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Dermatology

KEY WORDS:
Dermatophytes, *Trichophyton* , *Epidermophyton, Microsporum*, tinea infections.

DERMATOPHYTOSIS IN MAN: SPECIES WISE INCIDENCE OF DERMATOPHYTES IN VARIOUS CLINICAL SAMPLES ISOLATED FROM PATIENTS.

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ABSTRACT

Dermatophytes are fungi that can cause infections of the skin, hair, and nails due to their ability to utilize keratin. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. These infections are known as ringworm or tinea, in association with the infected body part. Occasionally the organisms do invade the subcutaneous tissues, resulting in kerion development. There are three genera ie, *Trichophyton, Microsporum* and *Epidermophyton*, that cause dermatophytosis in human beings but there are several species present in these genera. If a particular species is known in diagnosis, the targeting of disease is more accurate during treatment. Hence the present study was conducted on 400 patients visiting the dermatology clinic of Dr. B.Ramesh from 2012-2014, to know the incidence of species in a particular clinical type of tinea infections. Therefore 400 clinical samples were collected from 400 patients who were suspected to be suffering from tinea infections. The most common infection of dermatophytosis is tinea corporis followed by tinea cruris. The frequent isolate is *Trichophyton rubrum* followed by *T. violaceum* in tinea corporis and again *T.rubrum* followed by *Microsporum gypseum*.

INTRODUCTION

Dermatophytosis is a mycological disease caused by dermatophytes. Dermatophytes are fungi that can cause infections of the skin, hair, and nails due to their ability to utilize keratin. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. These infections are known as ringworm or tinea, in association with the infected body part. Occasionally the organisms do invade the subcutaneous tissues, resulting in kerion development. There are 3 genera ie, *Trichophyton, Microsporum* and *Epidermophyton*, that cause dermatophytosis in human beings but there are several species present in these genera. Dermatophytes show distinct clinical manifestations in different parts of the body. Each focus of infection is due to local inoculation of the fungus. The lesions show maximum inflammation in the margins leaving a clear central zone. The clinical features of tinea infections depend on the ability of dermatophytes in keratin utilization and host immunity responses resulting inflammations. The variations in clinical conditions vary from species to species and it also depends on the size of inoculums, site of infection, the immune status of host etc.

Depending upon the site of infection these infections are categorized in to tinea corporis (body), tinea cruris (groin region), tinea pedis (foot), tinea facie (face), tinea anguium (nail), tinea mannum (hands) and tinea capitis (head).

METHODS AND MATERIALS:

Sample collection: The present study was conducted on 400 patients to know the incidence of species in a particular clinical type of tinea infections. Therefore 400 clinical samples were collected from 400 patients who were suspected to be suffering from tinea infections. The site or organs of infection and the nature of isolated specimen is very much important for the diagnosis of dermatophytosis (2, 3) The lesions caused by the dermatophytes in tinea infections usually spread outwards in a concentric form with a healing central region. Hence clinical samples from the lesions were collected with the help of a scalpel blade, by scrapping the skin outwards from the edges of the lesions. The samples from the scalp were collected with scalpel by scraping the lesion so that they include hair stubs, contents of follicles and scales. The infected hairs were plucked from the base with the hair follicle intact by using epilating forceps. Before plucking the hairs are selected by wood's lamp. Nail specimens were collected with a nail cutter by clipping the nails from the free edge by using.

The samples collected from the patients were transported immediately under aseptic conditions, processed appropriately and subjected to different tests as early as possible i.e., on same day. The specimen materials were apportioned into 2-3 parts and used for different tests as described below. The diagnosis of fungal infections of the skin is usually based on the location and characteristics of the lesions and on the following laboratory examinations

Table -1: Category of clinical samples collected for laboratory diagnosis

Type of samples collected	No. of samples	Percentage
Skin scrapings	306	76.5
Nail clippings/skin scrapings	50	12.50
Hair stubs	44	11.00
Total	**400**	**100**

Direct microscopic examination: (7,8)

For direct microscopic observations the samples were processed as described below. The samples collected were cleared in 10-30% KOH solution. Small and thin pieces of material were used for examinations. Within 30 minutes the materials was cleared and clearing was hastened by subjecting to gentle heating in a watch glass covered by an upturned petridish and allowed for 24 hrs to elapse. All the samples were observed under microscope for the presence of hyphae and arthroconidia. After clearing the samples were placed on a clean glass slide and a cover slip was placed over the preparation and pressed against the slide so that the material is flattened and spread uniformly. The excess fluid was wiped using a filter paper and the slide is kept for 10-15 minutes depending on the thickness of the scales. The wet-mount preparation was then examined under a microscope (×400) with back-and-forth rotation of the focus knobs. This technique aids the visualization of hyphae (branching, rod-shaped filaments of uniform width with lines of separation (septa). In tinea capitis, the hair shaft may be uniformly coated with minute dermatophyte spores. The slide was examined for the detection of the type of hair infection (ectothrix, endothrix) and/or septate hyphae and spores of dermatophytes. The hyphae, which appear were highly refractile, hyaline, septate branching threads interspersed with the epithelial cells. Older hyphae may have septa and were provided with arthroconidia.

In our study out of total of 400 cases of clinical dermatophytosis, a total of 119 isolates were obtained in culture representing the three genera of dermatophytes, namely *Trichophyton, Epidermophyton* and *Microsporum*. Out of 119 samples 14 were negative by KOH but yielded growth on culture ('+' positive & '-'negative).

Culture medium

For macroscopic and microscopic identification of dermatophytes Sabouraud dextrose agar medium is being used for culturing.(Raymond Jacques Sabouraud 1864-1938). Bacterial contaminants were controllable only by the using an acid medium, such as Sabouraud dextrose agar at pH 5.5, and with the addition of antibacterial antibiotic like broad spectrum chloromphenicol and cyclohexamide which inhibits the growth of saprophytic fungi.

The cultural characters include both macroscopic and microscopic characters of dermatophytes. The macroscopic characters are visible to naked eye and involve the colony characters of cultures as appeared in the plates and slants.The colony morphologies include: colour, consistency, topography and presence of pigment on the obverse and reverse side(4,5). The microscopy includes the type of hyphae, presence and structures of microconidia, macroconidia , conidiospores and arthroconidia.

The microscopic observations of organisms were made by making a slide mount with lactophenol. Observations were made both in low power and high power. Wherever it was necessary photo-micrographs of the slides were taken. The identification of different dermatophytes was made by using standard manuals (Murray1999).

Cultural characteristic of different dermatophytic species of clinical isolates

The three dermatophytes have their own specific variations both macro and microscopically. The *Trichophyton* was identified from *Microsporum* and *Epidermophyton* based on the macroconidia which were clavate to cigar shaped, thin to thick walled and smooth (1,Ananthanarayan R., 2005).

Microsporum was identified from other dermatophytes in having spindle shaped, multicellular and echinulated or rough walled macroconidia. The *Epidermophyton* was identified from *Trichophyton* and *Microsporum* by prominent absence of microconidia. Moreover these were unable to perforate hair, hence these were not found in the clinical samples which were extracted from tinea capitis infections.

The dermatophytic growth on their specified medium is slow, hence the cultures swere incubated for at least 4 weeks before discarding them as sterile. Usually the growth of the fungi is seen within 7-10 days of duration. The cultures were examined for growth daily for the first week and twice a week for subsequent period. If the cultures are threatened by the problem of overgrowth or contamination by saprophytic fungi, the subculturing should be done. Based upon cultural characteristics, colony morphology and micromorphology of mycelia the species were identified (9).

Table 2: Species wise Incidence of dermatophytes in various clinical samples (culture+ve)

Dermatophytic species	Sample from skin	Sample from hair	Sample from nail	Sample from skin+ hair	Sample from skin+ nail	Sample from hair+ nail	Sample from skin+ hair+ nail	Total	Percentage (%)
T. rubrum	19	2	3	5	4	3	4	40	33.61
T.mentagrophytes	7	2	1	2	3	2	2	19	15.96
T. violaceum	5	3	-	1	1	3	2	15	12.60
M. gypseum	3	3	-	2	-	3	1	12	10.04
E. floccosum	3	-	5	-	1	-	-	9	7.56
M. audouinii	3	3	-	1	-	-	1	8	6.72
T.schoenieinii	2	-	-	2	1	-	-	5	4.20
M. canis	5	-	-	-	-	-	-	5	4.20
T.tonsurans	2	2	-	-	-	-	-	4	3.36
T.verrucosum	2	-						2	1.68
Total no . of cases	***51***	**15**	**9**	**13**	**10**	**11**	**10**	**119**	**100**

Table -3: Incidence of various dermatophytic species in tinea corporis

Dermatophytic species	No. of isolates in tinea corporis	percentage
T. rubrum	13	35.13
T. mentagrophytes	5	13.51
T. violaceum	7	18.91
M. gypseum	1	2.70
E. floccosum	2	5.40
M. audouinii	4	10.81
T.schoenleinii	4	10.81
M. canis	-	-
T.tonsurans	-	-
T.verrucosum	1	2.70
Total No. of cases	**37**	**100**

Table -4: Incidence of various dermatophytic species in tinea cruris:

Dermatophytic species	No. of isolates in tinea cruris	percentage
T. rubrum	8	42.10
T. mentagrophytes	3	15.78
T. violaceum	1	5.26
M. gypseum	4	21.05
E. floccosum	-	-
M. audouinii	1	5.26
T.schoenleinii	1	5.26
M. canis	1	5.26
T.tonsurans	-	-
T.verrucosum	-	-
Total No. of cases	**19**	**100**

RESULTS AND DISCUSSIONS:

The present study was conducted on 400 patients. Therefore 400 clinical samples were collected from 400 patients who were suspected to be suffering from tinea infections. The data pertaining to the sample collection was summarized in Table-1.

The critical analysis of Table-1, showed that out of a total of 400 patients 306 (76.5) samples were collected from skin in the form of scrapings. The samples of nail clippings and skin scrapings comprised of 50 (12.50%) in number and the collected hair stubs were 44(11.00%) out of 400cases.The reason for increased percentage of the skin scrapings was attributed to the high prevalence of tinea corporis and tinea cruris.

The isolated species of dermatophytes from various clinical samples in different clinical conditions of tinea infections were recorded in Table-2. The critical analysis of Table-2 revealed that the *T .rubrum, T.mentagrophytes* and *T. tonsurans* were isolated from all the three types of samples i.e., skin, hair and nail. Whereas *M. audouinii, M. gypseum, T. violaceum* and *T.schoenleinii* were isolated from skin and nail samples only. *E.floccosum* was isolated from skin and nail samples only.

M. canis and *T. verrucosum* was isolated from skin specimens. Thus this study revealed that *Trichophyton* species could infect skin, hair and nails. *Microsporum* species could infect skin and hair and *Epidermophyton* species infects skin and nail only. Table-2, summarizes the different species of dermatophytes isolated and their incidence in different clinical types of tinea.

A critical analysis of Table-3 revealed that in tinea corporis, out of 37 culture positive cases the highest prevalence was recorded for *T.rubrum* with 13 cases (35.13%) followed by *T. violaceum* with 7 cases (18.91%).The least incidence was recorded for *T. verrucosum* and *M.gypseum* with 1 case (2.70%) each. The incidence of *T. mentagrophytes* was in third position with 5 cases (13.51%) and *M. audouinii* and *T. schoenleinii* were in fourth place with 4 cases (10.81%) each. No case was reported for *T.tonsurans* and M. *canis.* The predominance of *T. rubrum* in our study report is similar to the findings of Kamothi, M N (2010) on a study report of prevalence dermatophytic infections in Rajkote district. According to his report, the *Trichophyton rubrum* was the most common species isolated. Tinea corporis was the most common clinical presentation and *T. rubrum* was the predominant fungus. In a study report by N. Sivakumar *et al* (2009) at Mallapuram district, Kerala revealed that the frequently isolated fungi was *T. rubrum* (in 22 cases), followed by *T. mentogrophytes* (in 18 cases*), E. floccosum* (in 3 cases), *Microsporum nanum* (in one case),and *T. violaceum*(in one case). The study report on *Trichophyton rubrum* – the predominant etiological agent in human dermatophytoses in Chennai, India by G. Venkatesan *et al* (2007) revealed that, *T. rubrum* was the predominant species responsible for the dermatophytoses, especially tinea corporis infections, followed by *T.mentagrophytes* (18.3%) and *M.gypseum* (1.4%).

Table-4, summarizes the incidence of different dermatophytic species in tinea cruris infections with culture positive cases. Out of 19 culture positive cases *T. rubrum* showed high incidence with 8 cases (42.10%) followed by *M. gypseum* with 4 cases (21.05%) and *T.mentagrophytes* with 3 cases (15.78%).Least incidence was recorded with *T. violaceum, M.audouinii, T.schoenleinii and M. canis* with 1 case (5,26%0 each. Nil incidence was reported for *E.flocossum, T.tonsurans* and *T.verrucosum*.

CONCLUSION:

The study report is similar to the findings of other reports. Venkatesan *et al* (2007) studied the predominance *of Trichophyton rubrum* in Chennai and reported that tinea cruris was the second predominant infection observed (26.8%). *T. rubrum* (22.6%) was the predominant etiological agent isolated from tinea cruris patients followed by *E. floccosum* (2.8%) and *M. gypseum* (1.4%). According to the study report on clinicomycological study of dermatophytosis in Bijapur by Peerapur *et al* (2004) in overall dermatophytic infections, *Trichophyton rubrum* (28, 43.7%) was the most frequent isolate followed by *T.mentagrophytes* (18, 28.1%), *Epidermophyton floccosum* (5, 7.8%) and *Microsporum* (4, 6.2%). In tinea cruris *T. rubrum* with 8 cases and *T. mentagrophytes* with 1 case was reported in their study at Bijapur. The identification of etiological agents of dermatophytosis helps in treating the disease with effective drug as each species has different cellular structure and different resistance system responding to the drug during treatment.

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