ORIGINAL ARTICLE

Prevalence of Dermatophytes in a Tertiary Care Center of Solapur, Maharashtra

Prachala Govind Rathod[1], Nasira Khalid Shaikh[1], Kishor Vishwasrao Ingole[1], Sapana Govindlalji Mundhada[1], Smita Maharudrappa Chakote[2]*

[1]Department of Microbiology, [2]Department of Skin & Venereal Diseases, Dr. Vaishampayan Memorial Government Medical College, Solapur-413003 (Maharashtra), India

Abstract:

Background: Skin infection due to dermatophytes has become a significant health problem in tropical and sub tropical countries including India. *Aim & Objectives:* The present study was undertaken with an aim to study the distribution and frequency of dermatophyte species according to the site of infection. Also, Dermatophyte Test Medium (DTM) and Sabouraud's Dextrose Agar (SDA) were compared as screening medium. *Material and Methods:* A total 162 specimen were collected from 150 clinically diagnosed cases during the period of December 2012 to July 2014. The identification of isolate was done by microscopy, culture and other physiological tests such as urease test, hair perforation test and rice grain test. *Results:* Commonest age group affected was 21-30 years. Tinea corporis was the most common clinical type. Out of 162 specimens, 90 (55.56%) specimen were positive on direct microscopy and 84 specimens showed dermatophytic growth on culture media. *T.rubrum* was the commonest isolate. As compared to DTM, SDA proved to be a slightly better isolation media. *Conclusion:* Dermatophytosis is very common in our region having favorable climate which in association with poor hygienic conditions plays an important role in the growth of these fungi. Laboratory diagnosis using conventional methods like direct microscopy and culture both are necessary as they are easy to perform but require skill and expertise for identification.

Keywords: Dermatophytes, Dermatophyte Test Medium, Sabouraud's Dextrose Agar, *T.rubrum*

Introduction:

During the last 40 years, studies of mycotic infections in humans and animals have increased significantly. The prevalence of superficial mycotic infections has risen to such a level that skin mycoses now affect more than 20–25% of the world's population, making them one of the most frequent forms of infections [1].

Skin infection due to dermatophytes has become a significant health problem affecting children, adolescent and adult especially in tropical and sub tropical countries including India, where moisture plays an important role in promoting the growth of these fungi [2, 3].

Dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissues of human and other animals to produce an infection, 'dermatophytosis', commonly referred to as 'ringworm [4]. They affect keratin rich tissues like skin, hair and nails producing dermal inflammatory response and intense itching in addition to a cosmetically poor appearance [5]. Recently fungal infections have gained the attraction of physicians and microbiologists which is mainly attributed to increase in number of immunodeficiency conditions like AIDS, patients receiving immunosuppressive drugs for malignancies and those under transplantation [2]. So also the widespread use of broad spectrum antibiotics has contributed to the increase in the occurrence of fungal infections. The immigration of labour, troop movements, emigrations and other traveling also play an important role in spreading this fungus [6].

The classical presentation of tinea infection is a lesion with central clearing surrounded by an advancing, red, scaly, elevated border (Fig. 1). This presentation though very typical of ringworm infection is very often confused with the other skin disorders, making laboratory diagnosis and confirmation necessary [7].

The present study was undertaken with a clinicomycological approach wherein correlation between the age, sex and occupation was studied and dermatophyte species isolation and identification was done using the standard techniques [8].

Fig.1: Typical "Ring" Lesion of Tinea Showing Elevated Inflammatory Border with Central Clearing

Material and Methods:

Study and duration:

This prospective study was carried out in the Department of Microbiology, during the period of December 2012 to July 2014.

Sample size:

From previous records there were 557 clinically diagnosed cases of dermatophytosis in one year. The Calculated sample size was 150. The samples were collected from one hundred and fifty clinically diagnosed cases on two days i.e. Monday and Friday. The cases were randomly selected by lottery method. On these two days all clinically diagnosed cases of dermatophytosis from all age groups and of both sexes, attending Dermatology and Venereology Outpatient Department of S.C.S.M Sarvopchar Rugnalaya Solapur, were taken for the study until the sample size is reached

Inclusion criteria:

Skin, hair and nail samples were taken from selected cases of dermatophytosis. A detailed history of selected cases was taken.

Exclusion criteria:

Patients already taking treatment were excluded from the study.

Specimen collection:

Skin specimen was collected by scraping with a sterile scalpel blade across the inflamed margin of lesion into the apparently healthy tissue. Nail specimen was collected by taking clippings of the infected part and scrapings beneath the nail. Hair specimen was collected by plucking with epilating forceps along with the base of the hair shaft around the follicle and by scraping the active border area with a scalpel blade.

Specimen processing for identification:

Direct microscopy:

Specimen collected was placed on a clean glass slide and few drops of potassium-hydroxide (KOH) were added to it. The material was then covered by glass coverslip and slide was screened under light microscope at 400x magnification for presence of fungal elements which appeared as highly refractile, hyaline septate branching filaments (Fig. 2). In case of hair, type and arrangement of the spore was noticed to classify it as ectothrix and endothrix hair infection [9].

Fig. 2: KOH Preparation of Skin Scrapping showing Fungal Element (400x Magnification)

Culture:

The culture media used were, Sabouraud's dextrose agar (plain), Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide and Dermatophyte test medium. All media were received from Himedia, Bombay. These media were incubated in duplicate at room temperature and at 37^0C for a period of 4 weeks. Any fungal growth was identified based on colony morphology, pigmentation, growth rate, microscopy (LPCB), slide culture, urease test and hair perforation test (Fig.3, 4, 5, 6). Potato dextrose agar was used for enhancing pigment production and sporulation of the fungus. Dermatophyte test medium was incubated at room temperature and observed for up to two weeks for growth and a color change from yellow orange to red (Fig.7).

Fig. 3: SDA Slants showing Growth of *T.rubrum* A-Obverse, B- Reverse

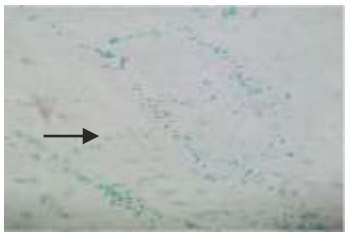

Fig. 4: LPCB of *T. rubrum* Downy Type showing Clavate microconidia with A "Bird on Fence Appearance" (400x Magnification)

Fig. 5: SDA Slants showing Growth of *T. mentagrophytes* A-Obverse, B- Reverse

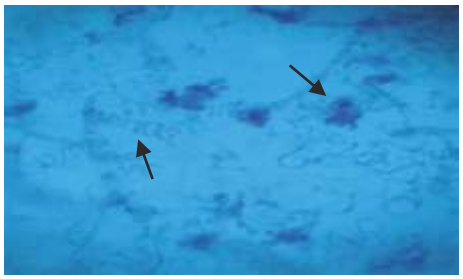

Fig. 6: LPCB of *T. mentagrophytes* showing Spherical Microconidia in Clusters and Abundant Spiral Hyphae (400x Magnification)

Fig. 7: Dermatophyte Test Medium A. Uninoculated B. Dermatophyte Growth with Red Colour Change in Medium

Fig.8: Hair Perforation Test showing Wedge Shaped Perforations (400x Magnification)

Physiological Tests:

Urease test:

It was done to differentiate between different species of *Trichophyton* (between *T. mentagrophytes and T. rubrum)* by using Christensen's urea agar slant (Hi Media, Bombay). The test strain was inoculated on the slant and incubated at room temperature for up to 14 days and observed for change in color of the medium from straw to pink which was considered positive

Hair perforation test:

Pre-pubertal hair sterilized by autoclave was placed on the filter paper (approximately 90mm in diameter) placed in sterile petridish and moistened by 10-15ml of sterile distilled water. One percent yeast extract was added; the test fungus was inoculated on the hair strands and incubated at room temperature. The hair strands were examined weekly for a period of four weeks under high power of microscope for presence of wedge shaped perforations (Fig. 8).

Rice grain test:

This test is used to differentiate *M. audouinii* from other *Microsporum* species. Sterilized polished rice grains were used for this test. Any isolate that was suspected for the *Microsporum* genus was inoculated on the rice grains. This was followed by incubation at 28^0C and observed for 7-10 days. *M. audouinii* grows poorly while other species shows growth.

Results:

Total 150 cases were in between the age range from 4 years to 75 years. Majority of (n= 43, 28.67%) affected patients were in 21-30 years age range. There was male preponderance with the male to female ratio of 1.8:1. *Tinea corporis* was found to be the most common clinical type affecting 78 (52%) cases followed by 34 (22.67%) cases of tinea cruris and 12 (8%) cases of multiple site infection. *Tinea faciei*, tinea manuum and tinea pedis revealed equal incidence of 3.33% each affecting 5 cases. Similarly, *Tinea capitis* and *Tinea unguium* affected equal number of cases, i.e 4 accounting for 2.67% each. The least common clinical type was *Tinea barbae* (1.33%) and *Tinea imbricata* (0.67%).

Occupation wise distribution of cases revealed that higher number of cases i.e. 79 (52.67%) belonged in labour class followed by households 36 (24%), students 20 (13.33%), sedentary job workers 12 (8%) and others 3 (2%). History of contact with infected family members was present in 21 (14%) cases and contact with animals was seen in 27 (18%) cases. Maximum numbers of cases were observed in the months of May to September.

Out of 150 cases, 88 (58.67%) cases were from low socio-economic group followed by 61 (40.67%) cases from middle income group and only single case (0.66%) was from higher socio-economic group.

A total of 162 specimens were obtained from 150 cases, out of which 90 (55.56%) specimens revealed presence of hyphae on direct KOH microscopic examination while 72 (44.44%) were negative. Out of 90 KOH positive specimens, fungal growth was observed in 80 (49.38%) specimens and 10 (6.17%) specimens were

culture negative. Amongst 72 KOH negative specimens, fungal growth was observed in 4 (2.47%) specimens and 68 (41.98%) were both KOH as well as culture negative. Among the 162 specimens, 84 specimens were isolated from SDA (51.85%), while 78 were isolated on DTM (48.15%).

Among the various dermatophyte species isolated, *T.rubrum* 43 (51.19%) was the most common species in our study followed by *T.mentagrophytes* 24 (28.57%), *T.tonsurans* 6 (7.15%), *T.verrucosum* 4 (4.76%), *T.violaceum* 3 (3.57%), *T.concentricum* 2 (2.38%) and *E.floccosum* 2 (2.38%). Out of 43 isolates of *T.rubrum*, 24 (55.81%) were of the downy type and 19 (44.19%) were of the granular type.

T.rubrum was isolated from all clinical types except tinea capitis, tinea barbae and tinea imbricata. *T.mentagrophytes*, *T.tonsurans* and *T.verrucosum* were isolated mainly from tinea corporis and tinea cruris. *T.violaceum* was isolated from tinea corporis, tinea faciei and tinea capitis. *T. concentricum* was isolated from *T. imbricata* and a clinically diagnosed case of tinea corporis. *E.floccosum* affected a case of tinea unguium and tinea corporis. The distribution of various isolated dermatophyte species in clinical types is as shown in Table 1.

Out of 150 cases, 6 cases (4%) presented with predisposing conditions. Diabetes mellitus was seen in 3 cases (2%) out of which 2 were culture positive. One case was affected by *T.rubrum* and the other by *T.mentagrophytes*. There was a single case (0.66%) of SLE which was affected by *T. concentricum* and a single case (0.66%) of pregnant women was positive for *T.verrucosum.*

Table 1: Showing Distribution of Dermatophyte Species in Clinical Types

Species isolated	*T. corporis*	*T. cruris*	*T. manum*	*T. pedis*	*T. faciei*	*T. capitis*	*T. unguim*	*T. barbae*	*T. imbricata*	MSI	Total Isolates
T. rubrum	19	6	1	3	3	-	1	-	-	10	43
T. mentagrophytes	11	3	-	-	1	-	-	1	-	8	24
T. tonsurans	2	2	-	-	-	-	-	-	-	2	6
T. verrucosum	3	1	-	-	-	-	-	-	-	-	4
T. violaceum	1	-	-	-	1	1	-	-	-	-	3
T. concentricum	1	-	-	-	-	-	-	-	1	-	2
E. floccosum	1	-	-	-	-	-	1	-	-	-	2
Total specimen	38	12	1	3	5	1	2	1	1	20	84

Discussion:

The higher incidence in our study group in the age group of 21-30 years may be due to increased physical activity and exposure to the hot and humid climate that is responsible for the excessive sweating. Our finding is comparable with other studies [4, 10-12]. Whereas Jain *et al* 2008 and Veer *et al* 2007 have reported in their studies that the most common age group was 31-40 years [13, 14].

In the present study males were more commonly affected than females with a male to female ratio of 1.8:1 which is comparable with the other studies [4, 10, 15]. Pires *et al* 2013, reported females to be more commonly affected with the male to female ratio of 0.62:1 [16]. Male preponderance of dermatophytosis in present study is because they are involved more in the physical activities for the living. Also, females approach to the medical facilities very less often as such skin disorders are considered to be one of the social stigmas. Many of them rely upon home remedies and seek medical advice in case of chronic conditions and for cosmetic purposes.

The most common clinical type encountered in our study was tinea corporis (52%) and they were in the age group of 21-30 years with male preponderance which could be attributed to increased sweating in young males due to vigorous outdoor activity. It is in accordance to the various studies [12, 15, 17]. The second most common clinical type encountered in our study was tinea cruris (22.67%) occurring in the age group of 11-20 and 41-50 years. Males were commonly affected than females. Similar findings were reported by Maity *et al* 2014 (12%) Kumar *et al* 2014 (24%) and Bhatia VK and Sharma PC 2014 (27%) [12,15,18].

In our study, there were 5 cases (3.33%) each of tinea faciei, tinea manuum and tinea pedis. Lower incidence of tinea pedis in our study could be explained by the fact that large sections of people in this area do not wear shoes regularly. The incidence rate of tinea faciei in our study correlates with the previous studies done by Kumar *et al* 2014 (3.2%), Bhatia VK and Sharma PC 2014 (3.4%) and Sharma M and Sharma R 2012 (1.1%) [15,18,19]. Children were more affected by tinea faciei, which could be due to contact with their infected parents.

In the present study, out of 150 cases tinea capitis was seen in 4 cases (2.67%) and was more common in the age group of 0-10 years. Our findings are similar to those of Kalla *et al* 1995 (4.43%) and Sharma M and Sharma R 2012 (5.6%) [19,20]. In our study, children were predominantly affected as they are involved more in sharing the objects like combs; caps etc. and are not so efficient in maintaining their personal hygiene. Also post pubertal changes in hormones resulting in acidic sebaceous gland secretions can cause decrease in the incidence with age [5].

Multiple site infection was seen in 12 cases, amongst which 11 cases (91.66%) had tinea corporis and tinea cruris and 1 case (8.33%) had *Tinea manuum* and *Tinea unguium*. Similar findings were reported by Kumar *et al* 2007 (4.27%) and Madhavi *et al* 2011 (9%) whereas Bhagra *et al* 2014 reported 23% of cases with multiple site involvement [3, 10, 21].

Majority of the cases in our study with multiple site infection were of low socioeconomic group who due to unawareness of the disease must have neglected the initial lesions and did not take any treatment so presented with lesions at multiple sites.

In the present study dermatophytosis was seen most commonly in labour class (52.67%) which included the farmers, daily wage labourers,

factory workers, field workers, servants etc. As the labour class is commonly exposed to hot and humid climate and come in contact with soil and animals, they are more prone to acquire dermatophytosis infection. Our findings are similar to that of Hanumanthappa *et al* [22].

Maximum cases of our study group belonged to the low income group (58.67%). Many factors are responsible for such type of distribution in the socioeconomic groups like the living condition, large family size and close contact, either directly or by sharing facilities, including combs and towels that are common between family members in low socioeconomic people.

Out of 162 specimens, 90 specimens (55.56%) were positive for fungal elements by direct KOH preparation and 84 specimens (51.85%) revealed growth on culture medium. Direct microscopy by KOH preparation plays an important role in diagnosis of fungal infections but culture gives definitive diagnosis. The higher direct microscopy positivity in this study is comparable with the other studies [4, 7, 15, 23]

In our study, 4 specimens that were negative in KOH examination revealed growth of dermatophytes on culture medium. This may be because the fungus could have been in an inactive sporulating phase that is difficult to be seen by microscopy but able to grow in appropriate media [24]. Of the culture negative cases, 10 specimen showed fungal elements on KOH mount but failed to grow in culture. Most of these cases had the history of previous treatment and this could be the reason for the non-viability of the fungi prior to inoculation.

In the present study, *T. rubrum* was the predominant isolate n= 43 (51.19%) followed by *T. menragrophytes* (28.57%), *T. tonsurans* (7.15%), *T. verrucosum* (4.76%) and *T. violaceum* (3.57%). The least common isolates were of *T. concentricum* and *E. floccosum* affecting 2 cases each (2.38%). We did not find a single case of Microsporum genus. Our findings correlate with the other studies (Singla *et al* 2013, Bindu V and Pavithran K 2002) *T. rubrum* as the commonest etiological isolate was also reported in many studies from India [10, 17, 25]. On the contrary, Bhatia VK and Sharma PC 2014 and Alkhafajii KA and Alhassnawei HH 2014 reported *T. mentagrophytes* as the predominant isolate, whereas Sharma *et al* 2012 found *M. audouinii* as the commonest dermatophyte species [18, 26, 27]. This variation in the distribution of dermatophyte species could be explained on the basis of different climatic conditions and geographic distribution. *T. rubrum* was isolated from all the clinical types namely *Tinea corporis, Tinea cruris, Tinea faciei, Tinea pedis, Tinea manuum, Tinea unguium* and MSI which is in accordance to the other studies [13, 15].

T. mentagrophyte was the second most common dermatophyte and was isolated from 11 cases of tinea corporis, 3 cases of tinea cruris, 1 case of tinea faciei, 1 case of tinea barbae and 4 cases of MSI. Similar results were reported by other studies [28, 29].

In the present study, out of 150 cases, 6 cases (4%) presented with underlying predisposing conditions. There were 3 cases (2%) of diabetes mellitus out of which 2 cases were culture positive, one for *T. rubrum* and the other for *T. mentagrophytes*. There was a single case (0.66%) of SLE which was positive for *T. concentricum* and a single case (0.66%) of pregnant women was positive for *T. verrucosum*.

The underlying predisposing conditions can cause immunosuppression. In immunosuppressed individuals with impaired cell mediated immunity, dermatophytes can cause extensive skin lesions and atypical clinical picture.

In our study, using SDA with antibiotic (Chloramphenicol and Cycloheximide), the culture positivity was n= 84 (51.85%) whereas DTM revealed growth of dermatophytes only on 78 (48.15%) of clinical specimen. Thus, DTM can be used as a very good screening medium as the isolate grows faster on it when compared to SDA. However, it can give false positive results as the saprophytic fungi also cause a color change. Madhavi *et al* 2011 has also reported similar findings [10]. Other studies reporting such comparative evaluations are those done by Singh S and Beena *et al* 2003 and Maity *et al* 2014 [7, 12].

Conclusion:

There is varying difference in the isolation of dermatophyte species from different parts of India. By and large, in our region *Trichophyton* species forms the commonest aetiological agent of dermatophytosis with *T.rubrum species* being the most common. The conventional methods for the fungal identification like direct microscopy and culture both are important in definitive diagnosis of dermatophytosis. The sensitivity of these diagnostic tests depends on the method of sampling, sample processing, failure rate of microscopy/culture, and final interpretation of results. Direct microscopy using KOH is a very good screening method for the preliminary diagnosis of fungi as it is quick, simple and inexpensive. However, fungal examination of fungal elements in KOH mount requires skill and expertise. Cultivation of fungi on different culture medium provides definitive diagnosis of dermatophytosis. Selective culture media are required as the contaminating moulds hamper the recovery of dermatophytes. DTM, being a selective medium can be used as a rapid screening medium for the rapid detection and isolation of dermatophytes. Supplementary tests viz. urease test, hair perforation test are required for complete identification of species. Use of single method i.e. direct microscopy or culture alone, may result in false negative reporting. The present study highlights the use of KOH mount examination and isolation of dermatophytes by using more than one culture medium viz: one simple and other selective medium.

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[*] ***Author for Correspondence:*** *Dr. Prachala Govind Rathod, Dr. Vaishampayan Memorial Government Medical College, Solapur, Maharashtra, India Email: prachala.rathod@gmail.com Cell: 9730889879*