

## PREVALENCE OF DERMATOPHYTIC INFECTION AND DETERMINING SENSITIVITY OF DIAGNOSTIC PROCEDURES

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### ABSTRACT

**Objective:** Dermatophytosis accounts for fungal skin diseases, the culprit organisms colonize the keratin tissues, thereby producing inflammatory reactions, restricted to the nonliving cornified layer of the epidermis. Additionally, it produces acid proteinases, elastase, keratinases, other proteinases reportedly act as virulence factors. The present work aims at evaluation of rate of incidences of dermatophytic infections (group wise), and etiological agents with associated symptoms. In addition, we also determine to test the sensitivity of present diagnostic procedures.

**Methods:** Samples from 150 case studies were collected and subjected to KOH test and culture test.

**Results:** Out of 150 case studies of clinically suspected patients, 74% (111) patients were males and 26 % (39) were females. Among the patient data, 70.27% (78 out of 111) males and 82.05% (32 out of 39) females were found to be infected on the basis of KOH test and culture test. Maximum infection was reported from groin 32.67% (49) followed by hands/legs 21.33% (32) and thighs 15.33% (23). 84% of the KOH positive samples showed positive test for culture, thereby indicating its high sensitivity as diagnostic tool. The maximum relative percent occurrence (RPO) was shown by *Aspergillus* sp. (29.33%) followed by *Trichophyton* sp. (21.33%), *Fusarium* sp. (12.67%), *Microsporum* sp. (11.33%) and *Cladosporium* sp. (11.33%).

**Conclusion:** Our finding showed that infection is more pronounced in male than female. However, the KOH test was positive in 126 cases and 118 cases were fungal culture positive. Sensitivity of the KOH test is 84% as compared with reference standard culture test. Our findings suggest that KOH test can be used for diagnosis of dermatophilic infections in remote conditions where rapid and low cost diagnosis is required.

**Keywords:** Prevalence, Dermatophytic infection, KOH test, Diagnosis and clinical isolates.

### INTRODUCTION

Skin infections are common diseases in developing countries, of which dermatophytosis are of particular concern in the tropics. A fungal skin infection is usually the result of the presence of some types of foreign fungus on the skin. These fungi grow best on those areas of the skin that are warm, dark and moist. Dermatophytes are parasitic fungi that infect the skin and cause infections of the skin, hair and nails because of their ability to obtain nutrients from keratinized material. These organisms colonize the keratin tissues and in response to their metabolic by products, host experiences inflammatory reactions. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Acid proteinases, elastase, keratinases and other proteinases reportedly act as virulence factors [4]. Dermatophytes cause fungal infections of keratinised tissues, e.g. skin, hair and nails. The organisms belong to 3 genera, *Trichophyton*, *Epidermophyton* and *Microsporum*. Dermatophytes may be grouped into 3 categories based on host preference and natural habitat. Anthropophilic species predominantly infect humans, geophilic species are soil based and may infect both humans and animals, and zoophilic species generally infect non-human mammals [6]. Diagnosis of these mycoses is made from mycological studies, direct examination, stains, isolation and identification of the fungi. Treatment includes systemic antifungal, topical antifungal and keratolytics [3]. Superficial mycoses are fungal infections limited to the stratum corneum and its adnexal structures. Diagnosis of these mycoses is made from mycological studies, direct examination, stains, and isolation, and identification of the fungi [3]. Tinea infections are among the most common dermatologic conditions throughout the world. To avoid a misdiagnosis, identification of dermatophyte infections requires both a fungal culture on Sabouraud's agar media and a light microscopic mycological examination from skin scrapings. Preventative measures of Tinea infections include practicing good personal hygiene, keeping the skin dry and cool at all times and avoiding sharing towels, clothing, or hair accessories

with infected individuals [6]. *Trichophyton rubrum* was the most prevalent fungal pathogen isolated from all cases of superficial fungal infections of the skin, except for *Tinea pedis*, where *Trichophyton interdigitale* was the most frequently isolated organism. Dermatophytes remain the most commonly isolated fungal pathogens isolated in toenail onychomycosis, whilst *Candida* species accounted for the majority of isolates in fingernail onychomycosis [16]. Though the climatic conditions are not favorable for the fungal growth during most of the time of the year in Rajasthan, but due to some injuries and inappropriate approach for the treatment of trauma situations in rural population the incidences of Dermatophytic infections are increasing.

Therefore, in the present study we aim to evaluate the rate of incidences of dermatophytic infection, to find its etiological agents and the associated symptoms in the study area. Simultaneously the sensitivity of KOH test and culture test for the screening of the infection was also determined.

### MATERIALS AND METHODS

**Sample collection:** A detailed record of patients susceptible for dermatophytic infection visiting Dr. B. Lal Clinical Laboratory, Malviya Nagar, Jaipur for clinical diagnosis was maintained. Patient Performa was filled during the collection of sample to obtain information on duration of the lesion, clinical picture, prior therapy as well as demographic data such as age, sex and duration of illness, etc. The final selected cases for this study were 150 cases consisted of 111 (74%) males and 39 (26%) females.

**Sample collection:** Two sample collection methods were used in this study: In one method, samples consisting of epidermal scales and infected hairs were scraped from the scalp/rim of lesions using a sterile scalpel blade following cleaning of the affected sites with 70v/v isopropyl alcohol. The scrapings were collected on a piece of sterile brown paper. In the other method, moist cotton swabs were used to collect pus from inflammatory lesions. The samples were divided into two portions: one for microscopic examination and one

for culture. The collected samples were transported to the laboratory within 2 hours for microscopic and cultural analysis [5].

### Sample processing

**Direct microscopic examination:** Direct microscopic examination of the scrapping placed on a microscope slide with one or two drop of 20% potassium hydroxide (KOH) and a cover slip was performed. The sample was warmed for 5 minutes over a flame [8]. Each treated slide was then carefully examined under low (X10) and high (X40) power objective for the presence of hyphae and/or arthroconidia.

**Fungal culture:** Each scraping was cultured into Sabouraud Dextrose chloramphenicol actidione agar [1]. A duplicate inoculation of the specimen was also cultured on Sabouraud's dextrose cycloheximide agar. The plates were incubated at 28°C for up to 4 weeks and examined at 2 to 3 day intervals for fungal growth. Fungal isolates were subcultured onto plates of Sabouraud's agar. The isolates were examined visually and microscopically for morphology of fungi using lacto phenol cotton blue by slide culture technique. The dermatophytes species were identified by gross and microscopic morphology and by *in-vitro* tests. Evaluation of the relative percent occurrence (RPO) of the fungi and sensitivity of KOH test (results of KOH test corresponds to culture test indicated the sensitivity of the KOH test) was done. The clinical isolates were further maintained in agar slants.

### RESULTS AND DISCUSSION

The fungal infections are termed as "Tinea". Dermatophytic fungal infections caused due to infestation of fungi in the skin, nails and hairs. These infections are generally predominant in areas having moist and warm climate which help in sustained growth of these organisms.

During the study for dermatophytic infection 150 patient samples susceptible for the infections were collected from Dr. B. Lal Clinical Laboratory, Jaipur. Among 150 samples collected and analyzed, it was found that 74% (111 out of 150) patients were males while the remaining 26% (39 out of 150) were females. Among the patient data collected, 82.88% (92 out of 111) males and 87.17% (34 out of 39) females were found to be KOH positive (Figure 1). Similar observations were recorded at the National Skin Centre, a tertiary referral centre for dermatological diseases in Singapore with more than 2,500 cases of superficial fungal infections annually. The majority of patients (n=9335) (72.3%) were males. Similar observations were recorded in anthropophilic species, the main cause of dermatophytosis in adults and are isolated more often in males than in females [15].

The patient data was categorized on the basis of age groups of 0-15 years to 60 years and above in groups of 15 years gap and it was found that the maximum incidence (47%) was observed in the age group of 16 to 30 years. (Figure 5) The prevalence of Tinea pedis in 15-year-old school children and 20-year-old males was found to be 4% and 6%, respectively [15].

Among all the cases showing infection at various sites in the body including scalp, groin, thighs, face, hands, nails, full body, hips, neck and others, the maximum infection 32.67% (49) was recovered from groin followed by 21.33% (32) from hands/legs and 15.33% (23) from thighs (Figure 2). These fungi are the causative agents of various types of dermatophytosis that attack various parts of the body and tend to the following conditions, Tinea capitis, Tinea cruris, Tinea corporis and Tinea pedis [14]. A total of two hundred and fifty samples were obtained from infected skin, hair and nails of individuals within Sokoto metropolis [13]. But feet are the site of infection in about 50% of the cases, toenails, glabrous skin and inguinal folds equally often in 42%, palms in 6% and the scalp in 2% [15]. The most common infection was Tinea pedis (n=3516) (27.3%), followed by pityriasis versicolor (n=3249) (25.2%) and Tinea cruris (n=1745) (13.5%) [16]. Similar kind of results were detected in a study conducted in which out of the 60 cases, 13% cases were healthy and 87% cases were found infected with one or more

fungi. In 95% of cases, the fungal species recovered were from the infected symptomatic area like inflammatory lesions redness, dry patches itching, flaky rings, and 6.7% of cases from pain. The pattern of distribution of site of infection was recorded maximum at internal parts (54%) followed by hand (15%), neck (12%) and leg (6%) [7]. During the infection maximum cases were found to show symptoms such as redness itching and dry patches while the duration of infection was variable ranging from a few days up to many years while some also complained about the infection to be seasonal and only confined to summers. To test and confirm the presence of fungal infection KOH result and culture findings were considered which revealed that KOH test is 73.33% sensitive (out of 150 cases, 110 were both KOH test and culture test positive). The data revealed that the KOH test was false positive (KOH positive and culture negative) in 16 cases and false negative (KOH negative and culture positive) in 08 cases studied but 16 cases were negative from both the test results, and were considered free from fungal infection. (Figure 3) On the basis KOH and culture test results 78 males (out of 110) and 32 (out of 39) females were found to be infected with fungal dermatophytic infection. (Figure 6) In a previous study, out of 155 patients clinically suspected with dermatophytosis, 105 specimens were skin scrapings and 50 were hair. Results of KOH microscopy and fungal culture were compared showing that KOH microscopy was positive in 70% cases while fungal culture showed positive results in 25.8% cases [4]. Diagnosis usually can be made with a focused history, physical examination and potassium hydroxide microscopy. Occasionally, Wood's lamp examination, fungal culture or histological tissue examination is required [2]. Whereas a study conducted indicates dermatophytosis is the most common skin disease in the rural population and around Sitapura and Sanganer area, Jaipur. Among the 200 suspected patients with clinical symptoms of dermatophytosis, 170 samples (85%) found to be positive by KOH examination and 120 (60%) confirmed in culture. *Tinea corporis* (infection of the glabrous skin) was the most common dermatophytosis reported followed *Tinea cruris*, *Tinea capitis*, *Tinea pedis* and *Tinea manuum*. *Tinea barbae* and *Tinea faciei* reported least among all the cases of dermatophytosis [7].

The incidences of infection were by various fungi such as *Trichophyton*, *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Curvularia*, *Microsporum* and *Fusarium*. Among these the maximum incidence was shown by *Aspergillus* sp. (29.33%) followed by *Trichophyton* sp. (21.33%), *Fusarium* sp. (12.67%), *Microsporum* sp. (11.33%) and *Cladosporium* sp. (11.33%). (Figure 4) In the test conducted on dermatophytes, that included clinical dermatophytes of the genera *Trichophyton*, *Microsporum* and *Epidemophyton* [2.12]. Four dermatophytes were identified to species level and one to genus level; they include *Trichophyton rubrum*, *Trichophyton mentagrophyte*, *Microsporum audouinii*, *Microsporum gypseum* and *Microsporum* species [13]. Two pathogenic dermatophytes *Trichophyton rubrum* and *Microsporum gypseum* were collected from dermatophytosis patients [10]. In Denmark, zoophilic species like *Microsporum canis* (from cats), *Trichophyton (T) verrucosum* (from cattle) and *T. mentagrophytes granulare* (from rodents) are the common causes of dermatophytosis and are seen in approximately 15% of all cases also *T. rubrum* (48%), *T. mentagrophytes interdigitale* (14%) and *Epidermophyton floccosum* (10%) are the species usually involved in infection [15]. *Trichophyton rubrum* was the most prevalent fungal pathogen isolated from all cases of superficial fungal infections of the skin [16]. Three hundred and thirty six (11.98%) of these children were positive for the dermatophytic infection. Infection was mainly due to *Microsporum audouinii*, *Chrysosporium keratinophilum* and *Trichophyton mentagrophytes*. Infected domestic animals constituted the apparent source of infection for most pupils. Playgrounds of children and animal fields were also source of infection for children and animals [9]. The incidence of *Aspergillus niger* (19%), *Cladosporium* sp.(14%), *Aspergillus flavus* (13%), *Trichophyton* sp.(13%) and *Microsporum* sp. (5%) was high and *Fusarium* sp., *Curvularia* sp., *Penicillium* sp. *Trichothecium roseum*, *Epidermetaphyton* sp., *Drechslera* sp. and *Alternaria* sp. was low [13].

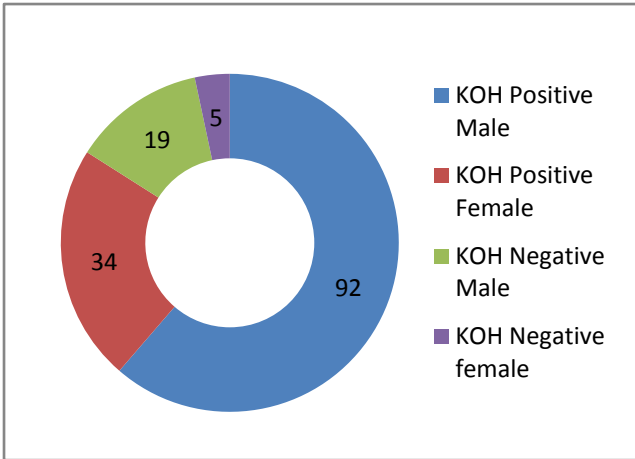


Fig. 1: Histogram showing sex wise incidences in total of 150 patients under study

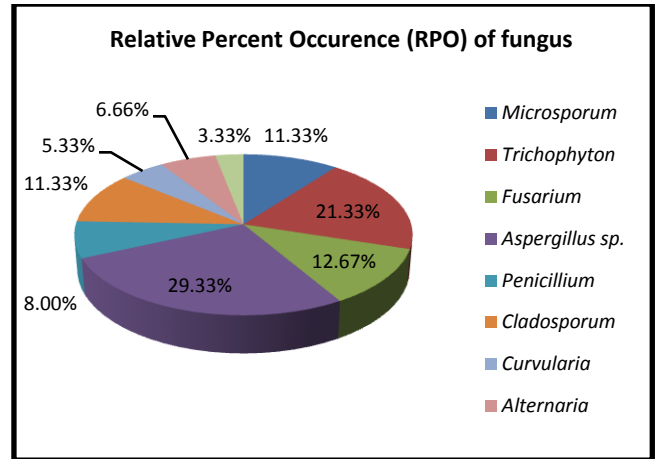


Fig. 4: Pie chart showing Relative Percent Occurrence (RPO) of different fungus from 118 patients under study showing positive test results

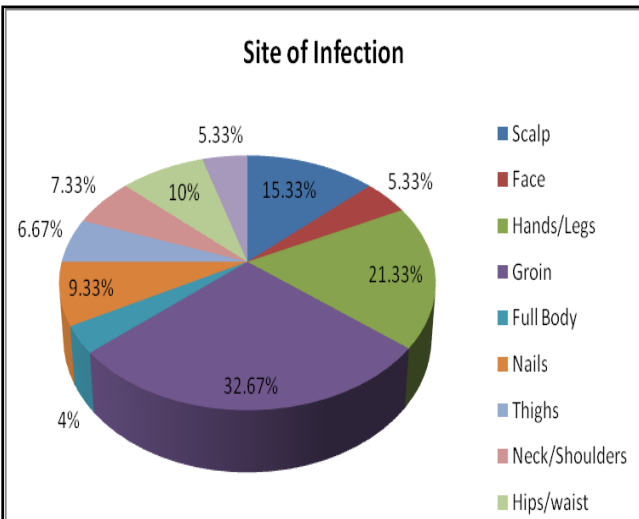


Fig. 2: Pie chart showing percent incidences of fungal infections at various sites in body of 150 patients under study

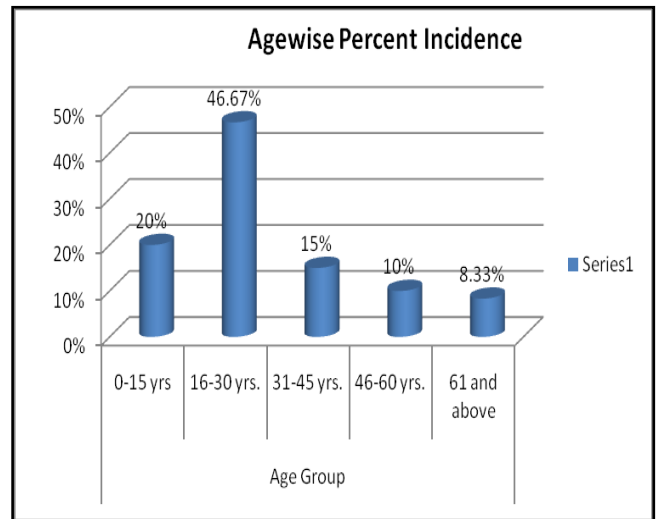


Fig. 5:- Histogram showing age wise percentage incidence in 150 patients under study

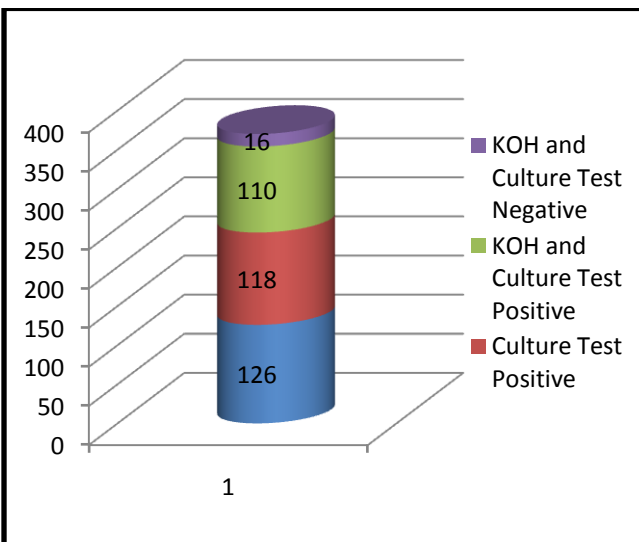


Fig. 3: Histogram showing sensitivity of KOH test versus culture test of 150 case study

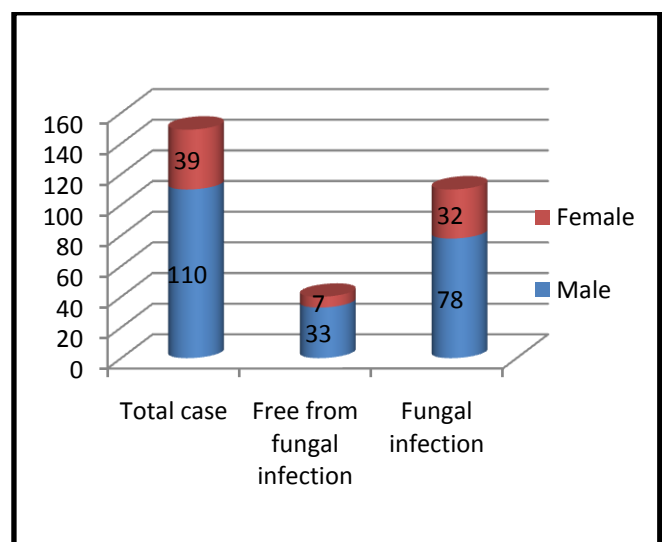


Fig. 6: Histogram showing incidence of fungal dermatophytic infection under case study

## CONCLUSION

The infection was pronounced in males as compared to females, based on 150 patient cases studied. Utmost infection was recovered from groin followed by hands, legs and thighs. Most frequently occurring fungus was *Aspergillus* sp. followed by *Trichophyton* sp., *Fusarium* sp., *Microsporium* sp. and *Cladosporium* sp. It can be inferred that KOH test was 73.33% and culture test was 100% sensitive for screening of infection. The data revealed the KOH test was false positive in 16 cases and false negative in 08 cases studied. Since the sensitivity of KOH test is significantly high with reference to culture test, it can be used as a definitive procedure for screening and diagnosis of dermatophytic infection. KOH test ensures cost effectiveness and require only microscopic facility with trained professionals. It can be recommended that if proper training is provided to the technical staff, precise diagnosis can be established with limited lab facilities even at remote places.

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