration of symptoms, trauma, occupation,
drugs, associated co morbid conditions, family and
personal history was taken. Enquiries were also made
as to exposure to animals, cases or any other suspected
sources. Collection and processing of the sample was
done following standard laboratory procedures.
Samples were collected from affected lesions. Whenever
the patients presented with lesions at
clinically different sites samples were collected from all
those sites and each of these were processed and
examined individually.

Collection of sample
Dermatophytes, as filamentous fungi, undergo
radial growth. The central clearing of the classical
annular lesion yields old poorly viable specimen. If the
annular lesion is well defined, collection is best made
from the advancing edges of the ring. Disinfection is
carried out with 70% alcohol in gauze and the lesion is
then scraped from centre outwards, crossing the annular
margin, using a sterile scalpel blade. If the lesions have
vesicles or bullae, the tops of the vesicles or bullae are
clipped and included in the sample. Suppurating lesions
are sampled with a swab when it is impractical to obtain
scrapings. Other skin dermatophytes, such as tinea
pedis and tinea manuum, are scraped in such a way that
the whole infected area is represented, since an
advancing margin is often not evident. Sample
materials are best transported in dry, strong dark paper
folded in the manner of a herbarium packet. Dark paper
allows easy visualization of small skin squames.

Microscopic Examination
Direct microscopy, although false negative in 5 to
15% of cases in ordinary practice, is a highly efficient
screening technique. Scrapings of skin were subjected
to 10% KOH solution. The preparation was kept at
room temperature for 30 mins. Subsequently
examination was done under low power objective (10x)
of the microscope for branching and septate hyphae and
confirmation was made by high power objective (40x)
of it.

Culture
Samples were inoculated after reducing the size of
the samples to approximately to 1 mm as it was
mentioned earlier. Inoculations were done at four (4)
sites at well spaced interval onto Sabouraud’s dextrose
agar slants with chloramphenicol (0.05mg/ml) and
cyclohexamid (0.5mg/ml). Chloramphenicol was
added to inhibit the growth of bacteria and
cyclohexamide was used to inhibit the growth of
saprophytic non-dermatophytic fungi. Inoculations of
specimens were also done on DTM slopes for isolating
dermatophytes where mixed pathogens were suspected.
The tubes were incubated in BOD incubator at 280
C and also at room temperature to achieve good growth of
some dermatophytes which prefer a little higher
temperature. Regular examination of the tubes for
detection and monitoring of fungal growth, were carried
out. Tubes without any growth were discarded at 6
weeks. Growth on SDCA or SDCCA was examined
for colony morphology, texture, pigmentation on
surfaces (obverse and reverse).

Microscopic examination of colony was done by
doing a lactophenol cotton blue mount to examine the
hyphal structure, different vegetative structures formed
by hyphal modifications, various reproductive
structures like microconidia, macroconidia and
chlamydoconidia. Urea hydrolysis was used to
distinguish some species of Trichophyton and
Microsporum. Hair perforation test was also performed
as an additional aid to confirm final identification of the
isolates.

Results
100 patients with dermatophytic skin infection
were further clinically diagnosed as tinea corporis
(58.0%), tinea cruris (6%), combined tinea corporis and
cruris(24%), tinea mannum(4%), tinea faciei (5%),
tinea pedis (3%). Male members were affected more
than the female (60.0%) and the M: F ratio was 3:2
[Table 1].

Clinical manifestation in relation to age showed the
dermatophytic fungal infection was predominantly
found in 21-30 years of age (35.0%) followed by 31-40
years (23.0%) as shown in Table 1.

Microscopic observation revealed presence of
fungal hyphae by KOH mount in 72.0% cases as shown
in Table 3. Total of 51.0% cases were positive in
culture for dermatophytes, 02.0% cases which were
KOH negative revealed positive culture.

Table 4 shows the distribution different sample in
relation to clinical manifestation and age.

Table 5 shows identification of dermatophytic
fungi. Among all the pathogens identified as
Microsporum audoidinii (20%) was the predominant
species followed by Trichophyton rubrum (10%) and
Trichophyton tonsurans. Some rare species like Microsporum fulvum were isolated in our study. Microsporum persicolor. Microsporum distortum.

Table 1: Dermatophytosis in relation to age and sex

<table>
<thead>
<tr>
<th>Age(years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>21-30</td>
<td>22</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>41-50</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>51-60</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>61-70</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>&gt;71</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>40</td>
<td>100</td>
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</table>

Table 2: Clinical types of dermatophytosis

<table>
<thead>
<tr>
<th>Clinical type</th>
<th>No of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea corporis</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Tinea cruris</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Tinea corporis+T.cruris</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Tinea pedis</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tinea faciei</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tinea mannum</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Results obtained in direct microscopy (KOH) and culture

<table>
<thead>
<tr>
<th>Culture</th>
<th>KOH positive</th>
<th>KOH negative</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>Culture positive</td>
<td>49</td>
<td>2</td>
<td>51</td>
</tr>
<tr>
<td>Culture negative</td>
<td>23</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>28</td>
<td>100</td>
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</table>

Table 4: Distribution of samples in relation to clinical manifestation and age

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Total no. of samples</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>&gt;61</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea corporis</td>
<td>58 (58.0)</td>
<td>12</td>
<td>22</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Tinea cruris</td>
<td>6(6.0)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tinea corporis+T.cruris</td>
<td>24(24.0)</td>
<td>2</td>
<td>9</td>
<td></td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tinea mannum</td>
<td>3(3.0)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tinea faciei</td>
<td>5(5.0)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tinea pedis</td>
<td>4(4.0)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100 (100)</td>
<td>18(18.0)</td>
<td>35(35.0)</td>
<td>23(23.0)</td>
<td>12(12.0)</td>
<td>7(7.0)</td>
<td>5(5.0)</td>
</tr>
</tbody>
</table>

Fig. 1: Tinea corporis. Psoriasiform lesion. Silvery scales covered this infiltrated maculopapular area. Some vesicles are evident in several places on the surface. The periphery was quite erythematous and inflamed.
Fig. 2: Tinea corporis. Plaque like lesion. The outer ring is infiltrated and has a red rolled border with a few vesicles. The remaining surface of the lesion is smooth, firm, infiltrated and plaque like.

Fig. 3: Tinea corporis on right lateral aspect of abdomen. Lesions are raised and erythematous caused by Trichophyton mentagrophyte var quinkeanum.

Fig. 4: M. audouinii, irregular crooked tip macroconidia (white arrow) are characteristic feature (LPCB mount X800).

Fig. 5: Colony morphology of Microsporum audouinii grown on SDCA, a) obverse b) reverse.
Table 5: Prevalence pattern of species of dermatophytes

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Total no of samples</th>
<th>Microsporum audouini</th>
<th>Trichophyton rubrum</th>
<th>Trichophyton tonsurans</th>
<th>Trichophyton schoenleinii</th>
<th>T. mentagrophyte</th>
<th>T. violaceum</th>
<th>Epidermophyton floccosum</th>
<th>M. fulvum</th>
<th>M. distortum</th>
<th>M. Racemosum</th>
<th>M. Persicolor</th>
<th>Total positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
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<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Tinea corporis</td>
<td>58 (25.0)</td>
<td>12</td>
<td>06</td>
<td>05</td>
<td>03</td>
<td>02</td>
<td>01</td>
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<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>34</td>
</tr>
<tr>
<td>Tinea cruris</td>
<td>6(5.0)</td>
<td>01</td>
<td>01</td>
<td>00</td>
<td>01</td>
<td>00</td>
<td>00</td>
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<td>00</td>
<td>00</td>
<td>00</td>
<td>03</td>
</tr>
<tr>
<td>Tinea corporis + Tinea cruris</td>
<td>24(24.0)</td>
<td>06</td>
<td>02</td>
<td>01</td>
<td>00</td>
<td>01</td>
<td>00</td>
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<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>10</td>
</tr>
<tr>
<td>Tinea manuum</td>
<td>3(3.0)</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>01</td>
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<tr>
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<td>00</td>
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<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>Tinea faciei</td>
<td>5(5.0)</td>
<td>01</td>
<td>00</td>
<td>00</td>
<td>01</td>
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<td>00</td>
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<td>02</td>
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<tr>
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<td>10(10)</td>
<td>6(6.0)</td>
<td>5(5.0)</td>
<td>02</td>
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<td>01</td>
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</table>
Discussion

Dermatophytosis has a wide geographical distribution; the species of dermatophyte causing infection may vary from region to region and are geographically restricted except some species like *Trichophyton rubrum* which have a cosmopolitan distribution. The present study was conducted to assess the clinical and culture profile of dermatophytic skin infection.

In the present study out of 100 samples processed 60% were from males and 40% were from female patients. Study showed that males were predominantly affected than females. Male preponderance may be correlated with the occupational hazards related to their nature of work, the frequent interaction with different people of the society, environmental conditions such as hot and humid weather, poor personal hygiene and illiteracy are other major factors that influence dermatophytosis.

In our study of 100 cases of tinea, *Tinea corporis* was the commonest clinical type observed (58.0%) followed by mixed infection of tinea corporis & cruris (24.0%) being the second commonest type [Table 2]. *Tinea corporis* as predominant clinical type is in line with the different studies in West Bengal, Kasmir, Manipal, Assam and other studies. *Tinea corporis* had been reported to be the most common clinical type even in few other countries like Spain and Brazil. Verma et al and Sardari et al found *tinea cruris* as the commonest clinical type which is in contrast to our study. Among mixed clinical types, *tinea corporis* with *tinea cruris* was highest (24 cases, 73.91%). Similar findings have been reported by Peerapur et al.

Majority (35.0%) of the infection has occurred between 21-30 years followed by age group of 31-40 years (23%) [Table 1]. The majority affected in our study were from the age group of 21-40 probably as they were employed as manual labourers and daily wage earners; due to the lower hygiene and sanitation facilities available to them. This agrees with results from other studies.

As in our study, fungal infection in clinical isolates was detected by KOH mount which later on confirmed by culture as shown in [Table 3]. Positivity rates of KOH mount was found to be 72.0%. Of which 49.0% of culture was positive for dermatophytes and remaining 20.0% of culture positive were KOH negative. This study is in accordance with the study of Doddamani PV et al who reported 65.0% KOH positive and 39.0% culture positive. 9.0% were KOH negative and culture positive. Singh S et al also found more positivity rate of detection by KOH mount (60.4%) than culture (44.6%) and only 3.8% were KOH negative and culture positive. Direct microscopic examination using KOH is very simple technique giving rapid presumptive diagnosis.

Distribution of dermatophytic fungi in different clinical patterns showed *Microsporum audouinii* (20%) was the commonest aetiological agent followed by *Trichophyton rubrum* (10%) which is contrast to most of the earlier studies. *Trichophyton rubrum* was the commonest isolate like Bindu V et al in 2002 (66.2%), Sumana V et al in 2004 (60%), Peerapur B V et al in 2004 (43.7%). In a study by Grover et al *Trichophyton tonsurans* was commonest isolate followed by *Trichophyton rubrum*. Although *Microsporum audouinii* was the most frequent established etiology of tinea capitis, in our study, none of them were isolated from tinea capitis as there was no such case of dual lesions causing clinical condition of ‘tinea capitis’ and ‘tinea corporis’ in the same patient.

Brush J et al found *M. audouinii* as isolate from *tinea corporis* in a German boy who had a history of animal exposure.

Antifungal drug resistance is becoming a common problem in patients and is inevitable due to wide availability and use of drugs. Establishment of a reproducible method of susceptibility testing will be important components of a strategy to limit the emergence of resistance to these agents.

Conclusion

The study shows Dermatophytosis is prevalent across all age groups even in Bengal. The most common clinical presentation was Tinea Corporis. Infection was predominant in males. The age group with highest prevalence was 21-40 years. The most common fungi isolated was *Microsporum audouinii* followed by *Trichophyton rubrum*. The study also concurred that diagnosis of Dermatophytosis clinically followed by KOH examination was the best strategy and Culture was the gold standard confirmatory test. We reiterate that even if culture facility not available, KOH examination should be performed on routine basis. Antifungal therapy selection should be based on the identity of the causative agent to ensure efficacy and safety.

References