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Isolation, Identification and Speciation of Dermatophyte Infectionin a Tertiary Care Hospital

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ABSTRACT

Introduction: The common fungal infections seen in human are Dermatophytoses. They are capable to invade keratinized tissues. Mainly affect the skin, hair and nail therefore called as kerotinophilic fungi. Dermatophytes falls under three Genus known as Trichophyton, Epidermophyton and Microsporum collectively called as dermatophytes. Unlike bacteria fungi are multicellular and are having different and specific morphology. They can be grown in the artificial media at room temperature and in human body temperature

Objective: Isolation and identification of fungi will help to select the appropriate antifungal management of dermatophyte infection.

Methodology: For isolation identification and speciation of dermatophytes the clinical specimens should be collected from skin, nail and hair which are obtained from patients attending the Dermatology department. Over a period ofone and half year. A total no of 180 samples are collected from the patients. Samples are examined under direct microscopy in 10%, and 40% KOH. The morphology of the fungal hyphae is studied. Samples are also inoculated on to SDA and incubated at 25C and 37C for14 days. The colony characteristics and microscopic morphology in LPCB preparations are also examined. Based on the results the fungiare identified. Further speciation was carried out by using hair perforation test and Urease test.

Results: All the 180 clinically suspected specimen of dermatophytes when examined results showed that most of the cases are seen between the age group of 21and 30 years. The predominant isolates belong to the genus Trichophyton (96.70%). Among the Trichophyton species mentagrophytes is the most common isolate (58.24%) T. rubrum is the next important species isolated.

Conclusion: Early identification of the dermatophytes and the timely given therapy will be of greater value in the prevention of fungal infection.

Key Words: Dermatophytes, Potassium Hydroxide, Urease test, Kerotinophilic fungus, Fungal culture medium, Fungal infection

INTRODUCTION

Dermatophytoses are the common types of superficial cutaneous fungal infections seen in human. They are caused by a group of closely related kerotinophilic fungi, which are capable of invading keratinized tissues of skin and its appendages like hair and nail. They belong to three mycelial fungal genera. Trichophyton, Microsporum, and Epidermophyton and are collectively known as dermatophytes.[1,2,3] Skin, hair and nail are infected by Trichophyton species. Skin and hair are infected by Microsporum species. Skin and nail are infected by Epidermophyton species. The diseases caused by non-dermatophytic fungi infecting skin are called dermatomycoses, whereas that of hair and nail are known as Piedra and onychomycosis respectively. The other frequently used terms like tinea and ringworm infections are synonym of dermatophytoses. Depending on the usual habitat (Humans, Animals or soil) dermatophytes are classified as Anthropophilic the fungal species exclusively infecting humans, Zoophilic infecting animals as well as birds, Geophilic the fungal species frequently isolated from soil.[4,5,6] Dermatophytosis is more common in tropical countries like India due to hot and humid climate. Poverty, overcrowding, poor peripheral circulation, poor personal hygiene, cancer chemotherapy, immunosuppressive therapy and immunocompromised conditions are the risk factors for Dermatophytoses.[7] Contact with the infected person / pet animals, fomites or auto inoculation from another body site are the mode of transmission.

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Predisposing factors include moist humid skin and tight-ill fitted underclothing. Fungus growth in skin showed in a centrifugal pattern in the stratum corneum leading to the formation of characteristic well-demarcated annular or ring-shaped lesions, hair may become brittle and areas of alopecia may appear,[8-10] in response to the host inflammatory reaction elicited by fungal antigen[11]. In 1958, Griseofulvin became available after break through experimental works of Gentles in guinea pigs. Dermatophytosis is cured with azole derivatives and allied group of antifungal drugs which was discovered in 1980's.[12-13] To reduce the emergence of resistant strains and morbidity, early diagnosis and initiation of treatment is essential.[14-16]

MATERIALS AND METHODS

This crosssection study was conducted in the department of Microbiology, KarpagaVinayaga Institute of Medical Sciences and Research Centre, Chinnakolambakkam, Kanchipuram District, Tamil Nadu, India. Over a period of one and half year between December 2016 and June 2018. A total number of 180 samples incudes Skin scrapings, hair and nail were collected from patients who were attended the Dermatology Outpatient department at Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Chinnakolambakkam. Both males and females of all age groups of clinically diagnosed dermatophytosis cases were included in this study, however patients with dermatophytic infection who are undergoing treatment and non dermatophytic skin infection patients are not included in this study. Details like age, sex, duration of complaint, distribution of lesion, history of previous similar complaints and treatment history, history fordiabetes, tuberculosis, neoplasms, HIV and surgeries. Exposure to animals, known cases, pets at home or any other suspected sources history were also collected and recorded.

METHOD: After disinfecting the affected site with 70% alcohol to remove surface contaminants. After the alcohol is dried, with the help of sterilized blunt scalpel, scraped the lesion from center to edge crossing margins and samples are collected

Hair Specimen: Taken basal root portion of hair by plucking and not by clipping, scraped scales and excavate hair for direct examination as well as culture.

Nail Specimen: Cleaned the affected nail with 70% alcohol and nail clippings are taken from an appropriate site depending on the type of nail infection. Collected samples are transported in sterile black paper packs to keep the specimen dry and to prevent bacterial contamination. Informed consent was obtained from the those who underwent the test procedure in this study further the institutional ethical committee approve this study.

Fungal identification

Direct microscopic examination of KOH wet mounts of keratinous material collected were treated with 10 – 40% KOH in a clean glass slide covered with a coverslip (Skin - 10% of KOH, Hair - 10% of KOH and nail - 40% of KOH). Flamed the slide slightly to clear the materials within 5-20 minutes and examined under both low and highpower objectives of light microscope for presence of hyphae. Fungal culture was carried out with the clinical specimens by inoculating on to fungal culture media Sabouraud's Dextrose Agar containing cycloheximide (0.05mg/ml) and chloramphenicol (0.05mg/ml) in duplicate. Irrespective of the findings of direct examination to detect the dermatophytes in the clinical sample, slopes were incubated at 25^0C and 37^0C for 4 weeks and examined daily during the first weekand twice a week thereafter for any fungal growth. Slopes not showing growth for 4 weeks were considered negative for growth**.** Fungal growth was identified using Lactophenol Cotton Blue (LPCB) mount preparation.

A bit of fungal colony was teased out from the culture tube and the LPCB mount was made on a slide and viewed under microscope to study the nature of the hyphae (such as septate or aseptate, hyaline or narrow or wide) and to study the Type of sporulation (conidia or sporangiaetc) for the purpose of Identification. Dermatophyte test medium was also used for the cultivation. Dematophytes on incubation at 25^0C, the dermatophytes turn medium red due to change in color of indicator phenol red by increased pH through their metabolic activity while other fungi and bacteria do not. Similarly, the species differentiation was determined by using hair perforation test. To differentiate between T.rubrum and T.mentagrophytes. It was also used to differentiate between M.canis and M.equinum. Hair perforation test result showed positive in Trichophyton mentagrophytes and Microsporumcanis but was negative in Trichophyton rubrum and Microsporumequinum. The other differential test that we did was urease test using Christensens medium. T.mentagrophytes strain, hydrolyse urea thereby medium turns bright pink color while T.rubrum shows negative results.

RESULTS AND DISCUSSION

Out of 180 isolates KOH positive is 145 (80.56%) and KOH negative is 35 (19.44%) Among the 180 samples, 114 (63.33%) are skin scrapings, 28(15.55%) are hair samples and 38 (21.12%) are nail samples. (Table 1). Whereas the culture positive showed 134 (74.44%) and culture negative is 46(25.56%). 72.13% are both KOH and culture positive three of culture positive samples showed no fungal filaments on direct KOH mount because of inactive form but able to grow in culture medium. In culture negative cases 15 showed fungal elements on KOH mount but failed to grow in culture.

Surenderan K A K et al.[17] study showed similar results. It may be due to nonviability of the fungi prior to inoculation, self-medication and inappropriate use of antimycotic treatment. The number of specimen used in this study showed in Table:1

Age wise distribution of dermatophyte infection showed that the common age group is 21-30 years (31.71%) followed by 31-40 years showed in table 2. The higher incidence in young males could be due to greater physical activity and increased sweating. Same results were shown in the studies of Smita Sharma et al.[18] (39%) US Agarwal et al.[19] (30.3%,), BV Peerapur et al.[20] Clarissa J. Lyngdoh et al.[21] (34.4%.) Surendran KAK et al.[17] showed 44% which is slightly higher than this study.

Male to female ratio showed a marginal variation which was 95 in male (52.78%) and 85 in female (47.22%) out of 180 cases.

Clinical presentation of dermatophytes infection showed in Table 3. It is observed that Incidence of Tinea corporis 68 (37.78%) and Tinea unguium 38 (21.10%) is high followed by Tinea cruris 27 (15%) and Tinea capitis 21 (11.67%). The common clinical types of dermatophytoses are Tinea corporis when compared with Suman Singh et al.[22] study (6.92%) showed a very low percentage than the present study. Tinea capitis is less common in India than in other countries, this may be attributable to the use of hair oils used by Indians. Hair oils have been shown to have an inhibitory effect on dermatophytosis. The prevalence of Tinea pedis (3.89%) in this study is comparable with G.Venkatesan et al.[23] (5.6%).

Among the180 clinical samples 134 fungi were isolated, in which 16 T.rubrum are isolated from skin samples, 20 from scalp hair and 15 from nail. 63 Trichophyton mentagrophytes from skin (47.01%) and 15 from nail clippings are isolated. (Table 4 T. mentagrophytes is the most common isolate from skin samples almost similar to Sundar Khadka and coworkers (39.6%) study and T. rubrum is the second common isolate.

T. mentagrophytes are found to be the commonest etiological agent (58.20%) isolated from tinea pedis, tinea corporis, tinea manuum tinea,barbae,tineafacei,tinea capitis, tinea unguium and tinea cruris, followed by T.rubrum (38.00%), Epidermophyton floccosum (1.50%), T.tonsurans (0.80%) and Microsporumgypseum (1.50%) (Table 5). A study by Sundar Khadka et al.[6] showed T.mentagrophytes (39.6%) the present study report showed a much larger number. Trichophyton mentagrophytes is the predominant dermatophyte isolated from Tinea corporis in this study. Similar results are alsoobserved in Sundar Khadka et al. study. Yet another study conducted by Suman Singh et al.[22] showed Trichophyton rubrum as the common isolate.

CONCLUSION

This study is done to isolate the Dermatophyte in patients suffering from Dermatophytosis. It is found that the age group of patients between 21 and 30 years are mostly affected. Reason for the high incidence may due to greater physical activity and increased moist body condition. Because of poor personal hygiene, illiteracy and environmental conditions. Males were affected slightly higher than females. More prone are family history of dermatophytic infection and diabetes mellitus. Among the samples collected, skin scrapings seem to be morecommon than hair and nail. Tinea corporis is the notable clinical condition followed by T. unguium, T. cruris and T. capitis. Five different species are isolated by culture and Trichophyton mentagrophytes was the predominant isolated dermatophyte followed by Trichophyton rubrum. Presently it is observed that fungi are becoming resistant to antifungal drugs therefore it become important that the early isolation and Specific identification of the dermatophytic species and timely given antifungal therapy based on the sensitivity pattern will be of greater value in treatment of dermatophyte infection.

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Confilict of Interest

None

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REFERENCES

1. ChanderJ(Ed). Text book of Mycology. 4th edition. Faridabad. Jaypee Brothers Medical Publishers. 2018.
2. Mathur M, Kedia SK, Ghimire RBK. "Epizoonosis of dermatophytosis": A clinico - mycological study of dermatophytic infections in central Nepal. Kathmandu Univ Med J. 2012;10(37):30–3.
3. Valdigen GL, Pereira T, Macedo C, Duarte ML, Oliveira P, Ludovico P, Souza-basto A, Leão C, Rodrigues F. A twentyyear survey of dermatophytosis in Braga, Portugal. Int J Dermatol. 2006;45:822-27
4. Bindu V, Pavithran K. Clinico - mycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol. 2002;68:259-61

5. Grover S, Roy P. Clinico-mycological profile of superficial mycosis in a hospital in North-East India. Med J Armed Forces India. 2003;59(2):114–6.
6. Khadka S, Sherchand JB, Pokharel DB, Mishra SK, Dhital S, Basista R. Clinicomycological Characterization of Superficial Mycoses from a Tertiary Care Hospital in Nepal, Dermatology Research and Practice, vol. 2016, Article ID 9509705, 7 pages.
7. Sahai S, Mishra D. Change in spectrum of dermatophytes isolated from superficial mycoses cases: First report from Central India. Indian J Dermatol Venereol Leprol 2011;77:335-6
8. Washington WJ, Stephen A, William J, Elmer K, Gary P, Paul, S. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 7th ed. Philadelphia: Lippincott Williams and Wilkins Publications. 2017
9. Negroni R. Historical aspects of dermatomycoses. Clin Dermatol.2010;28(2):125–32
10. Qadim HH, Golforoushan F, Azimi H, Goldust M. Original papers Factors leading to dermatophytosis.Annals of Parasitology 2013;59(2):99–102.
11. Khosravi AR, Shokri H, Mansouri P. Immediate hypersensitivity and serum IgE antibody responses in patients with dermatophytosis. Asian Pac J Allergy Immunol. 2012;30(1):40–7.
12. Fernanda M, Gavazzoni R, Bernardes-filho F, Schechtman RC, Quaresma-santos MVP, Gutstein A. Update on therapy for superficial mycoses : review article part I. 2013;88(5):764–74.
13. Fernanda M, Gavazzoni R. Treatment of superficial mycoses: review - part II. An Bras Dermatol.2013;88(6):4-6.
14. Ameen M. Epidemiology of superficial fungal infections. Clin Dermatol.2010;28(2):197–201.
15. Nowrozi H, Nazeri G, AdimiP,Bashashati M, Emami M. Comparison of the activities of four antifungal agents in an in vitro model of dermatophyte nail infection. Indian J Dermatol. 2008:53(3):125-8.
16. Moriarty B, Hay R, Morris-Jones R. The diagnosis and management of Tinea. BMJ .2012;345:e4380
17. Surendran K, Bhat RM, Boloor R, Nandakishore B, Sukumar D. A Clinical and Mycological Study of Dermatophytic Infections. Indian Journal of Dermatology. 2014;59(3):262-267. 20.
18. Sarma S, Borthakur AK. A clinico-epidemiological study of dermatophytoses in Northeast India. Indian J Dermatol Venereol Leprol 2007; 73:427-8
19. Agarwal US, Saran J, Agarwal P. Clinico-mycological study of dermatophytes in a tertiary care centre in northwest India. Indian J Dermatol Venereol Leprol 2014; 80:194 22
20. Peerapur BV, Inamdar AC, Pushpa PV, Srikant B. Clinico-mycological Study of Dermatophytosis in Bijapur. Indian J Med Microbiol.2004;22(4):273-274
21. Lyngdoh CJ, W. Lyngdoh V, Choudhury B, Sangma KA, Bora I, Khyriem AB. Clinico-mycological profile of dermatophytosis in Meghalaya. International Journal of Medicine and Public Health. 2013;3(4):254-256.
22. Suman Singh S, Beena P M. Profile of dermatophyte infections in Baroda. Indian J Dermatol VenereolLeprol 2003; 69:281-3.
23. Venkatesan G, Singh AJAR, Murugesan AG, Janaki C, Shankar SG. Trichophyton rubrum – the predominant etiological agent in human dermatophytoses in Chennai, India. 2007;(May):9–12

Table 1: No of Specimen Skin Hair and Nail Teste

S. NO	SPECIMEN	%
1	SKIN(n=114)	63.33
2	HAIR(n=28)	15.56
3	NAIL(n=38)	21.11

Table 2: Age Wise Distribution of Dermatophyte Infection

S. NO	AGE GROUP	MALE	FEMALE	TOTAL	%
1	1-10	2	4	6	3.30
2	11-20	20	12	32	17.78
3	21-30	34	23	57	31.71
4	31-40	24	24	48	26.70
5	41-50	6	10	16	8.81
6	51-60	3	6	9	5.00
7	61-70	3	5	8	4.40
8	71-80	3	1	4	2.30

Table 3: Dermatophytosis Clinical Presentation (n = 180)

S. NO	SPECIMEN	FUNGAL LESION	NO OF CASES	%
1	SKIN(n=114)	Tinea corporis	68	37.78
		Tinea cruris	27	15.00
		Tinea mannum	5	2.78
		Tinea pedis	7	3.89
		Tinea facei	7	3.89
2	HAIR(n=28)	Tinea capitis	21	11.67
		Tinea barbae	7	3.89
3	NAILS(n=38)	Tinea unguium	38	21.10

Table 4: Distribution of Dermatophytes in Clinical Specimens (n134).

SPECIMEN	ISOLATES	NO OF CASES	%
SKIN	T.mentagrophytes	63	47.01
	T.rubrum	16	11.94
HAIR	T.rubrum	20	14.93
	T.tonsuran	1	0.77
	M.gypseum	2	1.49
	T.rubrum	15	11.19
NAIL	T.mentagrophytes	15	11.19
	E.floccosum	2	1.49

Table 5: Dermatophytic Species Frequencies (n=134)

ISOLATES	NO OF CASES	%
T.mentagrophytes	78	58.20
T.rubrum	51	38.00
T.tonsurans	1	0.80
E. floccosum	2	1.50
M.gypseum	2	1.50