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Mycological study of Dermatophytosis in rural population

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ABSTRACT

100 clinically suspected cases of dermatophytosis were subjected to mycological examination with microscopy and culture using 10% KOH and Sabouraud's Dextrose Agar (SDA) and Dermatophyte test medium(DTM) .Causative agents were identified macroscopically and microscopically from the growth obtained from SDA. Direct microscopy revealed fungal elements in 43% cases , Of which 58% were positive on culture. Commonest age group affected is between 21-30 years and males outnumbered females 1.3:1. 32% gave history of possible sources of infection. Tinea corporis was the commonest lesion (27%) followed by Tinea pedis (17%) and Tinea unguium (17%) lesions in mixed sites are seen in 9% cases .Tricophyton rubrum was the commonest aetiological agent (51.72%), followed byT.mentagrophytes(31.0%), T.violaceum(6.89%), T.verrucosum (3.4%)and Microsporum gypseum (6.89). DTM is more useful as a general screening medium as opposed to an identification medium in the isolation of dermatophytes.

Key words: Dermatophytosis, Dermatophyte test medium, Trichophyton rubrum.

INTRODUCTION

Dermatophytosis or ring worm infection or tinea is by far the most common disease in human beings.It is an infection of the skin , hair or nails by any of a group of keratinophilic fungus called 'Dermatophytes '.The etiologic agents of dermatophytosis are classified into three genera based primarily on differences in microscopic morphology and modes of sporulation as - Epidermophyton, Mirosporum and Trichophyton .[1] Dermatophytosis is common in tropical countries like India and may reach epidemic proportions in areas with high rate of humidity and over population and poor hygienic conditions . The present study was undertaken with a view to find out

a) clinical pattern of dermatophytosis and the species prevalent in this part of the country
b) to evaluate dermatophyte test medium(DTM) as a screening medium for isolation of dermatophytes in comparison with Sabouraud's Dextrose Agar (SDA).

MATERIALS AND METHODS

One hundred patients who were clinically diagnosed as cases of dermatophytosis formed the study group. The cases studied were patients who attended the out patient clinic of the Department of Dermatology at Bhaskar General Hospital Moinabad, R.R District, A.P, India ,between September 2008 and February 2009.The Hospital is situated in a village in the out skirts of Hyderabad .

First the details and history of the patients were recorded which included age, sex , duration of the complaints, distribution of the lesions and history of treatment of similar episodes in the past. Enquiries were also made as to exposure to animals, cases or any other suspected sources. 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 examined and processed individually.

Collection of samples from the skin :
The affected area was swabbed with 70% alcohol and the active edge of lesion scraped with a flame sterilized blunt scalpel. The scrapings were collected from the margins of the lesion without injuring the skin surface .

From the scalp: The same procedure was followed as for scrapings , in addition a few affected hairs were also epilated and collected with a pair of flame sterilized tweezers . Care was taken to collect the basal portion of the hair as the fungus was usually found in this area.

From the nails : The affected nails was swabbed with 70% alcohol after which the nails were scraped deeply enough to obtain recently invaded nail tissue. The samples were collected in paper sachets for transport to the laboratory. The specimens were processed by microscopy and culture.

Miccroscopic examination was done using 10%KOH solution to see hyphal segments in skin scales or either ectothrix or endothrix invasion of infected hairs [1]. The samples were then inoculated on two slopes of Sabouraud's Dextrose Agar containing chloramphenicol (50mg/liter) one of which also contained cycloheximide (500mg/liter) [2] and a plate of Dermatophyte test medium (DTM).Himedia with supplement . Dermatophyte Test Medium (DTM) is a selective medium recommended for the isolation and cultivation of pathogenic dermatophytic fungi. It is a modification of a commercial formulation made by Taplin et al in 1969.[3] .Nitrogenous and carbonaceous compounds essential for microbial growth are provided by soy peptone. Dextrose serves as the energy source for metabolism. Chloramphenicol acts as a broad spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide is added to inhibit saprophytic fungi. Phenol red, the pH indicator, in the medium is affected by the presence of dermatophytes (*Epidermophyton*, *Microsporum*, and *Trichophyton* spp.), which all produce alkaline metabolites. Production of alkali results in the medium changing from yellow-

orange to red in color. Other organisms that may grow on the medium can be recognized as non-dermatophytes by their color and colony morphology. Contaminating saprophytes can turn Dermatophyte Test Medium from its yellow-orange color to red, but can be ruled out due to the green to black hyphae produced. Dermatophytes typically produce white aerial hyphae.

The slopes and plates were incubated at room temperature and examined at intervals for evidence of fungal growth . Slopes and plates not showing growth for four weeks were discarded..Any visible growth from SDA was examined for colony morphology, texture, surface pigmentation and pigmentation on the reverse.

Microscopic examination of colony was done by doing a lactophenol cotton blue mount to examine the hyphal structure, microconidia and macroconidia .Slide culture was also done to study the undisturbed morphology of the fungal structures .Urea hydrolysis was used to further distinguish T. mentagrophytes from T. rubrum.

RESULTS AND DISCUSSION

In the present study 100 cases of clinically suspected dermatophytosis were subjected to mycological examination. Highest incidence (31%) of the patients were in the third decade of life. Males outnumbered females with a ratio of 1.3:1.

Table 1: **Age, sex, duration of symptoms in patients with Dermatophytosis**

Parameter	**Mean ± SD**
Age (years)	28.5 ± 6.32
Number of cases at mean age	16.67 ± 8.14
Sex Male Female	 7.13 ± 5.57 6.14 ± 4.18
Duration of Symptoms (months)	14 ± 6

These observations are similar to findings of other authors like SS Sen et al , SS Singh et al and BV Peerapur et al. [4], [5],[6] .The higher incidence in young males could be due to greater physical activity and increased sweating.

36% of the cases gave history of contact with possible sources of infection like history of contact with cases 11%, history of contact with animals 15%, history of contact with animals and cases 4 % , history of similar episodes in the past 6% .This confirms that dermatophyte infections are transmitted from person to person by sharing common house hold clothes and fomites.

Duration of symptoms ranged from 5 days to 5 yrs. Mean duration - 2 months .

Tinea corporis was the commonest lesion(Table 2) accounting for 27% cases , followed by Tinea pedis (17%) and Tinea unguium (17%) . lesions in mixed sites are seen in 9% cases .Tinea capitis(14%) is commonly seen in children . These findings are in agreement with other workers, SS Sen et al, DDBelurkar et al and V Bindu et al who also found Tinea corporis to be the commonest lesion [4], [7], [8]. Since the study was done in rural population which included illiterate people of low socioeconomic group who are unaware of the disease, neglected the

initial lesions and did not take any treatment so presented with lesions at multiple sites. This also explains the chronicity of lesions in some cases.

Direct microscopy revealed fungal elements in 43% of the cases .Of these 29(58%) were culture positive . Out of the 29 culture positive, 4 cases (13.7%) were negative on microscopy. Thus out of 100 samples studied 33 (33%) did not show evidence of the fungi either on direct microscopy or culture These results are mostly comparable with the results of Sen SS, Rasul Assam [4] , which showed- Forty nine (49%) cases were positive for fungal elements by direct microscopical examination. Culture was positive in 51 (51%) cases and of these four had no evidence of fungus by direct microscopy while two out of 49 culture negative cases were positive by direct microscopy.

Most of the isolates obtained are species of Trichophyton namely rubrum (51.72%), mentagrophytes(31.0%), violaceum (6.89%), verrucosum (3.44 %) , one species of Microsporum namely gypseum (6.89%) was isolated. T.rubrum was the predominant isolate (Table 2) accounting for 51.72% of the isolates which is in conformity with other reports[4],[9], [10] T.rubrum which also the one mostly affecting multiple sites .

Table 2:Dermatophytes isolated from different clinical types

Species	T.rubrum	T.mentagrophytes	T.verucosum	T.violaceum	M.gypseum	Total
Tinea corporis	5	5	-	-	1	11
Tinea capitis	1	-	-	2	-	3
Tinea cruris	3	2	-	-	-	5
Tinea pedis	1	1	1	-	-	3
Tinea unguium	1	-	-	-	-	1
T.mannum	2	-	-	-	-	2
T.cruris & corporis	-	1	-	-	-	1
T.capitis & corporis	1	-	-	-	-	1
T.pedis & mannum	-	-	-	-	1	1
T.corporis&mannum	1	-	-	-	-	1
Total (%)	15(51.72%)	9(31.0%)	1(3.44%)	2(6.89%)	2(6.89%)	29

George[11] has suggested that both the predominantly chronic nature of the infection and the adaptation of the dermatophyte to the human skin can explain the higher predominance of T. rubrum in India Present study also showed the isolation of M. gypseum (geophilic dermatophytes), which could be accounted due to the patient's interaction with soil and domestic animals (Ramesh and Hilda, 1998). Ranganathan et al., (1997) [12] [13] reported the isolation of M. gypseum from the dermatophytoses of domestic and pet animals in and around Chennai.

Two media were used for the culture of the samples DTM and SDA DTM was found as efficient as SDA in primary isolation of dermatophytes [Table 3] All .the isolates were isolated on SDA while 93.10 % . were isolated on DTM . The comparative evaluation of the isolation of dermatophytes on SDA and DTM has been reported by Singh S, Beena PM et al[5] found SDA to be 96.55% and DTM 98.27% effective in isolation of dermatophytes., Mashkoor Ahmed et al reported SDA 100% and DTM 75% effective in isolation of dermatophytes from onychomycosis cases , while Yavuzdemir [15] found no significant difference in the isolation rates of these

media.. The effectiveness of SDA was 93.5% and that of DTM was 95.4% in his study of 225 samples.

Table 3 : Analysis of isolates on SDA and DTM

Isolates	Total isolates	On SDA	On DTM
T.rubrum	15	15	15
T.mentagrophytes	9	9	9
T.verrucosum	1	1	-
T.violaceum	2	2	2
M.gypseum	2	2	1
Total	29	29	27

86.2% of dermatophytes were isolated on DTM in the first week after inoculation while 47.5% of the dermatophytes were isolated on the SDA after the first week .The maximum incubation period was more than a week for SDA, where as DTM gave positive results on culture within a week of inoculation . SDA required to be incubated at least for three weeks before being reported as negative.

CONCLUSION

The present study gives an insight about the etiological agents of dematophytosis in this part of Andhra Pradesh in India which being rural population and lack of knowledge about the disease differed from other areas in chronicity and involving mixed sites . In case of commonest lesion ,species isolated and other variables it is similar to other parts of India .

DTM is more useful as a general screening medium as opposed to an identification medium and the isolation of dermatophytes is rapid when compared to SDA. However it is recommended that biochemical and/or serological tests be performed on growth from SDA for complete identification.

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