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Research Article

TO STUDY CLINICO MYCOLOGICAL STUDY OF DERMATOPHTES ALONG WITH DIAGNOSIS AND MANAGEMENT IN TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. Apart from the resistance of the causative organisms, there are many modifiable environmental factors contributing to this sudden pandemic. The prevalence of the disease and the associated environmental factors need to be evaluated further. Opportunistic fungi of Dermatophytes are most infected in skin infection primarily disturbing superficial layers of integument with rare systemic involvement. The higher popularity in India because of their hot and humid climatic condition, occupation and low socio-economic status. Recent studies in fungal infections are as a result of increased prevalence of immunosuppressive state. To determine the incidence of different fungal species associated with dermatophytosis and organization of dissimilar clinical parameters with fungal species, if any. The present cross-sectional observational study was conducted during September 2018 to February 2019 at SLIMS, Pondicherry and BMCH, Chennai. Samples were taken from 225 patients with clinically diagnosed dermatophytosis. Turn on the site of lesion, specimen collected from skin, hair or nails were taken. These samples were than examined phenotypic methods. Out of 225 patients, 65% samples were positive by Potassium Hydroxide (KOH) mount while 86% samples were positive by culture. Most frequent species of dermatophytes recognized was Trichophyton rubrum followed by Trichophyton mentagrophytes. dermatophytic infection mainly occurrence in Agricultural workers Males (61%) were more commonly affected than females (39%). Dermatophytosis is infections seen generally in people who work in hot and humid conditions and those who indulge in strenous work. Clothing patterns and personal hygiene also play an important role. By taking proper precautionary measures, the incidence and disease burden can be minimized. Our study, tinea corporis was initiate to be the most frequent clinical type with T. rubrum being the commonest isolated species. Significant the resistance pattern of antifungal drugs will lead the family physicians and medical officers working in peripheral regions to choose the proper empirical therapy for better patient ending.

Keywords :- Dermatophytosis, minimum inhibitory concentration, Superficial mycoses, KOH.

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INTRODUCTION

Superficial fungal infections of the hair, skin, and nails are a major cause of morbidity in the world. Dermatophytoses are the most common cause of fungal infection in men, although candidiasis and pityriasis versicolor are also examples of major superficial mycoses. In the recent times, dermatophyte infections are

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emerging as a serious concern for dermatologists. Apart from the etiological factors, several environmental factors are contributing to the current pandemic. Usually skin is the broadest organ of the body and also a biological role for microbiota. This is the first barrier against antagonism from surrounding harmful particles and infection causes microorganisms. It is act as a dynamic system represented by the skin inhabitant immune system that is essential to control an infection, determine damage and continue tissue homeostasis. Amid one of the common human skin infections likes ringworms (dermatophytoses), Which signify the 4th cause of disease with a worldwide occurrence to be 25% within the healthy individual [1-2].

Dermatophytosis is a superficial fungal infection caused by dermatophytes. Which are a group of fungi that have particular facility to get nutrient from keratin layer of skin, hair and nail Even though, the infection is not always life threatening, it may direct to local allergic reactions like pruritus, erythema, pustular lesions and can help to secondary bacterial infections.

These fungi are classified into three genera: 1) *Trichophyton*; 2) *Epidermophyton*; and 3) *Microsporum*. The distribution of the dermatophytosis and their aetiological agents vary from one ecological niche to another based on several factors, such as lifestyle, socio-economic status, occupation, and climatic conditions, therefore some species are widely distributed whereas others are geographically restricted [3]. In distinction, the immunosuppressed inhabitant's mainly cell-mediated immunity deficiency settings for example HIV-AIDS, transplant, neoplasia, diabetes, or corticosteroid therapy is mostly inclined to these infections viewing widespread superficial lesions that are repeatedly insensitive to predictable antifungal treatment [4-6]. This was freshly observed in India where there was a significant increase in treatment-intractable persistent and chronic dermatophytosis most likely because of unsystematic use of antibiotics and corticosteroid drug combination [7].The current scenario calls for further delving into understanding of pathogenesis and epidemiological data of the dermatophyte infection in an attempt to treat the infection effectively.

A fastidious dermatophyte species could create lesion at multiple anatomic sites. Also clinically equivalent lesions may be produced by different species [8]. It is now well recognized that proper mycological identification of clinically suspected cases of dermatophytosis is vital before initiation of antifungal therapy. Diagnosis of the dermatophyte up to the species level it is helps in epidemiological estimation in addition to guide in therapy, predominantly when long duration treatment is planned [9]. Hence, this study is intended observance in mind the that this systemic study might give us more clear picture of different aspects of dermatophytosis i.e. correlation with age, gender, occupation, clinical and mycological types etc, Therefore this study was designed to find the incident of dermatophytic infections in Puducherry region to study socio-demographic profile of dermatophytosis patients attending tertiary care health centre in Puducherry with correlation of site concerned and causative agent responsible.

Our aimof the study is to determine the incidence of different fungal species associated with dermatophytosis, to estimate the possible organization of dissimilar clinical parameters with fungal species, if any, to find out the connection between the site of involvement and the causative agent, to investigate minimum inhibitory concentration for antifungal agent.

MATERIALAND METHODS

The present study was conducted during September 2018 to February 2019 at SLIMS, Pondicherry and BMCH, Chennai. Present study is to study socio-demographic outline of clinically diagnosed case of dermatophytosis and associate site of infection and causative Dermatophytes. The data was collected in prescribed proforma and later analysed.

Inclusion Criteria

Patients of all ages and of both the sexes who are clinically suspected with dermatophytic infection of skin, hair or nails and who are not using any antifungal treatment for at least one week. Patients who gave informed consent for required investigations.

Exclusion Criteria

Who are used with antifungals or topical steroids in the recent past,Those have superficial fungal infections other than dermatophytes, such as pityriasis, versicolor and Candidiasis and secondary bacterial infection, Patients with subcutaneous and deep fungal infection, Patients with Diabetes, chronic diseases and immunocompromised and immuno suppressive etc.

Sample Collection

Samples were collected from skin scraping, hair and nail on the site on the lesion of fungi, Skin scraping: swabbed affected area with 70% alcohol and allowed to dry and collected by scrapping the active margin of the lesion with help of blunt edge of sterile scalpel, Hair: Hair was plucked with sterile forceps from basal portion of the hair where fungus is usually found, Nail: cleansed with 70% alcohol on the affected area of nail and clippings and scrapings beneath the nail.

These samples were screened for the presence of fungal element and keep into the *potassium hydroxide (KOH) for wet mount* preparation of various

concentrations (10%, 20%, & 40%) depending upon the type of clinical specimen likes skin,hair,nail respectively.

Sabouraud's dextrose agar (SDA)contain 0.05% chloramphenicol and 0.5% cycloheximide and add dermatophyte test medium (3 test tubes). The first two test tubes are incubated at 280 C for 2-4 weeks and was observed periodically for growth. If no growth was found after 4 weeks, its negative. The third test tube will be incubated at 280 C for up to ten days and observed for colour change. Fungal isolates were identified based on colony morphology, pigmentation, growth rate and microscopy (LPCB mount) CMA was used to differentiate *Trichophyton rubrum* from *Trichophyton mentagrophytes* based on pigment production on the media. In addition, hair perforation studies were accepted out to distinguish between these two species [10-11]. Identification of the organisms were done by growth of fungal colony on culture plate and microscopic appearance of organism by using Lactophenol Cotton Blue (LCB) and slide culture method.

Preparation of inoculum

Seven to eight days old grown of dermatophytes species on potato dextrose agar slants at 27°C were used to prepare inoculums. The clear suspension of inoculum having conidia was transferred to fresh tube, and its optical density was set equal to 0.5 McFarland standards. The final inoculum was set from 1×10^3 to 3×10^3 colony forming units per ml which was used in the sensitivity testing. Here we are performed Antifungal susceptibility test broth microdilution method as per Clinical Laboratory Standards Institute (CLSI) approved standard M38-A2 guidelines suggested for molds. [12-14] Quality control isolates *Aspergillus flavus* ATCC 204304, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included. MIC50 and MIC90 values for isolates were also recorded.

In present study we used antifungal agents were fluconazole, itraconazole, ketoconazole, and terbinafine in powdered form. Stock solutions of itraconazole, ketoconazole and terbinafine were prepared in dimethyl sulfoxide, and fluconazole was dissolved in distilled water. Two-fold dilutions of stock solution were further prepared in RPMI 1640 with L-glutamine without sodium bicarbonate and were buffered at pH of 7.0 ± 0.1 with 0.165M 3-(N-morpholino) propanesulfonic buffer along with 1N NaOH. Concentration used for fluconazole was from 0.125-64 μg/ml, and for other drugs was 0.03-16 μg/ml.

RESULTS

A total of 225 specimens were collected from patients with clinically suspected tinea infection out of which 175 were from skin, 26 from nails and 24 were hair samples. Out of them 187 (83.1%) samples were positive by KOH mount [Table/Fig-1] and 137 (60.8%) showed culture positivity [Table-1]. Sample analysis shows that most common age group was 21-30 years (46.5%) followed by 31-40% (23.5%) with mean of 28 years. Male: female ratio was 3:2 [Table/Fig-3]. The samples were further analysed depending upon the clinical manifestations. Sample analysis shows that most common age group was 21-30 years (38.6%) followed by 31-40% (20.4%) with mean of 28 years. Male: female ratio was 3:2 table2. The samples were further analysed depending upon the clinical manifestations Greater part of the patients (42.76%) presented with <1 month disease duration. Family history was positive in 48.8% of cases. In the present study 225 dermatophyte species isolates 96 cultures were *T.rubrum* (42.6%) 45 isolates were *T. mentagrophytes* (20%)39 isolates were *E. flocosum* (17.3%) and 12 were *T. tonsurans* (5.3%). Most common isolate from hair was *T.tonsurans* although from nail and skin was *T. rubrum*. All the three cases of *Fusarium* were isolated form nails [Table-3].

Our study showed that isolates with MIC values of >2 μg/ml for fluconazole and >1 μg/ml for itraconazole, ketoconazole and terbinafine were classified as resistant [13-14]. Isolates resistant to fluconazole and itraconazole were 52.8% and 51.5%, respectively. While isolates which were sensitive to fluconazole and terbinafine were 47.1% and 48.4%, respectively. MIC values for itraconazole and ketoconazole were <1 μg/ml for 100% of isolates [Table 4].

Table 1: To determine the KOH and culture positive of clinical samples of dermatophytes

Site	No of cases	KOH	Culture positive	Both KOH and culture positive	Culture positive
Skin	175	25(14.2%)	09(5.14%)	68(38.8)	73(41.7)
Nail	26	10(38.4%)	0	6(23.0)	10(38.4)
Hair	24	6(25%)	3(12.5%)	4(16.6%)	11(45.8)
Total	225	41(18.2%)	12(5.3%)	78(34.6)	94(41.7%)

Tables: 2 Age and gender distribution of various cases

Age group	Number	Male	Female
0-10	11(4.8%)	5	6
11-20	46(21.3%)	28	18
21-30	87(38.6%)	56	31
31-40	46(20.4%)	27	19
41-50	18(8%)	12	6
51-60	10(4.4%)	7	3
>60	7(3.1%)	4	3

Table:3 Incidence of different species of dermatophytes and its isolation from different clinical samples.

Species	No of cases (%)	Skin	Hair	Nail
T. rubrum	96(42.6%)	56	12	23
T.mentagrophytes	45(20%)	41	-	4
T.tonsurans	12(5.3%)	5	8	-
M.canis	7(3.1%)	7	-	-
M.gypseum	9(4%)	6	3	-
E.flocosum	39(17.3%)	29		10
Candida albicans	10(4.4%)	6	-	4
fusarium	7(3.1%)	-	-	7
Total	225	150	23	48

Table: 4 Table showing number of isolates as per cut-off value

Anti-fungal	No. of isolates below cut-off value	No. of isolates above cut-off value
Fluconazole	106(47.1%)	119(52.8%)
Itraconazole	225(100%)	0
ketaconazole	225(100%)	0
Terbinafine	109(48.4%)	116(51.5%)

DISCUSSION

Dermatophyte infections in humans encompass a spectrum of conditions, ranging from superficial and localized to potentially deep and invasive. Recognizing the signs and symptoms associated with each type of infection is crucial for accurate diagnosis and effective management.Dermatophytes constitute a worldwide problem and are more prevalent in the developing world. Hot and humid environment of various parts of India considered to be best suited for the dermatophyte infections. The overall male and female ratio in both groups is approximately 1:1. High prevalence among male patients has also been reported by other studies done in India contrary to our study, as there was approximately equal number of cases among both groups [14-15]. Dermatophytosis has an extensive ecological division and its prevalence varies from one region to another region. India is the one of the hot and humid climatic condition it is considered conducive for dermatophytosis and other factors like socio-economic condition, occupation and population density also influence its prevalence [15]. Our study focused on identifying the demographic distribution, clinical subtypes of suspected cases and identification of the species in confirmed cases of dermatophytosis.

An evaluation of the direct microscopy and culture results showed that direct KOH mount is good screening test for dermatophytosis because 86.5% samples were positive in KOH mount while 61.5% were positive in culture. These results correlated with Doddomani PV et al [16]., KOH positivity was 65% while culture positivity was 48% Sudha M et al [17], there KOH positivity was 86% and culture positivity was 77%. Culture positivity was highest in hair (45.8%) followed by skin (41.7%) and nails (38.5%) correlated by Doddamani PV et al., also culture positivity was maximum in hair (100%) followed by skin (50%) and nails (15.7%).

Our study, it was showed that 38.6% cases of dermatophytosis were in the age group 21-30 years while 20.4%cases were in the age group 31-40 years. Which is correlated with Dhayagude S et al [18], also observed that the frequent age group concerned in dermatophytosis was 21-40 years. The present observation similarity with previous publications by Phudang RT et al.[19], and Konda C et al. [20] and Sudha M et al. [21]. Commonest (40.76%) age group was between 30-40 years. Usually adults in the age group of 20 -40 years are most physically active resulting in increased perspiration. Because of a hot, humid, environment in the body,

favouring the growth of dermatophytes. In present study, the male: female ratio was 3:2 which correlates with other studies by Dhayagude S et al., Sudha M et al., and Doddamani PV et al.,[22]. Higher prevalence in males might be as a result of greater physical and outdoor activity. In the present study, 88% cases were agricultural workers and labourers working outdoors leading to profuse sweating which in turn resulted in increased dermatophyte infection.

Our study showed T. rubrum was the predominant 42.6%isolate followed by T. mentagrophytes 20% E. floccosum 17.3%and T.tonsurans (5.3%) between all culture confirmed cases of dermatophytosis correlated with Dukare A et al. [23], and Jain N et al [24] studies also establish the T. rubrum as the most widespread isolate [9,12-15]. This may be due to adaptability to survive in varying climatic condition, overcrowding and unhygienic conditions [15]. Some research showed Guruprasad KY et al [25]., and Phudang RT et al [26], obseverd T. mentagrophytes as the most regular species.

In the present study, Itraconazole and ketoconazole had lower MIC for all species of dermatophytes, which indicates that these drugs could be the better choice for successful treatment of dermatophytic infections. Pathania S, et al [27]and Aktas AE, et al [28] have reported similar findings with itraconazole and ketoconazole.

119 isolates (fifty two.Eight%) confirmed higher MIC against fluconazole (i.E. Reduce-off MIC > 2 μg/ml) and 116 isolates (51. Five %) in opposition to terbinafine (i.E. Reduce off MIC >1 μg/ml). Patients with those isolates were switched over to itraconazole, as it carried fewer unfavorable consequences as compared to others. No patient turned into switched over to ketoconazole. Patients with isolates having decrease MIC values for fluconazole or terbinafine have been suggested to hold equal remedy and were suggested to maintain employees hygiene and affected vicinity dry. With implementation of above strategies all remedy failure cases of dermatophytosis have been treated successfully [29-30].

However, a comprehensive data on the most common dermatophytes agents is prevalent in a particular area, the risk factors associated with it, and the availability antifungal susceptibility profile of common dermatophytes to the commonly used drugs will go along way in providing holistic therapy to the patients and preventing antifungal resistance. However, more studies are needed to correlate the antifungal MIC with clinical response to the antifungals so that susceptibility breakpoints may be arrived at.

CONCLUSION

This study attempts to highlight the clinicoepidemiological features of dermatophytic infections and the various social and environmental factors associated with it. Dermatophytosis is a familiar superficial mycotic infection. Males are more frequently infected by dermatophytes. Middle age group especially 3rd decade is more vulnerable to Dermatophytosis.In present study most common isolate being *T. rubrum.* This is more common people in rural area, low socioeconomic status and poor hygiene.

Therefore, present study reveals all the clinically diagnosed tinea infections need to be confirmed by laboratory analysis and also differentiated dermatophyte species. Significant the resistance pattern of antifungal drugs will lead the family physicians and medical officers working in peripheral regions to choose the proper empirical therapy for better patient ending. Dermatophytes take long time to grow, they may not be isolated in all the cases of dermatophytosis and putting up antifungal susceptibility testing routinely may not be feasible. Studies should be taken up to correlate the clinical condition with their most common pathogen and the best antifungal to treat these infections. Since most of the clinicians do not send a sample, rather treat these infections empirically, these studies will go a long way to help the clinicians in choosing the most appropriate therapy. However, more studies will go a long way to help the clinicians in choosing the most appropriate therapy.

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