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ORIGINAL RESEARCH

To Study Incidence and Prevalence of Dermatophytes in Patients Attending a Teaching Hospital in Jharkhand

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ABSTRACT

Aim: The purpose of this study is to determine the incidence and prevalence of dermatophytes in patients who are treated at a teaching hospital in the state of Jharkhand.

Materials and Methods: This cross-sectional study was carried out in the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India. Total of 100 patients showing lesions typical of dermatophytes infection based on the clinicians' preliminary diagnosis from outpatient Department of Dermatology.

Results: Among them tinea corporis was predominant in 61% patients followed by tinea cruris in 17% patients. Tinea unguium was found in 12% patients, tinea manuum was observed in 3% patients, tinea pedis was seen in 2% patients and tinea capitis, tinea facei were 2% and tinea barbae were seen in 2% and 1% patient respectively. Based on the conventional and molecular methods, out of 100 clinical samples, 68 (68%) were positive for dermatophyte growth. T. rubrum was predominant with 40 isolates (40%) followed by T. mentagrophytes with 23 isolates (23%). Other isolates were T. tonsurans 2 (2%) isolates, M. gypseum 1 (1%), one M. canis (1%) and E. floccosum (1%).

Conclusion: In conclusion, dermatophytoses are widespread and may be found in all parts of the globe, with an increasing frequency particularly in tropical nations like India. Infection with dermatophytes may be caused by a number of variables, including age, gender, lack of education, improper hygiene, and socioeconomic status. Tinea corporis was the clinical location that dermatophytes were isolated from the vast majority of the time.

Keywords: Dermatophytes, Tinea cruris, Tinea corporis, Tinea unguium.

INTRODUCTION

Fungal infections of the skin's upper layers are the most frequent kind of skin illness, affecting millions of individuals in every region of the globe. [1] Dermatophytes are by far the most important fungus because of the extensive participation of the population as a whole and the ubiquity of their presence all over the globe. [2] It is expected that between 10 and 20% of people will get infected with dermatophytes at some point in their lifetimes1. Infections

brought on by dermatophytes are often referred to by a variety of common names, including "ring worm," "tinea," and "dermatophytosis." The term "dermatophytosis" refers to an infection that may affect the skin, hair, and nails and is caused by a group of keratinophilic fungi that are closely linked to one another. All types of Dermatophytes are capable of producing the enzyme keratinase.[2] Tinea infections often manifest as lesions that have a centre clearing that are surrounded by an expanding red, scaly, raised border. This is the classic appearance of tinea infections.[3] Dermatophytes are becoming more important in both developed and developing countries, in particular as a result of the development of immunosuppressive drugs and various conditions such as organ transplantation, lymphoma, leukaemia, and infections and diseases caused by the human immunodeficiency virus (HIV).[4] The study of dermatophytosis in a community is essential because it may represent the climatic conditions, customs, sanitary conditions, and socioeconomic standing of the individuals in that group.[5] The purpose of this research was to analyse the clinico-epidemiological profile of dermatophytic infection as well as to identify dermatophytes and other fungal agents from clinical specimens. The purpose of this research was to investigate the incidence and prevalence of dermatophytes in patients who were receiving treatment at a teaching hospital in the state of Jharkhand.

MATERIALS AND METHODS

After receiving clearance from both the protocol review committee and the institutional ethics committee, this cross-sectional research was carried out during October 2016 to September 2017 at the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

According to the preliminary diagnostic made by the physicians at the outpatient Department of Dermatology, a total of 120 patients had skin lesions that were indicative of a dermatophytes infection.

Methodology

Patients exhibited a variety of tinea diseases, including tinea corporis, tinea capitis, tinea cruris, tinea pedis, tinea unguium, tinea faciei, and tinea manuum. Tinea faciei was also detected. It was necessary to scrape the lesions from the centre outwards of the diseased region. Other types of tinea infections, such as tinea pedis and tinea manuum, were scraped in such a manner that the whole affected region was captured on film. Tinea capitis and tinea barbae need the use of sterile tweezers to remove the hair together with the basal root component of the hair, followed by epilation of tiny pieces of the hair roots. In the case of an infection caused by tinea unguium, the debris from under the distal end of the nails as well as scrapings from near the nail bed were gathered. In areas where scraping the infected nail end was not feasible, close trimming of the diseased nail end was done. A heavy black chart paper was used for the collection of the samples, which were then folded and transported. The presence of septate hyphae was determined by examining scrapings and hair that had been mounted in fresh 10% KOH with Parker ink and seen at 400x magnification. Because the substance is difficult to digest, fresh 20% or 40% KOH mixed with Parker ink/India ink was employed to process the nail clippings.

Sabouraud's dextrose agar with cycloheximide was employed as a semi-selective medium for the initial isolation of dermatophytes from clinical samples. This is because cycloheximide inhibits the growth of non-dermatophytic fungus, which is necessary for the process. A shift in colour from green to red on the dermatophyte test media shows that alkalinity has been produced by dermatophyte development. This medium was utilised for all of the samples. The samples were inoculated in triplicate in Sabouraud's dextrose agar with cycloheximide

and dermatophyte test media, and then they were incubated at 25 degrees Celsius and 37 degrees Celsius, respectively.

The LPCB mount was covered with a clean glass coverslip, the environment was heated gradually, and observations were carried out at 100 and 400 magnifications.

The method of slide culture was used to all of the isolates, even those from LPCB, for which the morphology was not quite obvious. The method of slide culture enables the study of the undisturbed connection between spores and hyphae via the lens of a microscope. The phenotypic approach was used to identify all of the clinical isolates, and then the genotypic method, utilising PCR-RFLP, was used to identify each of the clinical isolates.

The phenol-chloroform procedure, with a few tweaks here and there, was used to extract DNA from each of the clinical isolates.[6] In a nutshell, the culture was suspended in 400 l of lysis buffer that consisted of 10 mM TRIS, (pH - 8), 1 mM EDTA (pH - 8), 3% SDS, and 100 mM NaCl. This solution was placed in a 1.5 ml microfuge tube. After adding around 20 l of proteinase K at a concentration of 1 mg/ml (merck genei), the mixture was heated to 56 degrees Celsius for half an hour. It was brought to a boil for one minute. After adding around 400 l of a mixture of phenol and chloroform (sigma) with a ratio of 1:1, the liquid was vortexed and centrifuged at a speed of 10,000 rpm for ten minutes. After transferring the aqueous layer to a fresh microfuge tube, an equivalent amount of chloroform was added, and the mixture was vortexed and centrifuged at a speed of 10,000 revolutions per minute for ten minutes. The aqueous layer was moved to a fresh microfuge tube after the transfer. In order to precipitate DNA, an equivalent amount of ice-cold isopropyl alcohol was used, and then the precipitate was washed twice with 70% ethanol. The pellet was dissolved in 40 l of nuclease-free sterile water and then kept at a temperature of -20 degrees Celsius until it was needed.

PCR was used to amplify the ITS1 and ITS 2 regions. The universal fungal primers ITS 1 (5' – TCC GTA GGT GAA CCT GCG G – 3') and ITS4 (5' – TCC TCC GCT TAT TGA TAT GC – 3') were used in the process. The reaction mix included 1 ul of template DNA, 25 ul of PCR master mix from Merck Genei, 50 pmol of universal fungal primers from Sigma (ITS-1 and ITS-4), 50 ul of PCR master mix, and the volume was brought up to 50 ul using nuclease-free water. The total volume was 50 ul. Amplification was performed for a total of 35 cycles at the following temperatures and times: initial denaturation at 95 degrees Celsius for five minutes, denaturation at 95 degrees Celsius for thirty seconds, annealing at 56 degrees Celsius for thirty seconds, extension at 72 degrees Celsius for thirty seconds, and final extension at 72 degrees Celsius for five minutes. Using the Mva I restriction enzyme, the PCR products were analysed for their degree of sequence restriction (thermo fishers). In the reaction mixture, there was 10: l of PCR product, 5 units of Mva I enzyme, and 2 l of enzyme buffer. The total volume of the mixture was brought up to 20 l with nuclease-free water. The reaction mixture was kept in an incubator at 37 degrees Celsius for one hour. After the agarose gel was produced in 1X TAE, 10mg/l of EtBr was added to it and 1 microliter of EtBr. At a voltage of 50 volts, the PCR products and RFLP products were separated by electrophoresis in agarose at concentrations of 1.5% and 2%, respectively, for forty-five to sixty minutes. Under ultraviolet light, the goods were analysed and evaluated.

RESULTS

From the study, it was found that, out of the 100 patients suspected with dermatophytosis, male were infected more (74) than female (26).

Table 1: Age and gender distribution

Gender	Number	%
Male	74	74
Female	26	26
Age		
below 20	8	8
20-40	65	65
40-60	20	20
60-80	5	5
Above 80	2	2

Table 2: Clinical manifestations of the patients

Clinical Manifestation	Number	Percentage
Tinea corporis	61	61
Tinea cruris	17	17
Tinea unguium	12	12
Tinea manuum	3	3
Tinea pedis	2	2
Tinea capitis	2	2
Tinea faceii	2	2
Tinea barbae	1	1

Dermatopyhytic infection was found more in the age group of 20-40 years with 65% patients, followed by age group of 40-60 years with 20% patients, age group of Below 20 years with 8% patients, old age group, 60-80 years with 5% patients, and very old age group (>81 years) with only 2% patient.

Samples were collected from patient's various anatomical sites such as epidermal layers of skin, hair and nail. Among them tinea corporis was predominant in 61% patients followed by tinea cruris in 17% patients. Tinea unguium was found in 12% patients, tinea manuum was observed in 3% patients, tinea pedis was seen in 2% patients and tinea capitis, tinea facei were 2% and tinea barbae were seen in 2% and 1% patient respectively.

PCR amplified ITS-1 and ITS-2 region of all 100 dermatophytes isolates using universal fungal primers ITS-1 and ITS-4. Amplicon size of 650-800bp was obtained from all 100 clinical isolates of dermatophytes. The restriction digestion of PCR amplicon using restriction enzyme Mva I was performed for all the clinical isolates, which yielded four to five bands in each isolates with different banding pattern which is unique to each species making it easy to distinguish one species from other.

Based on the conventional and molecular methods, out of 100 clinical samples, 68 (68%) were positive for dermatophyte growth. T. rubrum was predominant with 40 isolates (40%) followed by T. mentagrophytes with 23 isolates (23%). Other isolates were, T. tonsurans 2 (2%) isolates, M.gypseum 1 (1%), one M. canis (1%) and E. floccosum (1%) (Table 3).

Table 3: Correlation of Clinical Manifestation with dermatophytes isolates.

Dermatophyte	Clinical manifestation						Total
	Tinea corporis	Tinea cruris	Tinea unguium	Tinea pedis	Tinea manuum	Tinea capitis	
T. rubrum	28	6	3	1	2	-	40
T. mentagrophytes	17	2	1	1	1	1	23
T. tonsurans	2	-	-	-	-	-	2
M. gypseum	1	-	-	-	-	-	1
M. canis	1	-	-	-	-	-	1
E. floccosum	1						1
	50	8	4	2	3	1	68

DISCUSSION

The study shows that dermatophyte infection is predominant in the adult age group (20 - 40 years). The reason for this may be due to the increased level of physical activity in the particular age group and this leads to excessive sweating which favours the growth of dermatophytes. Socialization with different people is also high compared to other age groups which eventually help in spreading of infection. This finding correlates with the earlier studies.[7,8,9]

Apart from India, tinea corporis had been reported as most predominant clinical type in Brazil and Spain.[10,11] Tinea cruris was the next dominant clinical type with 17% samples, followed by tinea unguinum 12%. Tinea cruris is more prevalent in men compared to women. The findings were backed by earlier studies.[12,13,14] This may be due to exhausting physical activity in open environment leading to excess sweating and the use of tightly worn synthetic clothes resulting in increased humidity and temperature of the body which makes skin as a suitable growth environment for dermatophytes.[12] These conditions are shown to be associated with the incidence of tinea corporis and tinea cruris.[5,13] Other clinical types such as tinea manuum, tinea pedis, tinea capitis, tinea faceii and tinea barbae were found less frequent. The details of sample with reference to the sex and the clinical manifestation have been shown in Table 2.

Trichophyton species have been a major causative agent of dermatophytosis than the other two genuses, Microsporum and Epidermophyton. In our study, among 100 dermatophytosis cases studied, T. rubrum was found to be the predominant etiological agent with 40 isolates out of 68 dermatophyte isolates, as only negligible number of isolates of Microsporum and Epidermophyton were grown. T. rubrum was the most predominant isolate (40% growth) like demonstrated by other studies earlier in India.[7,14,15,16] In recent years, prevalence of T. mentagrophytesis increasing gradually but in our study we have obtained 23% isolates and is second most common isolate next to T. rubrum.[7,14,15] This finding is in slight variation to the previous study, although T. mentagrophytes was again the second most common in all the previous studies, the number of isolates were very less compared to T. rubrum.[7,12,15] Apart from T. rubrum and T. mentagrophytes, T. tonsurans was also isolated from 5 samples. Microsporum was represented by two M. gyseum and one M. canis isolates. E. floccosum was represented by only one isolate. Compared to Trichophyton, the other two genuses were very few to represent. Generally, Microsporum and Epidermophyton are accounted for very low percentage compared to Trichophyton species.[13,16] Correlation between the etiological agents with clinical manifestation of infection is indicated in Table II.

The increased incidence of dermatophytoses could be due to environmental conditions such as humid weather and hot temperature of the geographical location. Apart from the environmental condition, poor personal hygiene along with poor illiteracy plays a major role in influencing the higher incidence of dermatophytosis.[5] The present study also shows that male are more prone than females. This can be correlated with the occupation of the person. On the other hand, social stigma present in the rural population of Jharkhand which influences non-reporting of female patients to the hospital may also be the factor for showing less frequency in females.[13]

CONCLUSION

In conclusion, dermatophytoses are widespread and may be found in all parts of the globe, with an increasing frequency particularly in tropical nations like India. Infection with dermatophytes may be caused by a number of variables, including age, gender, lack of education, improper hygiene, and socioeconomic status. Tinea corporis was the clinical location that dermatophytes were isolated from the vast majority of the time.

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