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Research Article

Dermatophytic

A mycological study of dermatophytosis in a tertiary health care centre in Central Karnataka

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Background: Dermatophytic infections are caused by keratinophilic fungi including genera Trichophyton, Epidermophyton, and Microsporum. The hot and humid climatic condition of central Karnataka promotes fungal infection. The incidence of dermatophytosis is increasing considerably over the last few years and many are becoming recurrent and chronic. This study was done to determine the most predominant species of Dermatophyte causing infection in this region, which helps in treatment. **Methodology:** The study was a cross-sectional study conducted in the Department of Microbiology for 6 months. All the patients who were clinically diagnosed with dermatophytosis were included in the study. Infected hair, nail or skin scrapings were taken as samples and KOH mount and fungal culture were done in all these samples. Slide culture was also done to speciate. **Results:** Out of 146 samples analysed for different dermatophyte species, only 140 samples grew dermatophytes in fungal culture and 145 samples were positive for fungal hyphae by KOH mount microscopy. Out of 140 culture grown dermatophytes, 118 (84.2%) were Trichophyton mentagrophytes, 18 (12.9%) were Trichophyton rubrum, 3 (2.1%) were Microsporum gypseum and one was Microsporum canis. **Conclusion:** The most common isolated dermatophyte species in our study was Trichophyton mentagrophytes which is the same as in many studies from India, a shift from Trichophyton rubrum to Trichophyton mentagrophytes. Since in Trichophyton mentagrophytes, many times antifungals are not sensitive, treatment should be done carefully in line with the literature on Trichophyton mentagrophytes antifungal susceptibility testing. Also, we recommend more studies with Antifungal susceptibility testing.

Keywords: Dermatophytes, Trichophyton, Microsporum, Epidermophyton

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Introduction

Dermatophytic infections commonly referred to as "tinea" or "ringworm", are most seen by dermatologists in outpatient departments [1]. These infections are caused by keratinophilic fungi called dermatophytes, which produce keratinase and invade skin, hair, and nail. *Trichophyton*, *Epidermophyton*, and *Microsporum* are the three genera of dermatophytes [2]. These infections are common in tropical countries like India due to several factors which include hot climate, humidity, poverty, overcrowding and poor hygienic living conditions [3]. Many dermatophytic infections are becoming increasingly in incidence and becoming chronic, recurrent, and unresponsive to treatment [1,2,3]. Dermatophytosis was an easily treatable condition for doctors previously with the use of common antifungals. Treatment resistance may be due to the use of Over-the-counter topical steroids [4,5]. It may also be due to host factors such as immunosuppression and non-compliance [6]. Also, there are reports of resistance to antifungal agents like fluconazole and terbinafine which are commonly used against dermatophytosis [7,8,9]. The emergence of dermatophytosis cases mainly due to *Trichophyton mentagrophytes* has also been postulated as a cause for the present scenario. Many recent studies also indicate the same trend of increasing cases due to *Trichophyton mentagrophytes* [2,3,4]. Hence this study was done to know the most common species causing the disease in our region, Central Karnataka. On literature search, we were unable to find a single study from Central Karnataka. Also, all over Karnataka, only a few studies are available. Knowing the most common etiological agent is important because it helps us to focus treatment on that fungus. This study involved the identification of different dermatophytes causing the disease to identify the predominant species responsible for dermatophytosis and to understand its influence on treatment.

Materials and methods

Setting, Duration and Type of study: This study took place in the Department of Microbiology, Basaveshwara Medical College and Hospital, Chitradurga, for 6 months with the permission of the institutional ethical committee. It was a cross-sectional study with the help of the department of dermatology, BMCH, Chitradurga.

Sampling methods: A total of 200 consecutive patients with a clinical history of dermatophytosis and who gave consent were included in the study.

Inclusion criteria: All the patients with a clinical diagnosis of dermatophytosis were included in the study.

Exclusion criteria: Patients not willing to undergo lab tests; patients whose skin scraping samples were negative for both KOH and fungal culture; cultures showing growth other than dermatophytes were excluded from the study.

Sample collection: The affected area was first cleaned, before the collection of samples. If the skin was involved, scrapings were collected from the edge of the lesion. In case of hair involvement, scalp scrapings were collected and a few affected hair strands along with their roots were epilated with help of forceps. In suspected cases of onychomycosis, nail clippings, and under surface scrapings were collected. Each specimen was divided into two parts: one for KOH mount and the other for fungal culture.

Direct microscopic examination: This was done using 10% KOH for skin, 20% for hair and 40% for nails, and fungal elements were looked for. In dermatophytes, we see thin septate hyaline hyphae with arthroconidia. Affected hair have arthroconidia on the surface of the shaft or within the shaft.

Isolation of dermatophyte on culture: The material was then inoculated onto two sets of SDA (Sabouraud's dextrose agar) slope, one with Chloramphenicol and Cycloheximide, and the other with only Chloramphenicol, and tubes were incubated at Room Temperature at 25^{0}C. They were observed for a growth period of 4-6 weeks before labelling it negative.

Species identification: Culture characteristics such as surface texture, topography and pigmentation are variable among different dermatophyte species. Hence along with growth rate and colony morphology, lactophenol cotton blue tease mount was also performed for speciation. Whenever morphological identification was doubtful, slide culture was performed and some biochemical tests such as the Urease test was done. *Trichophyton mentagrophytes* and *Trichophyton rubrum* were differentiated by the urease test and hair perforation test also. *Trichophyton mentagrophytes* produces both a positive hair perforation test and a positive urease test.

Microscopic morphology on LPCB mount: This is the most employed method for species identification among dermatophytes. This can be done directly on grown colonies or after slide culture. After slide culture, conidiation is better for speciation, which is required in difficult cases. Dermatophytes have either microconidia, which are small, unicellular or have macroconidia which are multicellular, septate. Special hyphae such as spiral hyphae, racquet hyphae and favic chandeliers are also present. In *Trichophyton*, microconidia are abundant, and macroconidia are rare, thin-walled, smooth and may appear pencil-shaped. *Trichophyton rubrum* colonies are usually velvety, with red pigment on reverse. Also microscopically, microconidia are plenty and teardrop-shaped. Macroconidia are few, long, pencil-shaped. Whereas *Trichophyton mentagrophytes* colonies are white to tan powdery with variable pigmentation. Microscopically, microconidia are numerous, but round to pyriform, as opposed to *T. rubrum* and macroconidia, are cigar-shaped. Another characteristic feature of *Trichophyton mentagrophytes* is spiral hyphae are seen in microscopy. In *Microsporum* species, microconidia are rare, but macroconidia are numerous, thick-walled, rough and spindle-shaped. Finally, in *Epidermophyton*, microconidia are absent and macroconidia are numerous, smooth-walled, and club-shaped.

Statistical analysis: Percentages were calculated and compared with other studies.

Results

200 patients were screened, out of which 28 patients were denied for processing samples for our study. So, a totally of 172 patients were included in the study, and among them, 26 more were excluded from reporting, because samples of 3 patients grew Candida spp., 2 patients grew Aspergillus niger, 1 patient grew Fusarium sp., and samples of 20 patients did not show any fungal elements in KOH mount or any growth in culture. Hence the results of 146 patients were analyzed. Of 146 patients, 97 were males, and 49 were females. The male to female ratio was almost 2:1. The infection was predominant in the age group of 21 to 40 years (53 patients), followed by <20 years (39 patients), 41 to 60 years (32 patients), and least common in > 60 years of age (22 patients). Clinical presentation is not mentioned here as the study was taken

As an exclusively microbiological study to know the predominant species of dermatophyte causing infection in our region and the importance of it. Out of 146 samples analysed for different dermatophyte species, only 140 samples grew dermatophytes in fungal culture and 145 samples were positive for fungal hyphae by KOH mount microscopy. Out of 140 culture grown dermatophytes, 118 (84.2%) were Trichophyton mentagrophytes, 18 (12.9%) were Trichophyton rubrum, 3 (2.1%) were Microsporum gypseum and one was Microsporum canis.

Discussion

In the present study of dermatophytosis, we have seen male preponderance with a male: female ratio of around 2:1. A similar finding has been reported by many other studies from different regions of India [1,2,3]. This is usually due to males working more outside leading to more perspiration and thus facilitating dermatophyte growth. In our study dermatophytosis is common in the age group 21-40 years which also correlates with other studies [2,3,4]. This may be because the younger working population works more outside predisposing them to more humid conditions prevalent in our region. Socialization of this population is also high leading to the spreading of this infection. In our study, as in other studies [2,3,4]. Trichophyton genus is most common (98%) compared to two isolates of Microsporum (2%) and no isolate of Epidermophyton. So, genera Microsporum and Epidermophyton are rare causative agents of dermatophytosis nowadays. In the study by Vineetha M et al [1], the predominant species was Trichophyton rubrum. Antifungal susceptibility test done in this study [1], showed increased MIC for fluconazole, terbinafine, and griseofulvin. In our study, the predominant organism responsible for dermatophytosis was Trichophyton mentagrophytes (84.9%). Our study depicts the change in the epidemiology of etiologic agents as many studies [2,3,4]. indicate i.e., from Trichophyton rubrum to Trichophyton mentagrophytes. Over the past 5 years, there has been a sudden, unexplained surge in dermatophytoses in India [9]. This epidemic has been characterized by recurrent, recalcitrant infections. Various techniques are available for antifungal susceptibility testing of dermatophytes but only the broth microdilution technique is currently accepted to determine

In vitro susceptibility of dermatophytes. As this technique is laborious and needs expertise, only a few mycology laboratories can perform this test [8]. In the present scenario of increasing resistance to the dermatophytes, there is a need to perform antifungal drug susceptibility tests at least in cases with chronic/recurrent dermatophytosis or treatment failure/relapse. As there is no Clinical Breakpoint (CBP) defined due to lack of data on the clinical correlation, pharmacokinetic/pharmacodynamic studies, or epidemiological cut off MIC values. Various biochemical mechanisms contribute to the phenotype of drug resistance in fungi. The most frequent ones involve a decrease in drug uptake, structural alterations in the target site, an increase in drug efflux or intracellular target levels, or biofilm formation [1]. In Trichophyton mentagrophyte complex (Trichophyton interdigitale) the increasing resistance to antifungals is being reported in many studies [9,10]. In the study by Poojary et al [8]. Median MIC-90 values for itraconazole were significantly higher in the Trichophyton mentagrophytes complex group as compared to the Trichophyton rubrum group. The median MIC-90 was again higher in the Trichophyton mentagrophytes complex group for terbinafine and ketoconazole. Hence, our study indicates the need for more studies with anti-fungal susceptibility testing to confirm multi antifungal resistance in Trichophyton mentagrophytes. Our study also indicates that since there is a change in the predominant causative agent of dermatophytosis in our region as in other parts of India, treatment should be properly calibrated according to the patterns of susceptibility of the predominant agent of dermatophytosis.

Conclusion

The most common isolated dermatophyte species in our study was Trichophyton mentagrophytes which is the same as in many studies from India, a shift from Trichophyton rubrum to Trichophyton mentagrophytes. Since in Trichophyton mentagrophytes, many times antifungals are not sensitive, treatment should be done carefully in line with the literature on Trichophyton mentagrophytes antifungal susceptibility testing. Also, we recommend more studies with Antifungal susceptibility testing. This study highlights the shift in most common isolated dermatophytes From Trichophyton rubrum to Trichophyton mentagrophytes in our area and antifungals to be chosen for treatment carefully in such cases.

Author contribution: Sumanta: Collection of data, preparation of the manuscript, **Jagadevi:** Data analysis, preparation of the manuscript, **Shubha:** Supervision, correction of manuscript, **Sudhindra**: Supervision, correction of manuscript, **Saipriya:** Collection of data.

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