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Original Research Article

An Observational Prospective Assessment of Prevalence of Dermatophytes in Patients Attending A Tertiary Care Facility

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Abstract

Aim: To Study prevalence of dermatophytes in patients attending a tertiary care facility.

Material and methods: This observational prospective study was carried out in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India for 12 months. Total of 120 patients showing lesions typical of dermatophytes infection based on the clinicians' preliminary diagnosis from outpatient Department of Dermatology.

Result: 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 73/120 (60.83%) patients followed by tinea cruris in 20/120 (16.67%) patients. Tinea unguium was found in 15/120 (12.5%) patients, tinea manuum was observed in 4 (3.3%) patients, tinea pedis was seen in 3 (2.5%) patients and tinea capitis, tinea facei were 2 and tinea barbae were seen in 1(1.67%) and one (0.83%) patient respectively.

Conclusion: Dermatophytoses are worldwide distributed with increased incidence especially in tropical countries like India. Several factors such as age, sex, illiteracy, poor hygiene and social economy influence the infection with dermatophytes.

Keywords: tinea corporis, dermatophytes, fungal infections

Introduction

Superficial fungal infections are the most common skin diseases, affecting millions of people throughout the world.[1] The dermatophytes are by far the most significant fungi because of their widespread involvement of population at large and their prevalence all over the world.[2] The estimated lifetime risk of acquiring a dermatophyte infection is between 10 and 20%1."Ring worm", "tinea" or "dermatophytosis", are common terms used for infections caused by dermatophytes. Dermatophytosis is defined as the infection of the skin, hair and nails caused by a group of closely related keratinophilic fungi called.

Dermatophytes all of which produce enzyme keratinase.[2] The classical presentation of tinea infection is a lesion with central clearing which is surrounded by an advancing red, scaly, elevated border.[3] Dermatophytes are assuming greater significance in both developed and developing countries particularly due to the advent of immunosuppressive drugs and various conditions like organ transplantation, lymphoma, leukemia, human immunodeficiency virus (HIV) infections and disease.[4] A study of dermatophytosis in a population is important as it may reflect the climatic condition, customs, hygienic and socio-economic status of people.[5] The present study was undertaken to isolate dermatophytes and other fungal agents from clinical specimens and to assess the clinico- epidemiological profile of dermatophytic infection.

Material and methods

This observational prospective study was carried out in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India for 12 months

Total of 120 patients showing lesions typical of dermatophytes infection based on the clinicians' preliminary diagnosis from outpatient Department of Dermatology.

Methodology

Different tinea conditions such as tinea corporis, tinea capitis, tinea cruris, tinea pedis, tinea unguium, tinea faciei, and tinea manuum were observed in patients. The lesions were scraped from centre to edge of the infected area. Other dermatophytoses, such as tinea pedis and tinea manuum were scraped in such a way that the whole infected area is represented. In tinea capitis and tinea barbae, the hair with basal root portion was plucked using sterile tweezers and small portions of hair roots were epilated. In tinea unguium infection, the debris from beneath the distal end of the nails, scrapings from near the nail bed were collected. Close clipping of the infected nail end was performed wherever scrapings were not possible. Samples were collected in a thick black chart paper, folded and transported. Scrapings and hair were mounted in fresh 10% KOH with parker ink and observed under 400x magnifications for septate hyphae. For nail clippings, fresh 20% or 40% KOH with parker ink was used, as the material is hard to digest.

For primary isolation of dermatopytes from clinical samples, Sabouraud's dextrose agar with cycloheximide was used as semi-selective medium, since cycloheximide reduces the growth of non-dermatophytic fungi. Dermatophyte test medium was also used for all the samples as a colour change to red indicates alkalinity generated by dermatophyte growth. The samples were inoculated in both Sabouraud's dextrose agar with cycloheximide and dermatophyte test medium in duplicates and incubated at 25°C and 37°C respectively.

The LPCB mount was covered with clean glass coverslip, heated gently and observed under 100 and 400 magnifications.

All the isolates for which the morphology was not clear in LPCB were subjected to slide culture technique. The slide culture technique permits the microscopic observation of the undisturbed relationship of spores to hyphae.

All the clinical isolates which were identified based on phenotypic method were subjected to genotypic method using PCR-RFLP.

DNA was extracted from all the clinical isolates by phenol: chloroform method with certain modifications.[6] Briefly, the culture was suspended in 400µl lysis buffer (10mM TRIS, (pH - 8), 1mM EDTA (pH - 8), 3% SDS and 100 mM

NaCl) in a 1.5ml microfuge tube. About 20 µl of proteinase K (1mg/ml) (merck genei) was added and incubated at 56ºC for 30 minutes. It was boiled for 1 minute. About 400µl of phenol:chloroform (sigma) (1:1) mixture was added, vortexed and centrifuged at 10,000 rpm for 10 minutes. The aqueous layer was transferred to a new microfuge tube and equal volume of chloroform was added, vortexed and centrifuged at 10,000 rpm for 10 minutes. The aqueous layer was transferred to a new microfuge tube. DNA was precipitated using equal volume of ice cold isopropyl alcohol and washed twice with 70% ethanol. The pellet was dissolved in 40 µl sterile nuclease free water and stored at -20ºC until use.

PCR amplification of ITS1 and ITS 2 region was carried out using universal fungal primers ITS 1 (5' – TCC GTA GGT GAA CCT GCG G – 3') and ITS4 (5' – TCC GCT TAT TGA TAT GC – 3'). The reaction mix contained 25 µl PCR master mix (merck genei), 50 pmol universal fungal primers, ITS-1 (sigma) and ITS-4 (sigma) each, 1 µl of template DNA and the volume made up to 50 µl with nuclease free water. Amplification was carried out for 35 cycles under following conditions: initial denaturation at 95ºC for 5 min, denaturation at 95ºC for 30 sec, annealing at 56ºC for 30 sec, extension at 72ºC for 30 sec and final extension at 72ºC for 5 min.

The PCR products were subjected to restriction analysis using Mva I restriction enzyme (thermo fishers). The reaction mix had 2 µl of enzyme buffer, 5 Units of Mva I enzyme and 10µl of PCR product, the volume was made up to 20µl with nuclease free water. The reaction mix was incubated at 37ºC for 1 hour.

Agarose gel was prepared in 1X TAE and 1µl of EtBr (10mg/ml) was added to it. The PCR products and RFLP products were electrophoresed in 1.5% and 2% agarose respectively for 45-60 minutes, at 50 V. The products were visualized under UV illumination.

Results

From the study, it was found that, out of the 120 patients suspected with dermatophytosis, male were infected more (80) than female (40). The sex distributions among various clinical types are tabulated (Table 1).

Table 1: Details of samples with reference to sex and clinical manifestation

Clinical Manifestation	Number of Samples	(%)
Tinea corporis	73	60.83 %
Tinea cruris	20	16.67 %
Tinea unguium	15	12.5 %
Tinea manuum	4	3.3 %
Tinea pedis	3	2.5 %
Tinea capitis	2	1.67 %
Tinea faceii	2	1.67 %
Tinea barbae	1	0.83
Total	120(100)	100.0 %

Dermatopyhytic infection was found more in the age group of 21-40 years with 100/120 patients, followed by age group of 41-60 years with 55/120 patients, age group of 11-20 years with 20/120 patients, old age group, 61-80 years with 15/210

patients, and very old age group (>81 years) with only one patient. Patients were from in and around Chennai district. Most of the patients were from West Chennai (70/120) and West suburbs of Chennai (60/120). Sixteen patients were from South Chennai. Eleven patients were from both Central Chennai and South-Western suburbs of Chennai. Two patients from both North Chennai and North suburbs of Chennai. 20 Patients were from outside Chennai District. 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 73/120 (60.83%) patients followed by tinea cruris in 20/120 (16.67%) patients. Tinea unguium was found in 15/120 (12.5%) patients, tinea manuum was observed in 4 (3.3%) patients, tinea pedis was seen in 3 (2.5%) patients and tinea capitis, tinea facei were 2 and tinea barbae were seen in 1(1.67%) and one (0.83%) patient respectively.

PCR amplified ITS-1 and ITS-2 region of all 143 dermatophytes isolates using universal fungal primers ITS-1 and ITS-4. Amplicon size of 650-800bp was obtained from all 143 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 120 clinical samples, 82 (68.33%) were positive for dermatophyte growth. T. rubrum was predominant with 45 isolates (37.5%) followed by T. mentagrophytes with 31 isolates (25.83%). Other isolates were, T. tonsurans 3 (2.5%) isolates, M.gypseum 1 (0.83%), one M. canis (0.83%) and E. floccosum (0.83%) (Table 2).

Table 2: 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	27	8	5	2	3	-	45
T. mentagrophytes	23	3	2	1	1	1	31
T. tonsurans	3	-	-	-	-	-	3
M. gypseum	1	-	-	-	-	-	1
M. canis	1	-	-	-	-	-	1
E. floccosum	1						1
Total	56	11	7	3	4	1	82

Discussion

The study shows that the dermatophyte infection is predominant in the adult age group (21 - 40 years). The reason for this may be due to 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.[7-9] 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 29 (13.86%) samples, followed by tinea unguinum 19 (9.04%). Tinea cruris is more prevalant 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.[15,13] 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 I.

Trichophyton species have been a major causative agent of dermatophytosis than the other two genuses, Microsporum and Epidermophyton. In our study, among 120 dermatophytosis cases studied, T. rubrum was found to be the predominant etiological agent with 45 isolates out of 82 dermatophyte isolates, as only negligible number of isolates of Microsporum and Epidermophyton were grown. T. rubrum was the most predominant isolate (48.95% growth) like demonstrated by other studies earlier in India.[7,14,16,17] In recent years, prevalence of T. mentagrophytesis increasing gradually but in our study we have obtained 44.75% isolates and is second most common isolate next to T. rubrum.[7,14,16] 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.[7,12,16] 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.[13,17] 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 in and around Chennai district. Apart from the environmental condition, poor personal hygiene along with poor illiteracy plays a major role in influencing the higher incidence of dermatophytosis.[15] The present study also shows that male are more prone than females. This can be correlated with the occupation of the person.[18] On the other hand, social stigma present in the rural population of Tamil Nadu which influences non-reporting of female patients to the hospital may also be the factor for showing less frequency in females.[13,14].

Conclusion

To conclude, dermatophytoses are worldwide distributed with increased incidence especially in tropical countries like India. Several factors such as age, sex, illiteracy, poor hygiene and social economy influence the infection with dermatophytes. In the present study we have attempted to understand the epidemiological status of the dermatophytes in and around India. Tinea corporis was the predominant clinical site from which dermatophytes were isolated. T. rubrum and T. mentagrophytes have been the major etiological agents and that has been evinced by our study.

References

1. Sara L. et al. Diagnosis and management of common Tinea infections. Am Fam Physician .1998 Jul 1; 58(1): 163-174.
2. Chander J. Textbook of Medical Mycology. 3rdEd. New Delhi: Mehta Publisher; 2009. Dermatophytoses. 91- 112.

3. Hainer BL. Dermatophyte infection. American Family Physician. 2003; 67: 101-9.
4. Singh S, Beena PM. Comparative study of different microscopic techniques and culture media for the isolation of dermatophytes. Indian J Med Microbiol 2003; 21: 21-24.
5. Ranganathan S, Menon T, Sentamil GS. Effect of socio- economic status on the prevalence of dermatophytosis in Madras. Indian J Dermatol Venereol Leprol 1995; 61: 16- 18.
6. Vijayakumar R, Giri S, Kindo AJ. Molecular species identification of candida from blood samples of intensive care unit patients by polymerase chain reaction - Restricted fragment length polymorphism. J Lab Physicians. 2012; 4:1-4.
7. Zaichick, V. (2022). Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods. Journal of Medical Research and Health Sciences, 5(3), 1808–1824.
8. Kumar K, Kindo AJ, Kalyani J, Anandan S. Clinico–Mycological Profile of Dermatophytic Skin Infections In A Tertiary Care Center– A Cross Sectional Study. Sri Ramachandra Journal of Medicine. 2007;1(2);12-5.
9. Verenkar MP, Pinto MJW, Rodrigues S, Roque WP, Singh I. Clinico-Microbiological study of dermatophytoses. Indian J Pathol Microbiol. 1991; 34(3):186-92.
10. Senthamilselvi G, Kamalam A, Thambiah AS. Scenario of chronic dermatophytosis. Mycopathologia. 1998; 140:129-35.
11. Chinelli PA, Sofiatti Ade A, Nunes RS, Martins JE. Dermatophyte agents in the city of Sao Paulo, from 1992 to 2002. Rev Inst Med Trop Sao Paulo. 2003; 45:259-63.
12. Fortuno B, Torres L, Simil E, Seoane A, Uriel JA, Santacruz C. Dermatophytes isolated in our clinics, 5-year study in Zaragoza. Enferm Infecc Microbiol Clin. 1997; 15:536-9.
13. Venkatesan G, Ranjit Singh AJA, Murugesan AG, Janaki C, Gokul Shankar S. Trichophyton rubrum– the predominant etiological agent in human dermatophytoses in Chennai, India. Afr J Microbiol Res. 2007;1(1);9-12.
14. Suman Singh, Beena PM. Profile of Dermatophyte infections in Baroda. Indian J Dermatol Venereol Leprol. 2003; 69:281-3.
15. Garg A, Venkatesh V, Singh M, Pathak KP, Kaushal GP, Agrawal SK. Onychomycosis in central India: a clinicoetiologic correlation. Int J Dermatol. 2004; 43:498-502.
16. Ranganathan S, Menon T, Sentamil GS. Effect of socioeconomic status on the prevalence of dermatophytosis in Madras. Indian J Dermatol Venereol Leprol. 1995; 61:16-8.
17. Balakumar, Srinivasan. Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamilnadu, India. Asian Pac J Trop Dis. 2012;2(4):286-9.
18. Kannan P, Janaki C, Selvi GS. Prevalence of dermatophytes and other fungal agents isolated from clinical samples. Indian JMed Microbiol. 2006; 24:212-5.