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RESEARCH ARTICLE

10% Potassium Hydroxide Mount of Skin Scraping- A Simple Tool for Diagnosing Dermatophytosis in Patients Attending a Dermatology Clinic in a Tertiary Care Hospital in Salem

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Abstract

Background: Dermatophytosis is also known as tinea infection, refers to a type of superficial fungal infection caused by fungi that invade keratin, which is found in skin, hair, and nails. Dermatophytosis is a major public health concern in India with an alarmingly rising trend in recent years. **Aim and Objectives:** In this study, the incidence of dermatophytosis in patients attending the dermatology clinic was measured. **Methods and Materials:** Skin scrapings were collected from patients suspected with tinea infection and examined under a microscope with a 10% KOH mount for early detection of fungal elements. The positive samples, which showed fungal elements, were cultured on SDA for species identification. **Results:** In this study, 170 patient samples were collected, among them, 68% were male and 32% were female, among all of them, diabetics were 26%. The most frequently identified fungus was Trichophyton mentagrophytes, found in 47% of cases and Trichophyton rubrum 33%. Clinically Tinea corporis was common presentation in this study. **Conclusion:** The detection of fungal elements using a 10% KOH wet mount is considered a crucial and effective method for the early diagnosis of Dermatophytosis. This KOH mount test often shows more positive results and helps to diagnose fungal infections earlier, compared to culture methods like SDA inoculation. Given the high rates of superficial fungal infections among people in rural areas who seek treatment at advanced healthcare facilities, it is essential to implement preventive measures and early screening practices

Keywords: LPCB- Lacto Phenol Cotton Blue, SDA- Sabouraud Dextrose Agar, *Tinea corporis Tinea cruris* Slide Culture, *T. mentagrophytes*, *T. rubrum*

1. Introduction

Dermatophytes are a group of fungi capable of penetrating and colonizing keratin-rich tissues, including hair, nails, and skin[1]. The causative organisms of dermatophytes belong to three asexual genera: Epidermophyton, Microsporum, and Trichophyton. These fungi are categorized within the anamorphic class Hyphomycetes, which is part of the Deuteromycota (Fungi Imperfecti) division[2]. The classification of genera primarily follows Emmons' system, which is based on the morphology and formation of conidia[3]. The prognosis differs among dermatophyte species as zoophilic species trigger intense inflammatory responses, frequently resulting in spontaneous recovery. In contrast, anthropophilic species typically

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cause less severe but persistent lesions. A remarkable aspect of dermatophytes as parasitic organisms is their exclusive targeting of dead keratinized structures. Despite ringworm infection triggering inflammatory responses in the dermis and Malpighian layer of the epidermis, the causative fungus thrives exclusively in the epidermal stratum corneum, surrounding keratinized hair shafts, and residing within nails. The fungus predominantly exists as mycelium and arthroconidia within these keratinized structures. In hair infections, the fungus penetrates the follicle from the adjacent stratum corneum, following specific growth patterns[4].

Dermatophytosis is locally referred to as ringworm, and various anatomical sites of infection are identified with names starting with the prefix 'Tinea', a Latin word[5], Dermatophytosis has become a major public health problem in the world today due to persistence, recurrence, and antifungal resistance[6]. It is especially prevalent in tropical and subtropical areas, such as India. The hot and humid weather there creates a perfect environment for this fungal infection to spread and thrive. The disease occurs more frequently in men than in women. Several factors may contribute to this increase, including excessive sweating, wearing tight clothing throughout the day[7], sharing clothes, and diabetes. This study aims to determine the incidence and contributing factors associated with dermatophytosis and to evaluate diagnosis through the efficacy of laboratory microscopic methods and culture techniques. The study emphasizes the importance of microscopic examination of skin scrapings with KOH mount, as it aids in initiating appropriate antifungal treatment earlier

2. Aim and Objectives

- To evaluate the clinical and mycological patterns of dermatophyte infections in patients arriving at the dermatology outpatient department.
- To correlate the formal clinical diagnosis with fungal identification, with KOH positivity and culture positivity.

3. Review of Literature

Dermatophytosis is a commonly encountered superficial fungal infection in the tropical and subtropical countries[1]. They are producing proteases that break down keratin, facilitating infection, invasion, and colonization of the skin's stratum corneum, hair shaft, and nail. They are molds belonging to the three genera of fungi imperfecti: Microsporum, Trichophyton and Epidermophyton[4]. Hot and humid climate, poverty, poor hygiene, social conditions like overcrowding are some of the factors favoring dermatophytoses in India. Immunocompromised states due to underlying malignancy, administration of steroids or immunosuppressive drugs, acquired immunodeficiency syndrome or endocrinological disorders such as diabetes mellitus and Cushing's disease can lead to generalized and atypical presentation that can be confused with other skin disorders[8].

The typical infections of dermatophytes are generally referred to as ringworm infections due to their ring like appearance. These infections are also known as 'tinea infections' and are named according to the location of the lesions on the body e.g. tinea capitis refers to ring worm infection of the head region[7].

The specimens collected include hair, nail clippings, and skin scrapings. Before the specimen collection, 70% alcohol is used to clean the lesions. Skin scrapings are collected from the lesion's active edge. Hair is collected from base in tinea capitis. Nail clippings are collected are collected in tinea unguium[9]

As previously mentioned, skin, hair, and nail samples were inoculated in Sabouraud dextrose agar. Four sites were inoculated, with intervals between each site. Sabouraud's dextrose agar slants containing cyclohexamide (0.5 mg/ml) and chloramphenicol (0.05 mg/ml). Cyclohexamide was used to inhibit the growth of saprophytic fungi, and chloramphenicol was added to inhibit the growth of fungus[9]. In order to achieve good growth of certain dermatophytes that prefer a slightly higher temperature, the tubes were incubated at 37 degrees Celsius as well as at room temperature. The tubes are checked for signs of internal growth at regular intervals, and the growth's progression is also recorded. After six weeks of incubation, culture tubes that show no growth are discarded. Colony morphology, texture, pigmentation on the surface (obverse), and pigmentation on the reverse of any visible growth on SDA are noted regularly[10]

Microscopic examination of colony is done by doing a lacto phenol cotton blue mount to examine the hyphal

structure, different vegetative structures formed by hyphal modifications, various reproductive structures like microconidia, macroconidia and chlamydoconidia. Urea hydrolysis is used to distinguish some species of Trichophyton and Microsporum. Slide culture is done to find the morphology of hyphae, microconidia and macroconidia[10]

4. Material and Methods

- Type of Study – Prospective and Study
- Study Period – 6 Months (May 2024-November 2024)
- Sample size - 169

Inclusion Criteria

- Patients attending the dermatology OPD with symptoms of itchy, scaly skin lesions who are suspected of having dermatophytosis.
- Patients with recurrent ringworm infections.

Exclusion Criteria

- Individuals who have had topical antifungals during the last four weeks and systemic antifungals within the last three months.
- Patients took a bath and applied any ointment before coming to the OPD (they were advised to come on the next day without taking bath or applying any ointment and skin scrapings were collected)
- Patients were informed about the purpose of the study and provided with a consent form in the local language. The samples were collected from willing patients and transported immediately to the microbiology laboratory for further processing.
- Sample Collection

Patients attending dermatology OPD with suspected dermatophytosis were mostly coming from rural areas, poor socioeconomic status, and poor literacy rate. A detailed history was collected regarding the onset of the disease, duration of symptoms, occupation, wearing tight dress for a long time in a day, medications used, any injuries, recurrence of the same illness, any co-morbidities, animal exposures, and both family and personal medical history.

- Sample was collected as per standard laboratory procedures. Tinea infections were found as scaly itchy lesions, mostly with radial growth – clear centre and extending periphery lesions. When the ring is clearly defined, it's best to collect samples from the outer edges that are still growing. Aseptic collection of specimens from affected locations was followed by 70% isopropyl alcohol cleaning and drying. Using a sterile blade or glass slide with a blunt edge, skin was scraped firmly over a black chart. For nail and subungual debris, clippings were collected, and infected hair was plucked from the base for analysis.
- Microscopic examination- "A Simple key to diagnose Dermatophytosis": All the skin scrapings were subjected to a 10% KOH mount, and Hair/Nail samples with a 40% KOH mount. In a sterile glass slide, skin samples were placed, and 2-3 drops of 10% Potassium hydroxide were added. A coverslip was placed over that and examined under a microscope, 10X and 40X, after 10-20 minutes. Potassium hydroxide dissolves the cellular elements (skin and debris) and makes the fungal elements to be visualised clearly. Samples of hair and nails were placed in a sterile glass tube with 0.5 ml of 40% KOH, left overnight at room temperature, and analyzed the following day. Two sets of test tubes containing Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide, were utilized for the dermatophyte culture to inoculate the specimen. One tube was incubated at 37° C, while the other was kept at room temperature to be observed for 2-4 weeks. Various dermatophyte species were distinguished based on their macroscopic appearance and microscopic features. Macroscopic appearance was observed by the colony colour and texture on the obverse and reverse. For microscopic examination, 2 LPCB* mounts were prepared and examined under low-power and high-power objectives of a microscope for the presence of hyphae, macroconidia, microconidia, and other accessory structures of vegetative hyphae, and the characters of each were noted.

*LPCB- Lacto Phenol Cotton Blue-fungal staining

- Slide culture

In a petri dish, a glass slide was kept on a 'V' shaped glass rod, with a piece of cotton in one side and sterilized in hot air oven. A Small square shape SDA was cut and kept on slide. Grown colonies from SDA media were taken with straight loop and placed in all 4 sides of this SDA squares and a coverslip was placed over them. This is observed for 5-7 days and coverslip was examined for hyphal and conidial morphology.

- Banana peel culture

Thick Banana peels were cut into medium-sized pieces, autoclaved in a glass petri dish. Over the inner surface of the banana peels, specimens or grown colonies from SDA agar with minimal media, were placed using sterile loop and covered with a coverslip. These were kept in room temperature and colony growth were observed. This method was used as an alternate for slide culture

5. Results

Among the study population of 169 suspected with dermatophytosis, 99 were males, 64 females and 6 were pediatric patients. The age and sex distribution of the study population summarized in Table 1. The common observed duration of illness was 3-4 weeks Among 72 patients; 33 patients were observed with recurrent illness in 3-4 months duration (male 21 and female 12). 3 patients were observed with extensive dermatophytosis and lesions were observed over front and back of the trunk and the thigh the distribution of various clinical types of dermatophytosis is detailed in Table 2. The prevalence of clinical type fungal species - *Tinea faciei* - 7 (2.3%), *Tinea cruris* - 47 (27.6%), *Tinea corporis* -102 (60%), *Tinea pedis* - 6 (3.5%) and *Tinea manuum* - 3 (1.7%) (Table 2).

Month-wise distribution of dermatophytosis cases showed a gradual increase during the study period, which is depicted in Figure 1. Direct microscopic examination using KOH mount was positive in 158 cases (92.9%), while fungal culture positivity was observed in 61 cases (35.8%). The correlation between KOH mount positivity and fungal culture results is illustrated in Figure 3. Among the KOH-positive cases, 34 patients (21.5%) were diabetic.

Table 1. Age and sexual distribution of the study participants

Age Group		Frequency (%)	
Adult	Male	99	(58.6%)
	Female	64	(37.9%)
Pediatrics (5 - 12 years)		6 (3.5%)	

Recurrence In Sexual Group	Frequency (%)	
Male	21	(63.6%)
Female	12	(36.4%)

Table 2. Clinical types of dermatophytosis

Clinical Type	Frequency (N = 169)	Percentage (%)
Tinea cruris	49	29.0
Tinea corporis	102	60.4
Tinea faciei	7	4.1
Tinea pedis	6	3.6
Tinea mannum	3	1.7
Tinea unguinum	2	1.2

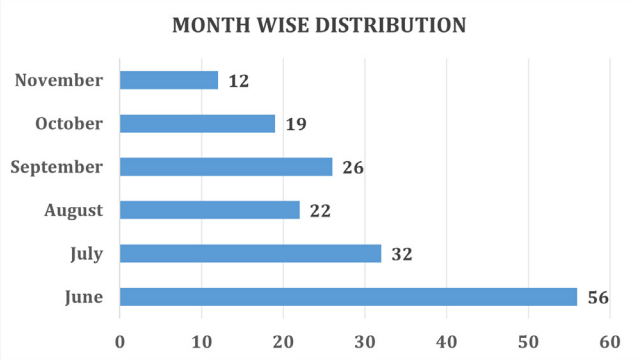

Figure 1. Month - wise distribution.

The distribution of isolated dermatophyte species is shown in Figure 2. *Trichophyton mentagrophytes* was the most commonly isolated species (47%), followed by *Trichophyton rubrum* (33%). The distribution of dermatophyte isolates across different clinical types of tinea is detailed in Table 3. Representative clinical images of dermatophytosis cases are shown in Figure 4. Microscopic findings demonstrating fungal hyphae in KOH mounts are illustrated in Figure 5. The colony morphology of dermatophytes grown on Sabouraud Dextrose Agar is shown in Figure 6, while characteristic microscopic features observed in LPCB mounts are depicted in Figure 7.

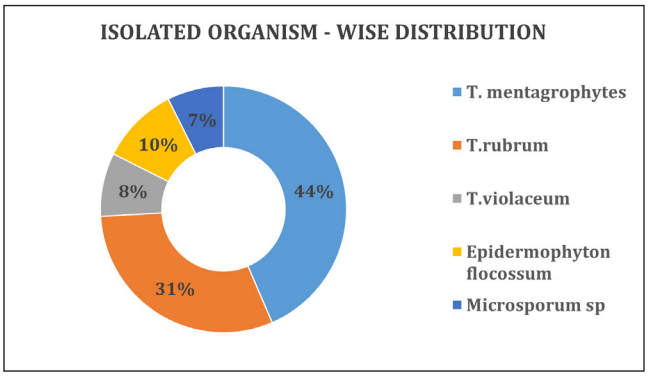

Figure 2. Isolated organism - wise distribution.

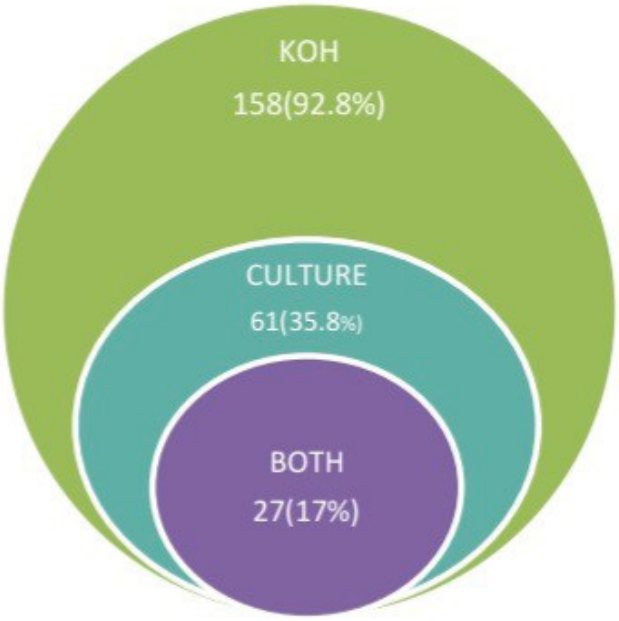

Figure 3. Positive and negative correlations between fungal culture and KOH.

6. Discussion

Recently, there has been a lot of interest in exploring dermatophytoses in India because of the increasing prevalence of mycotic infections that are difficult to treat worldwide. Dermatophytosis and the increasing prevalence of antifungal drug resistance are both currently on rising in India, although they probably have not received enough scientific attention[5].

The age groups of 21–30 and 31–40 years old had the largest numbers of participants in our study (39% and 41.7%, respectively). Similar results have been reported in studies by Basaket *et al.,*[3], Walke H R *et al.*,[11] Poluriet *et al.*,[12]., the highest incidence of dermatophytosis was observed in the age group of 21-40 years, dermatophytosis was more common in the age group of 21-30 years (19.2%) followed by 31-40 years (21%), Misra M[13] and Sen SS *et al.,*[14] Reluctance to seek medical assistance, particularly in remote regions where there may be a shortage of female doctors or specialists, may be the reason for the lower prevalence among females

In this study, 13.3% of participants reported a period of illness of less than one month, 22.7% reported more than six months, and 45.9% reported one to two months of duration. This matched the results of Ghuse *et al.,*[15] who found that the majority of patients (43.3%) had an infection lasting one to two months, and just 6.7% had the illness for a year. In contrast to the study by Gupta et al.,[16] where the majority of patients had a longer duration of sickness (58.8% of patients experienced disease for more than six months), our observations were rather different *Tinea corporis* (60%) was the most common clinical type found in the present study, followed by *Tinea cruris* (27.6%). The other clinical types reported were *Tinea faciei* (4.1%), *Tinea pedis*

Table 3. Dermatophyte isolates on culture of different clinical types of Tinea

Dermatophyte isolate on culture	*Tinea corporis*	*Tinea cruris*	*Tinea faciei*	*Tinea pedis*	*Tinea mannum / unguinum*
T. mentagrophytes	9	6	1	0	0
T. rubrum	3	5	0	0	0
T. violaceum	0	0	1	0	0
Epidermophyton flocossum	0	0	0	0	2
Microsporum sp	0	0	0	3	1

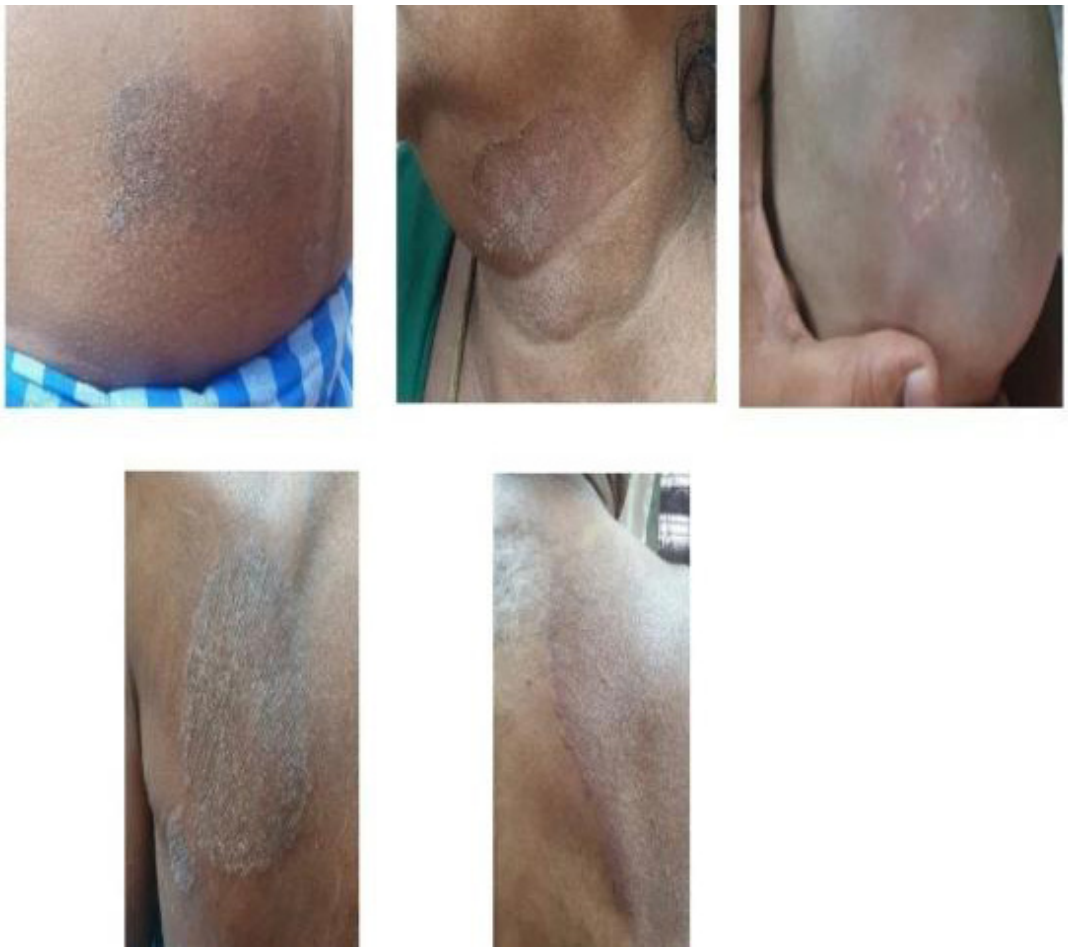

Figure 4. Clinical pictures of Tinea infection.

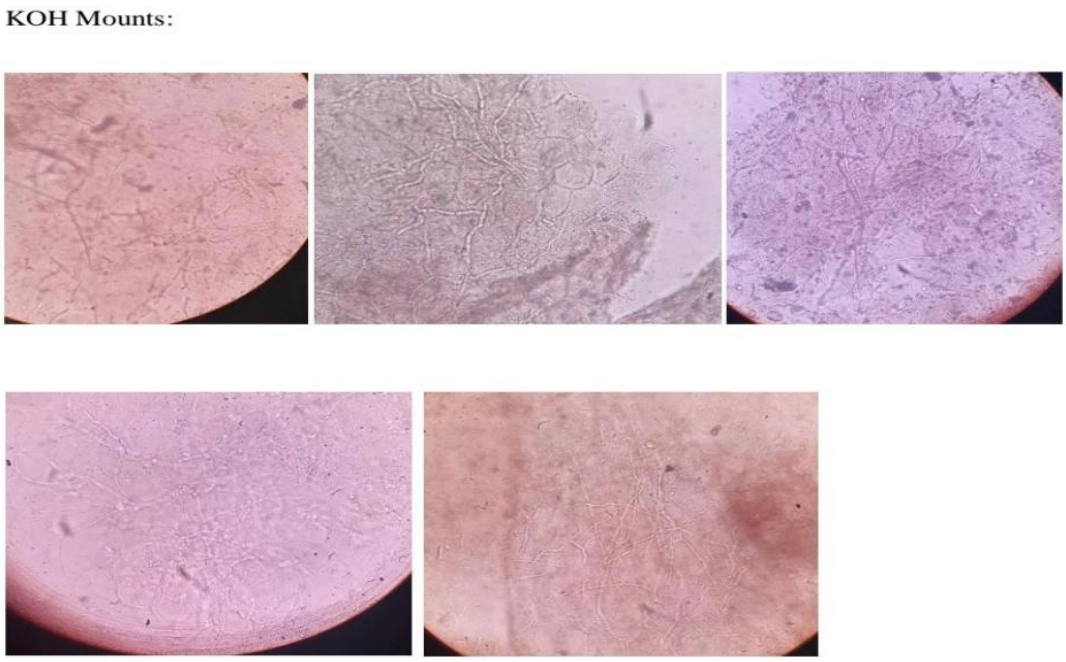

Figure 5. KOH mounts.

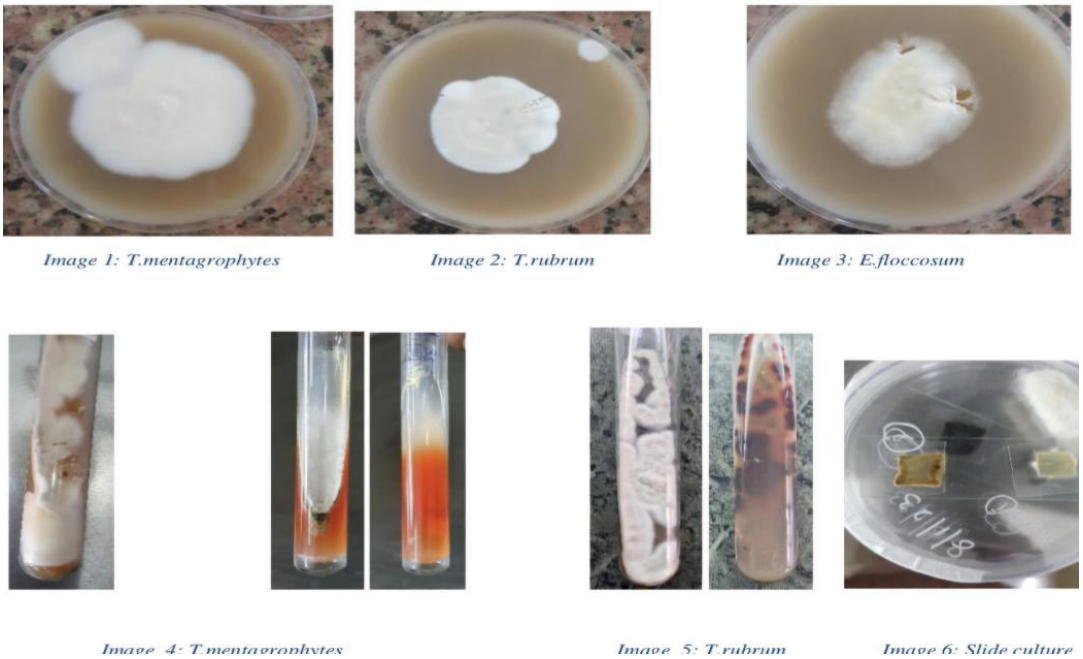

Figure 6. Colony morphology in SDA.

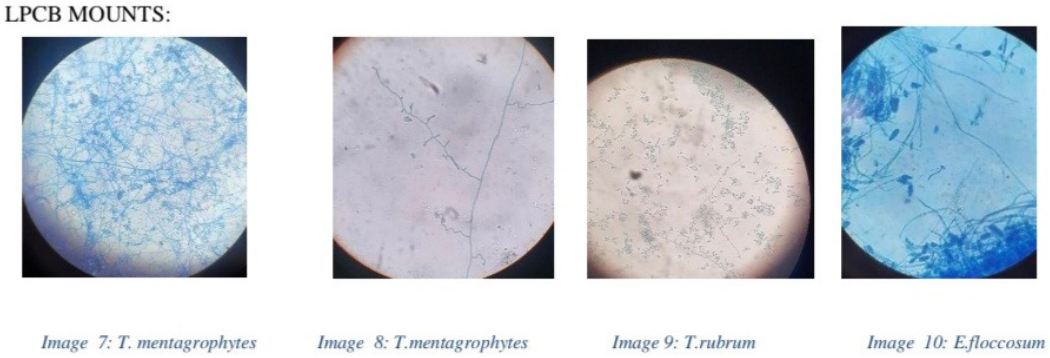

Figure 7. LPCB Mounts.

(3.5%) and *Tinea manuum* (1.7%) *Tinea unguinum* (1.6%). This was per the findings of Basak *et al.*,[3] *Tinea corporis* (52.65%) was the most common clinical presentation followed by *Tinea unguium* (14.1%) and *Tinea cruris* (12%) and Kucheria *et al.*,[4] where the most common clinical presentation was *Tinea corporis* (31%) followed by *Tinea unguium* (21%). Studies by Walke HR *et al.*,[11] and Nagaral *et al.*,[17] showed *Tinea corporis* as the most common clinical type, followed by *Tinea cruris*. Agarwal *et al.*,[18] also reported that the most common clinical types observed were *T. cruris* (40%) followed by *T. corporis* (34.3%). However, studies by Gupta CM *et al.*,[19] and Ghosh RR *et al.*,[9] showed *Tinea unguium* as the most common clinical presentation, followed by *Tinea corporis* and *Tinea capitis*.

In this study, 92.8% of the samples tested positive by direct microscopy using KOH mount, 35.8% tested positive by culture, and 17% tested both KOH and culture. Clinically diagnosed superficial mycotic infections were 74.7%. in our study all KOH positive cases were diagnosed as dermatophytosis cases and treated with anti-fungal drugs. The current study's direct microscopy and culture results are correlated with those of studies by Mahale RP *et al.*,[20] (61.01% culture positive) and Dhyaneswari GP *et al.*,[6] (72.6% KOH positive). In the study by Yadav SP *et al.*,[21] out of 66 clinically diagnosed cases of dermatophytosis, 52 cases (78.79%) were positive for fungi, either by KOH and/or culture. 35 cases (53.03%) were positive by both KOH and culture, 13 cases (19.70%) were positive by

KOH and negative by culture, 4 cases (6.06%) were negative by KOH but culture positive, 14 cases (21.21%) were negative by both KOH and culture, which is comparable with other studies done by Singh S *et al.,*[22] and Verma S *et al.,*[23]. This discrepancy may result from non-viability of fungal elements in certain situations, inadequate sample because of small non scaly lesions, and unreported partial antifungal drug treatment. Similar to the work by Poluri *et al*[12], where KOH positive was 58.18% and culture positivity was 56.36%, the current study had KOH positivity of 55.3% and culture positivity of 44.7%. Nonetheless, similarities were seen with the Basak *et al.*,[3] study 59.8% of the samples were culture positive and KOH-positive 35.8% cases, with both KOH and culture positive only 17%.

This shows that direct microscopy by KOH mount is a good screening test and a simple key in the laboratory diagnosis of dermatophytosis. Additionally, in other cases, the authors observed development on culture but no fungal elements under direct microscopy. This may be because there were very few fungal elements present that were not visible under direct microscopy, or it could be because there were fungal elements present in an inactive sporulating state that were invisible under direct microscopy and grown in media with nutritional availability.

In our study the isolations were *Trichophyton mentagrophytes* (47%) was the most often isolated dermatophyte, followed by *Trichophyton rubrum* (33%), *Trichophyton violaceum* (9%), *Epidermophyton floccosum* (11%) and Microsporum species (8%). In Basak *et al.*, study[3], *Trichophyton mentagrophytes* (57.5%) was the most often isolated dermatophyte, followed by *Trichophyton rubrum* (30.1%), *Trichophyton tonsurans* (8.1%), and *Microsporum anuum* (4.3%). The most prevalent species was *T. mentagrophytes*, according to Shivam SG *et al.*,[24] *Trichophyton mentagrophytes* (50.7%) and *T. tonsurans* (29.9%) were isolated. Another study showed *Trichophyton mentagrophytes* 81.8%, *Trichophyton rubrum* 11.36% with Bhatia VK *et al.*,[7] *Trichophyton mentagrophytes* 63.5%, *Trichophyton rubrum* 35.1% with Sahai *et al.*, studies. This was in contrast to the study conducted by Yadav SP *et al*[21]. According to Vineetha *et al.,*[26] *T. rubrum* (38.6%) was the most frequently isolated species in the first episode and in chronic dermatophytosis, followed by *T. mentagrophytes* (33.33%), *T. tonsurans* (17.95%). Similar results to our study were also reported by Agarwal *et al.,*[18] and Bindu *et al.,*[27], who found that *T. rubrum* was the most prevalent species.

7. Conclusion

T. corporis was the most prevalent clinical type identified in the study, which revealed a male preponderance. The majority of patients arrived between one and six months, and they were in their third decade. The most common species isolated from patients was *T. mentagrophyte.*

Additional investigation is required to validate the findings. Even with the present shifting patterns of the causative organisms and the resistance to different antifungals that it has evolved, dermatophytosis does not receive the attention that it deserves, thus, research on antifungal susceptibility should be expanded. Public awareness to avoid self-medication and to follow hygienic practices is also an important tool to prevent resistance and recurrence. Dermatologists and other medical professionals who treat dermatophyte infections need to be sensitized to the changing characteristics of the fungus and their upcoming resistance pattern

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