MYCOLOGICAL STUDY ON TRICHOPTHYTON INTERDIGITALE ISOLATED FROM CLINICALLY DIAGNOSED CASES OF DERMATOPHYTE INFECTION

Archana Dukare¹, Dr. Ravindra Khadse*¹, Smita Boyar², Sonal Chavan¹, Sharmila Raut¹

¹Indira Gandhi Government Medical College, Nagpur.
²Government Veterinary College, Nagpur.

*Corresponding Author: Dr. Ravindra Khadse
Indira Gandhi Government Medical College, Nagpur.

ABSTRACT

Introduction: Dermatophytosis is one of the most commonly encountered cutaneous fungal infections worldwide. Recently, marked increase in the isolation of Trichophyton interdigitale. The changing epidemiology of fungal infection is not only of public health interest as a “new” pathogen to an area but also is of clinical and microbiological importance. Material and Methods: It is the clinico-mycological study of 250 dermatophytoes cases to find out trichophyton species as causative agent. The isolation and identification of fungus is performed by conventional methods like direct microscopy and culture on sabouraud’s dextrose agar, dermatophyte test medium. The T. interdigitale is differentiated from T. mentagrophytes by MALDI-TOFMS technique. Results: The dermatophytes were isolated in 132(52.8%) cases of suspected dermatophytoses. Trichophyton 113(85.6%) is the commonest isolated dermatophytes in which T. rubrum 40(30.3%), T. mentagrophytes 37(28.02%) followed by T. tonsurans 23(17.42%), T. schoenlenii 7(5.30%). Out of 37 isolates of T. mentagrophytes, 10(27.02%) were T. interdigitale. The history of contact with animals observed in 50% cases The commonest age group affected by T. interdigitale was 16-30 years with male preponderance. T. interdigitale isolated from cases of tinea unguum (4), tinea pedis (3), and each one with tinea corporis, tinea barbae, tinea capitis. Conclusion: It is imperative to be aware of the changing patterns of causative fungi for making adequate strategies for prevention and treatment. The conventional methods had failed to identify T. interdigitale strains, while MALDI-TOFMS analysis enabled the distinct classification as trichophyton species of T. interdigitale and helpful for the identification of dermatophyte species.

KEYWORDS: Dermatophytosis, Trichophyton interdigitale, MALDI-TOF.

INTRODUCTION

Dermatophytosis is also called ringworm infections and it is the most common cause of fungal infections worldwide. Dermatophytes are grouped into three genera namely Trichophyton, Microsporum and Epidermophyton. Dermatophytes are also classified according to their habitat, being either anthropophilic associated with humans, zoophilic associated with animals or geophilic associated with soil. Anthropophilic species are responsible for the majority of human infections and tend to be chronic with little inflammation. Infection caused by zoophilic and geophilic species are associated with acute inflammation.[1] The distribution of the dermatophytosis and their etiological agents varies with geographical location and depends on several factors, such as lifestyle, type of the population, migration of people and climatic conditions, therefore some species are widely distributed whereas others are geographically restricted.[2]

Previously, Trichophyton rubrum was the predominant pathogen causing dermatophytosis,[2,3,4,5] but now there is change in trend of disease causing agent. However, dermatophytosis caused by T. interdigitale, an emerging pathogen have been observed in the past five years. It is the second-most commonly isolated fungus causing tinea infections in humans and one of the most common fungi that cause zoonotic skin diseases.[6] It belongs to T. mentagrophyte complex. T. mentagrophyte complex consists of several anamorphs and three teleomorphs (Arthroderma vanbreuseghemii, A. benhamiae, and A. simii) and are usually isolated from pets, such as guinea pigs and rabbits. This fungus can cause inflammatory tinea corporis, tinea facie and tinea capitis in humans.[7]

According to recent molecular studies, the species T. mentagrophyte is synonymous with only the zoophilic subspecies T. mentagrophyte var. quinckeum. The anthropophilic subspecies of T. mentagrophytes, as well as many of the zoophilic strains, formerly differentiated as var. mentagrophytes or var. granulosum, are
indistinguishable and are now designated T. interdigitale. The morphological differentiation between T. interdigitale, T. mentagrophytes, and its anamorph of A. benhamiae is impossible. In such cases, molecular identification is helpful for the confirmation of species. Rapid identification of dermatophyte species and knowledge of their host preference and ecology play an important role in epidemiology, public health issue and infection control. The availability of scanty data on T. interdigitale prompted us to present study which was conducted to know the prevalence, etiology and common clinical presentations of dermatophytosis and use of MALDI-TOF as diagnostic laboratory methods for the purpose of species identification.

MATERIALS AND METHODS

This is a cross-sectional and observational study over a period from January 2017 to September 2017 conducted at Indira Gandhi Medical College & Hospital, Nagpur in central India. The study population comprised of 250 clinically suspected cases of ringworm infection, attending Dermatology outpatients department. 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.

These samples were collected and processed as per standard guidelines. The affected area of all types of lesions were swabbed with 70% alcohol. The active edge of skin lesion scraped with sterile blunt scalpel. The nails were scraped deeply enough to obtain recently affected nail tissue as well as nail clippings were also collected. The scrapings were taken from scalp lesions and few affected hairs were also epilated and collected with sterilized forceps. Usually basalportion of hair (hair stub) was collected as the fungus was found in this area. The scrapings were collected on a piece of sterile brown paper and transported to the laboratory within 2 hours for microscopic and cultural analysis. The samples were divided into two portions; one for direct KOH examination and remaining part was used to inoculate onto Sabouraud dextrose agar with chloramphenicol (SDCA), Sabouraud dextrose agar with chloramphenicol & cyclohexamide (SDCCA) and Dermatophyte test medium (DTM) with supplement to isolate the causative dermatophytes.

The identification and specification of dermatophytes were carried out by standard procedures. Skin and hair specimens were subjected to 10% KOH solution and kept at room temperature for 30 minutes while nail clippings and scrapings were kept in 40% KOH solution for overnight. Subsequent examination was done for branching and septate hyphae. The samples were inoculated on Sabourauds dextrose agar (SDA) with chloramphenicol (0.05mg/ml) & cyclohexamide (0.5mg/ml) and on DTM for isolating. The tubes were incubated at 37°C and also at room temperature 25°C to achieve good growth. The tubes were examined at regular intervals for evidence of fungal growth for four weeks. Any visible growth on SDCA or SDCCA was examined for colony morphology, texture and pigmentation on obverse & reverse surface.

The slide culture technique (Riddel Slide culture Method), urease test, rice grain test and hair perforation test performed for fungus identification and lactophenol cotton blue mount was prepared for colonies to examine the hyphal structure, different vegetative structures formed like microconidia, macroconidia and chlamydoconidia, were performed.

The identification of T. interdigitale from T. mentagrophytes was performed by MALDI-TOF. The dermatophyte proteins were extracted using the procedure for molds. A piece of mycelium was gently scraped from the culture plate with a scalpel and suspended in 900 μl absolute ethanol and 300 μl HPLC water. The sample was centrifuged at 13,000 g for 10 min, with the resulting pellet re-suspended in 12.5 μl of 70% formic acid and incubated for 5 min at room temperature. Subsequently, 12.5 μl of 100% acetonitrile was added during 10 min at room temperature and then centrifuged at 13,000 g for 10 min. One μl of supernatant was spotted onto a MTP 384 target plate polished steel TF and allowed to air dry. Then, the spot was covered with 1 μl matrix solution [alpha-cyano-4-hydroxycinnamic acid] saturated in 50 acetonitrile: 25 HPLC water: 25 10% TFA and was allowed to air dry. The standard was used for instrument calibration. The MALDI-TOF MS assays were performed on an UltraFlex (BIOMERIEUX) mass spectrometer, according to the manufacturer’s instructions.

RESULTS

A total of 250 samples were collected from patients with clinically suspected tinea infection. The dermatophytes were isolated in 132 (52.8%) cases. Among these dermatophytes, trichophyton 113(85.6%) is the most common genus, in which T. rubrum(30.3%) is most common species followed by T. mentagrophyte (28.02%), T. tonsurans (17.42%), T. concentricum (3.03%), T. verrucosum (1.51%), T. schoenleinii (5.30%).

Table 1: No. of Trichophyton strains isolated from samples (n=113).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>No. of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. rubrum</td>
<td>40 (30.30%)</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>37 (28.02%)</td>
</tr>
<tr>
<td>T. tonsurans</td>
<td>23 (17.42%)</td>
</tr>
<tr>
<td>T. concentricum</td>
<td>4 (3.03%)</td>
</tr>
<tr>
<td>T. verrucosum</td>
<td>2 (1.51%)</td>
</tr>
<tr>
<td>T. schoenleinii</td>
<td>7 (5.30%)</td>
</tr>
</tbody>
</table>

Out of 37 isolates of T. mentagrophyte, T. interdigitalis identified in 10(27.02%) samples by MALDI-TOF. T.
interdigitale isolated from cases of tinea unguium (4), tinea pedis (3), and each one with tinea corporis, tinea barbae, tinea capitis. They were in the age group of 16-30 years with male to female ratio 1:0.43. The characteristic of zoophilic strains of T. interdigitale was in 5(50%) cases with history of contact with animals. They presented with 5-8 weeks history of skin lesions showing inflammatory signs along with scaling and numerous yellow crusts attached to the lesions’ surface.

Table 2: Details of cases of infection with T. interdigitale (n=10).

<table>
<thead>
<tr>
<th>Duration of illness in weeks</th>
<th>Occupation Related History</th>
<th>H/O contact with animals</th>
<th>Diagnosis</th>
<th>KOH Mount</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Student</td>
<td>Dog(pet)</td>
<td>T. pedis</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Farmer</td>
<td></td>
<td>T. unguium</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Farmer</td>
<td>Indirect contact</td>
<td>T. corporis</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Student</td>
<td>Cat(pet)</td>
<td>T. unguium</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Auto-Driver</td>
<td></td>
<td>T. unguium</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>House Maid</td>
<td></td>
<td>T. capitis</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>Student</td>
<td>Dog(pet)</td>
<td>T. unguium</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>Student</td>
<td>Dog(pet)</td>
<td>T. barbae</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Student</td>
<td></td>
<td>T. pedis</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Figure 1: Trichophyton interdigitale: white, flat, radiating thallus on Sabouraud’s dextrose agar.
Figure 2: Reverse pigment shows yellow-brown pigment.
Figure 3: Trichophyton interdigitale; powdery, granular surface on the thallus.
Figure 4: Urease activity on a urea agar.

**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. In our study of 250 cases, Trichophyton is the most common genus, in which T. rubrum is most common species. This is comparable to the previous studies conducted by Sara Asticoli et al, A laksmman et al, Doddamani PV et al, Lakshmi poiuri et al.[2,4,5,6]

Recently, there was a marked increase in the isolation of Trichophyton interdigitale. The changing epidemiology of this fungal infection is not only of public health interest as a “new” pathogen to an area but also is of clinical and microbiological importance to suggesting an increasing incidence. T. interdigitale was previously known as T. mentagrophyte. But now on the basis of molecular biological analyses of dermatophyte DNA, T.interdigitale is classified under separate new species.

In order to confirm results obtained from cultures, we use MALDI-TOF (matrix-assisted laser desorption/ ionization time-of- flight) mass spectrometry. Now a days VITEK MS Plus, bioMerieux, Nurtingen, Germany, a software and database, constitutes a quick and specific procedure for the identification of bacteria and fungi.[14] Here, samples are prepared and analyzed without prior purification, eventually yielding an unequivocal fingerprint mass spectrum of the microorganism. This fingerprint is individual and therefore, used in the identification of species, subspecies, and ultimately even strains. Protein mass spectra allow for the detection of genus, species, type and strain specific signals. The sample material used is fungal colonies of dermatophyte embedded into a matrix and subsequently desorbed and ionized by means of a laser. Acceleration of ions generated in the gaseous phase by an electromagnetic field is then followed by time-dependent detection after a flight distance of 1.2 meters. Flight times may then be matched with molecular masses according to prior calibration. This method offers an easy and outstandingly accurate way to differentiate all clinically relevant
dermatophytes and even rare species, provided they have
been entered into the database.

The anthropophilic strains of T. interdigitale tend to
cause tinea unguium and tinea pedis, and less often, tinea
corporis. The morphological differentiation between
anthropophilic and zoophilic strains of T. interdigitale is
often problematic. In present study, five strains were
macroscopically characterized by a beige, powdery
surface and presented microscopically with numerous,
thin walled clavate macroconidia and round microconidia. These morphological features, combined
with the strong inflammatory legion, indicate the
zoophilic characteristics of the isolate in this case, which
can originate from the dog and cat or may be indirect
contact with animals in farmers. The study from China
observed significant increase in T. interdigitale infection
associated with animal. In Germany as well
Trichophyton interdigitale is the second most common
dermatophyte infection. Remaining five strains
macroscopically showed white, cottony, flat, radiating
thallus like growth, indicating the anthropophilic strain.

This study showed predominance of younger patients
which was observed previously in studies from India. The higher incidence of toe nail infection in
younger population could be attributed to the higher
occupational exposure and sports related trauma and use
of occlusive footwear. In addition, younger population is
usually cosmetically conscious and therefore seeks early
and frequent dermatologic consultation. In our study,
males were affected more than females. Our findings are
in accordance with those reported by Vijaya et al. Higher incidence in male might be because of more
pronounced outdoor activity in men resulting in higher
incidence of trauma which thus increases susceptibility
to infection caused by anthropophilic and zoophilic strain
of Trichophyton interdigitale.

We reported T. interdigitale isolates maximum from 4
cases of tinea unguium followed by 3 cases of tinea
pedis. A study from Delhi observed that T. interdigitale
is most common etiological fungus for onychomycosis. Kai Wen Zhuang et al reported T.
interdigitale to be the most isolated dermatophyte from
Tinea faciei cases. The zoophilic strain of Trichophyton interdigitale causing of tinea barbae had been reported by R. Trotha et al.

CONCLUSIONS
There is a continuous change in the clinical pattern as
well as the geographical region of infection. Therefore, it
is imperative to be aware of these changing patterns and
causative fungi for making adequate strategies for
prevention and treatment of the infections. The
conventional culture methods had failed to identify these
strains, while MALDI-TOFMS analysis enabled the
distinct classification as trichophyton species of T.
interdigitale and helpful for the identification of
dermatophyte species. Although MALDI-TOF is
extremely useful in unequivocally identifying
trichophyton species of T. interdigitale colonies, the
differentiation between anthropophilic and zoophilic
strain of T. interdigitale, is not possible.

REFERENCES
1. Mahale RP, Rao MR. Clinicomycological profile of
Dermatophytosis in a teaching hospital. Int. J. of
2. Asticcioli S, Sara Di Silverio, Adriano Sacco, Laura
Fusi, Ilaria Vincenti, Luca Romero, Egidio.
Dermatophyte infections in patients attending a
tertiary care hospital in northern Italy. The new
3. Suruchi B, Sunite AG, Anil K, Nand LS.
Myological pattern of Dermatophytosis in and
Around Shimla Hills. Indian Journal of
Dermatology, 2014; 59(3): 268-70.
4. Ganeshkumar P, Mohan Sr, Hemamalini M,
Madhavan R, Lakshmanan A. Epidemiological and
clinical pattern of dermatomycoses in rural India.
5. Doddamani, P V Harshan, K H Kanta, R C
Gangane, R Sunil, K. B.Isolation, Identification and
Prevalence of Dermatophytes in Tertiary Care
Hospital in Gulbarga District. People’s Journal of
Scientific Research, 2013; 6(2).
6. Poluri LV, Indugula JP, kondapaneni SL.
Clinicomycological study of dermatophytosis in
7. Zaias N, Rebell G "Clinical and mycological status of
the Trichophyton mentagrophytes (interdigitale)
syndrome of chronic dermatophytosis of the skin
8. Kai Wen Zhuang, Ya Ling Dai, Jve Ping Ran, Jebina
Lama, Yi Ming Fan, Tinea faciei on the right
eyebrow caused by Trichophyton interdigitale An
Trichophyton mentagrophytes sive interdigitale? A
dermatophyte in the course of time Journal der
Deutsch enDermatologischen Gesellschaft, April
2007.
10. Laronne DH. Dermatophytes. In: Medically
Washington DC: American Society for
Medical Mycology, 3rd Edn; Mehta publication,
New Delhi, 2009; 134-141.
12. Emmons CW, Chapman HB, John PU:
Dermatophytoes. In: Medical Mycology. 2nd, Edn;
Henry Klipon Publishers, Great Britain, 1970;
109-120.
13. Padhye AA, Weitzman I: The Dermatophytoes. In:
Topley& Wilson’s Microbiology and Microbial
Infection (Medical Mycology ;vol. IV) L Ajello, RJ
Hay, L Collier, A Balows, M Sussman (Eds.); 9th
Edn.; Arnold publication, Great Britain, 1998;
215-225.


