Original Article

Comparison of KOH with Culture in the Diagnosis of Dematophytic Fungal Infection in a Tertiary Care Hospital

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ABSTRACT

Skin mycoses also called dermatophytosis is the most common fungal infection affects 20-25% of World population. Its prevalence varies place to place with climatic changes, time to time with age, sex and lifestyle of the population. Dermatophytoses could be caused by dermatophytes and non-dermatophytes, their frequency of isolation differs with geographical variation.

Objectives: To study various clinical presentations of skin mycosis and compare KOH with culture in the diagnosis of dermatophytic infections in a tertiary care hospital.

Material & Methods: A total of 98 skin scrapings collected from consecutive OPD patients from 1st June 2016 to 31st May 2017 examined using KOH and culture on modified Sabouraud's dextrose agar medium. The isolated fungi were identified by morphology, lactophenol cotton blue, slide culture and biochemical tests.

Results & Discussions: T.corporis(40.82%) was the most common clinical presentation followed by T.cruris(17.35%), T.pedis(15.31%), T.capitis(8.16%). Fungal infections were demonstrated in 52/98(53.06%). The male to female ratio of the positive cases was 15:9. The most affected age group in males 30-40yrs and females 40-50yrs. KOH positive were 43.87%(43/98). The samples which were positive in both KOH and culture were 17.35%(17/98), those positive in KOH and culture negative were 26.53%(26/98) and KOH negative and culture positive were 7.14%(7/98). Out of 24 positive cultures, 21 were dermatophytes and three were non-dermatophytes. The most common dermatophytes were T.mentagrophytes(62.5%) followed by T.rubrum(20.8%) and M.gypseum(4.33%).

Conclusion: Skin mycoses is caused mainly by dermatophytes (T.mentagrophytes followed by T.rubrum) but non-dermatophytic infections do occur. Therefore, fungal culture is imperative for correct diagnosis and proper treatment.

Keywords: Dermatophytes, Skin Mycoses, Non-Dermatophytes, Lactophenol Cotton Blue Preparation, Tinea Spp.

Introduction

Skin mycoses the most common fungal infection affects 20-25% of World population (WHO 2005).[1] Its prevalence varies from place to place with climatic changes and time to time with age, sex and lifestyle of the population. It is more prevalent in tropical and subtropical countries like India where the heat and humidity are high for most part of the year. Dermatophytes thrive at surface temperatures of 25–28°C and infection of human skin is supported by warm and humid conditions. For these reasons superficial fungal infections are relatively common in tropical countries and are exacerbated by the wearing of occlusive clothing. In addition, the frequency of dermatomycoses is greater in communities with low socioeconomic status: crowded living conditions provide multiple opportunities for skin-to-skin contact and close proximity to animals, where hygiene may be suboptimal. Moreover, superficial skin infections show a low tendency to self-limitation and absence of/poor medical care further increases the spread of skin mycoses.[2]

Despite regional characteristics and predispositions for dermatophytic infections, the spectrum of dermatophytes is not static. Booming mass tourism, international sports activities and increasing migration mean that less common or forgotten species are being imported and disseminated.[2] Dermatophytoses could be caused by dermatophytes (Genera *Trichophyton*, *Microsporum* and *Epidermophyton*) and non-dermatophytic fungi (*Candida*, *Fusarium*, *Trichosporon* and *Scytalidium*) and their frequency of isolation also differs with the geographical variation. Over the decades, non-dermatophytes, as agents of superficial fungal infection in humans, produce lesions that are clinically similar to those caused by dermatophytic infections. [3,4] These fungi grow best on those areas of the skin that are warm, dark and moist. These organisms are assuming greater significance due to the excessive use of immunosuppressive drugs for controlling serious as well as non-infectious conditions. They produce keratinases which degrade the keratin and thus, invade the superficial skin tissue. They are usually restricted to non-living 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.[5]

Since, these infections are often confused with other skin disorders, it is therefore, necessary to make early laboratory diagnosis for better management of these conditions. The present study was conducted to study the prevalence of different dermatophytic and non-dermatophytic fungi causing skin mycoses in a tertiary care hospital and also done for comparison of KOH microscopy as compared to culture in early diagnosis of fungal infections.

Material and Methods

Study design: Descriptive study. **Study Area:** Department of Microbiology, Tertiary care hospital. **Study Population:** Patients presenting from skin department of a Tertiary care hospital. **Study duration:** One year study. **Sample Size:** On an average 10 skin scrapings/month from skin department are received in the department of Microbiology. As the study period was one year all the skin scrapings had been included in the study. **Sampling technique:** Non-probability consecutive sampling technique.

A total of 98 skin scrapings collected from same number of consecutive patients who visited OPD of dermatology and VD department of tertiary care hospital from 1st June 2016 to 31st May 2017 were examined by making KOH preparation and culture on modified Sabouraud's dextrose agar medium.

The assessment of the participants consisted of an interview, clinical examination and collection of specimens for microbiological studies. All patients completed a questionnaire that contained demographic data, patient and family history and specific data related to risk factor for dermatophytosis and non-dermatophytoses such as age, gender, physical activities, occupation, predisposing diseases and contact with animals and clinical diagnosis.

Sample collection and transportation: the suspected site was cleaned with 70% ethyl alcohol and material was transported in black paper and transferred to the laboratory as soon as possible for direct microscopic examination and culture.

Microscopic examination of specimens: was performed with an aqueous solution of 20% potassium hydroxide (KOH) mixed with 5% glycerol.

Culture: All specimens were cultured on modified Sabouraud's dextrose agar medium (SDA along with chloramphenicol and cycloheximide), both with and without antibiotics and incubated at 25°C, 37°C for 21days.

Examination and identification of fungus isolates: The isolated fungi were identified by morphology, lactophenol cotton blue preparation, slide culture and biochemical tests (hair perforation test, urease test and growth characteristics on bromocresol purple agar).

The study was approved by authorisation committee of our hospital and was carried out in accordance with previously reported recommendations on the design and conduct of diagnostic accuracy assessments.

Statistical analysis

The data pertaining to sociodemographic and other clinical variables was entered in the form of data matrix in Microsoft ® Excel ® and analysed using IBM® SPSS® v 20.0.0. The descriptive statistics for categorical variables was represented in the form of frequencies and percentages and as means and standard deviations for continuous variables.

Results

Out of the total 98 clinically suspected specimens, the most common infections were *Tinea corporis* 40 (40.8%), followed by *Tinea cruris* 17 (17.35%), *Tinea pedis* 15 (15.3%), *Tinea capitis* 8 (8.2%), *Tinea cruris* et *corporis* 4 (4.1%), Recurrent Tinea, *Tinea facei* and *Tinea mannum* 3 (3.1%) each, *Tinea cruris* et *pedis* and *Tinea incognita* 2 (2%) each and *Tinea mannum* et *pedis* 1 (1%) (Figure 1). Figure 7 shows erythematous patches with active borders near shoulder and groins. Out of 98 clinically suspected cases of skin mycoses, 58 were males and 40 were females (Figure 2). Fungal infections were demonstrated in 52/98(53.06%) (Figure 3,4). The male to female ratio of the positive cases were recorded as 15:9. KOH positive samples were 43.87% (43/98). The samples which were positive in both KOH and culture were 17.35% (17/98), those positive in KOH preparation and culture negative were 26.53% (26/98) and KOH negative and culture positive were 7.14%(7/98) (Figure 5). The most affected age group in males were 30-40yrs and in females 40-50yrs (Table 1). Out of 24 positive cultures, 21 were dermatophytes and three were non-dermatophytes. The most common dermatophytes were *T. mentagrophytes* (62.5%) followed by *T. rubrum* (20.8%) and *M. gypseum* (4.33%) (Figure 6). Figure 8- *Trichophyton mentagrophytes* showing spiral hyphae and clusters of microconidia. (LCB * 400). *T. corporis* was found in 40 clinically suspected cases and out of which 24 were KOH positive and on culture 6 isolates were *T. mentagrophytes, 4* were *T. rubrum* and one isolate was *Fusarium* sp. *T. cruris* were found in 17 clinically suspected cases, 7 were KOH positive for fungal infections and 4 *T. mentagrophytes* and one isolate was *T. rubrum* (Table 2). Culture is gold standard test done for fungus

but other biochemical tests are helpful in identification of fungus. Sensitivity, specificity, PPV and NPV of KOH in comparison to Culture were 70.83%, 62.16%, 37.78% and 86.8% (p=0.0017 test is significant) (Table 3).

Table 1: Age wise distribution of clinically suspected cases in relation to culture (n=98).

AGE IN YEARS	MALE- TOTAL (CULTURE POSITIVE)	FEMALE- TOTAL (CULTURE POSITIVE)
0-10	3 (0)	2(1)
11-20	6 (2)	2(1)
21-30	21 (3)	7(1)
31-40	11 (4)	7(2)
41-50	8 (3)	13(3)
51-60	4 (2)	7(1)
61-70	4 (1)	2(0)
71-80	0 (0)	0(0)
81-90	1 (0)	0(0)
TOTAL	58 (15)	40(9)

Table 2: Comparison of types of skin mycosis and Culture results (n=98).

Types of skin mycosis	Total clinically suspected cases	KOH positive	Fungus isolates
T. corporis	40	24	6m*, 4r**,1f***
T. cruris	17	7	4m*, 1r**
T. pedis	15	7	3m*, 1 a*****
T. capitis	8	0	0
T. cruris et corporis	4	2	1m*
Recurrent tinea	3	1	1m*
T. mannum	3	1	1g****
T. facei	3	0	0
T. cruris et pedis	2	1	1 a*****
T. incognita	2	0	0
T. mannum et pedis	1	0	0

NOTE----m*-T. mentagrophytes, r**-T. rubrum, f***-Fusarium sp., g****-M. gypseum, a*****-Aspergillus sp.

Table 3: The results of various biochemical tests performed for identification of dermtophytes.

	Hair perforation test	Urease test	Bromocresol purple agar
T. mentagrophytes	Positive	Positive	Alkaline
T. rubrum	Negative	Negative	Alkaline
M. gypseum	Positive	Positive	No change

Table 4: Showing sensitivities and specificities of KOH in comparison to Culture.

CULTURE POSITIVE NEGATIVE TOTAL				
KOH	POSITIVE	17	26	43
	NEGATIVE	07	48	55
	TOTAL	24	74	98

Statistically significance (McNemar's Test) p value= 0.0017 (Statistically significant) **Sensitivity** *70.83% [95% CI 48.91-87.38%],* **Specificity** *64.86% [95% CI 52.89-75.61%],* **PPV** *39.53% [95% CI 30.43-49.43%]* **NPV** *87.27% [95% CI 78.24-92.90%].*
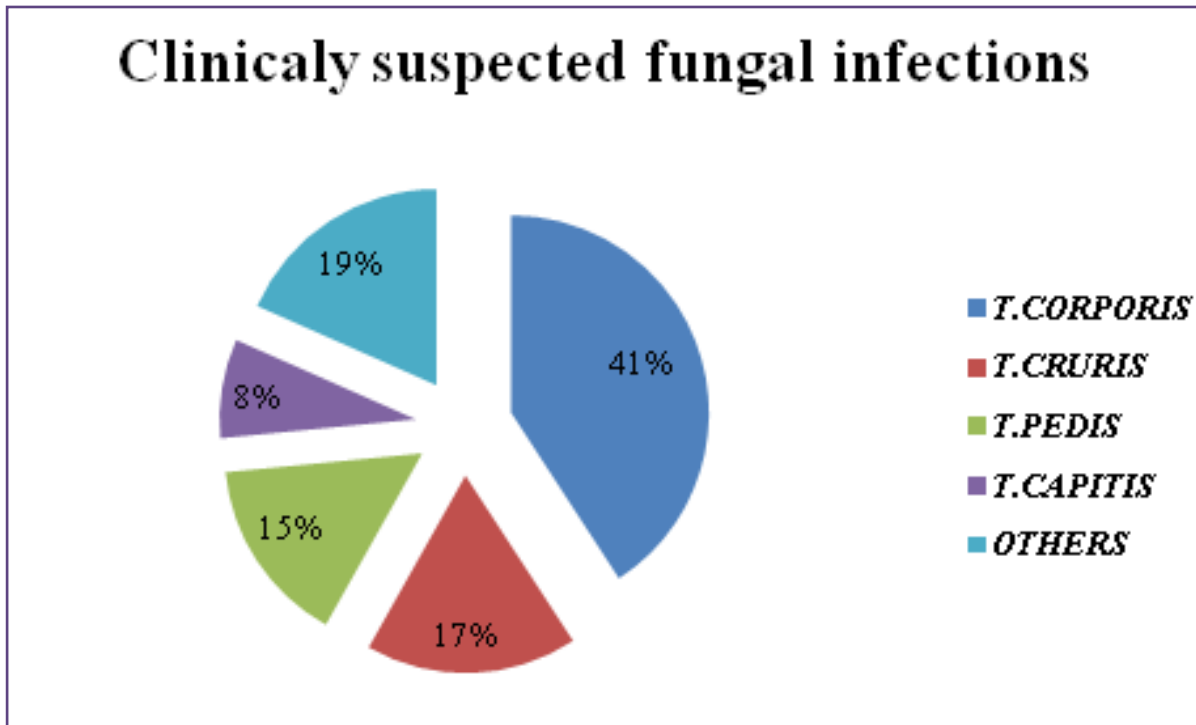
Fig. 1: Pie chart showing distribution of clinically suspected fungal infections (n=98).
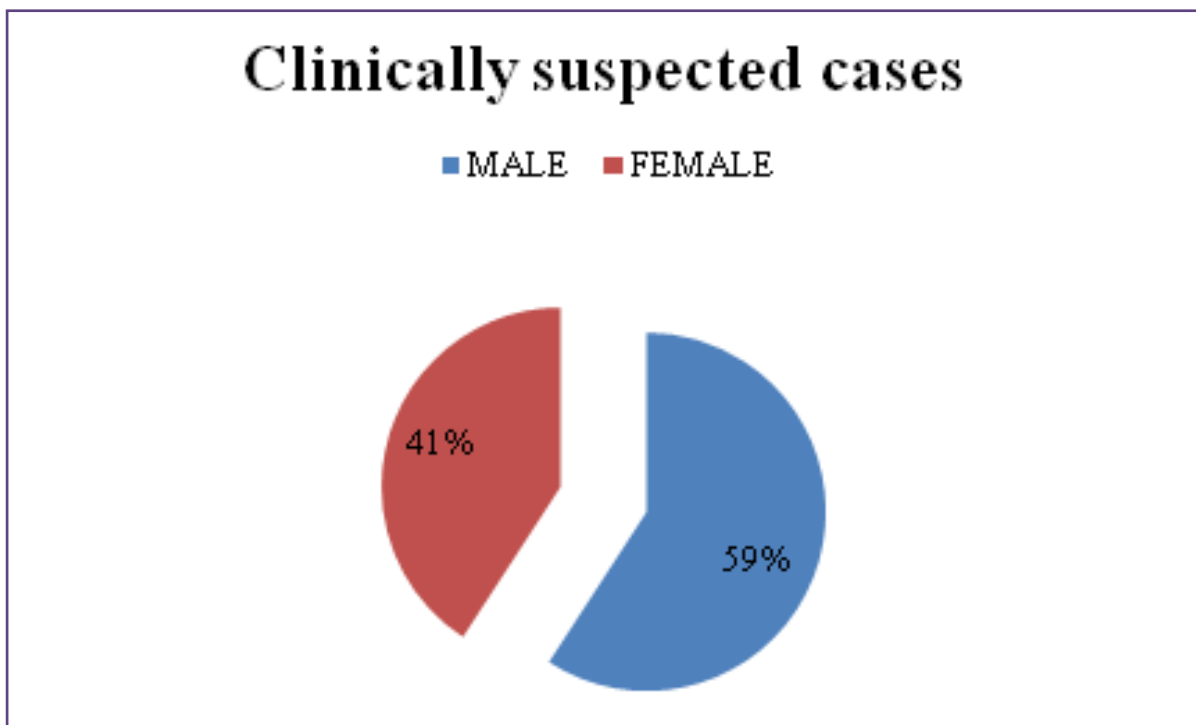

Fig. 2: Pie chart showing sex wise distribution of clinically suspected cases.
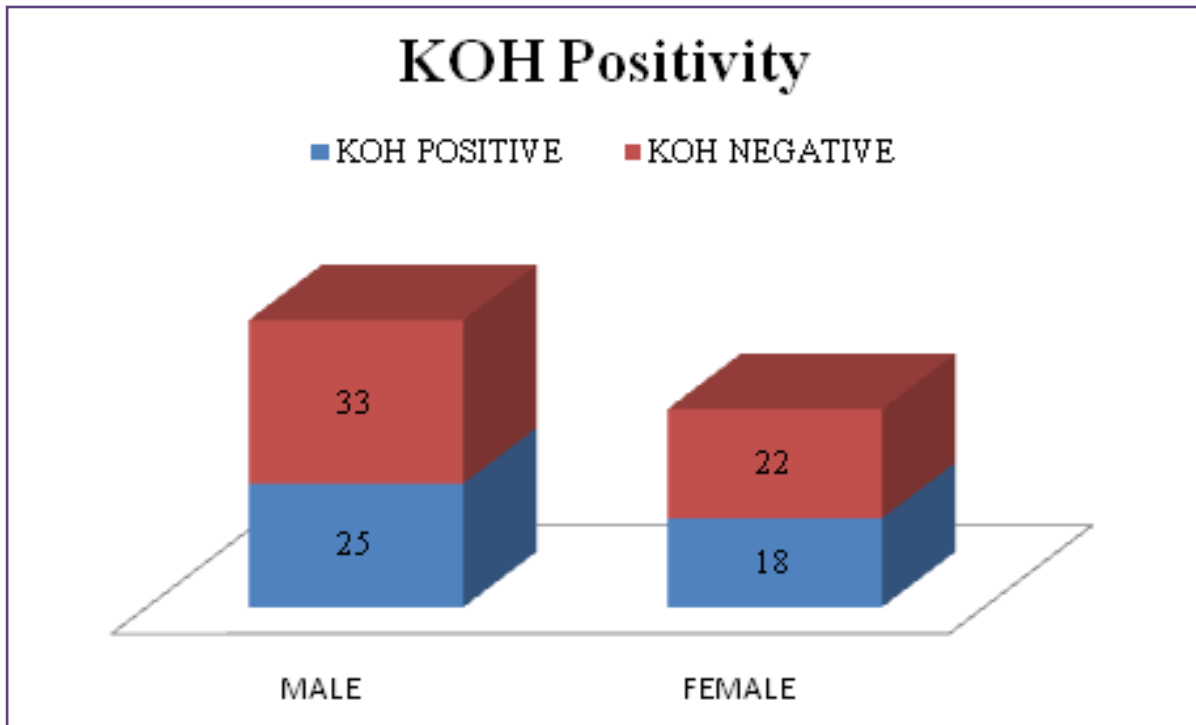
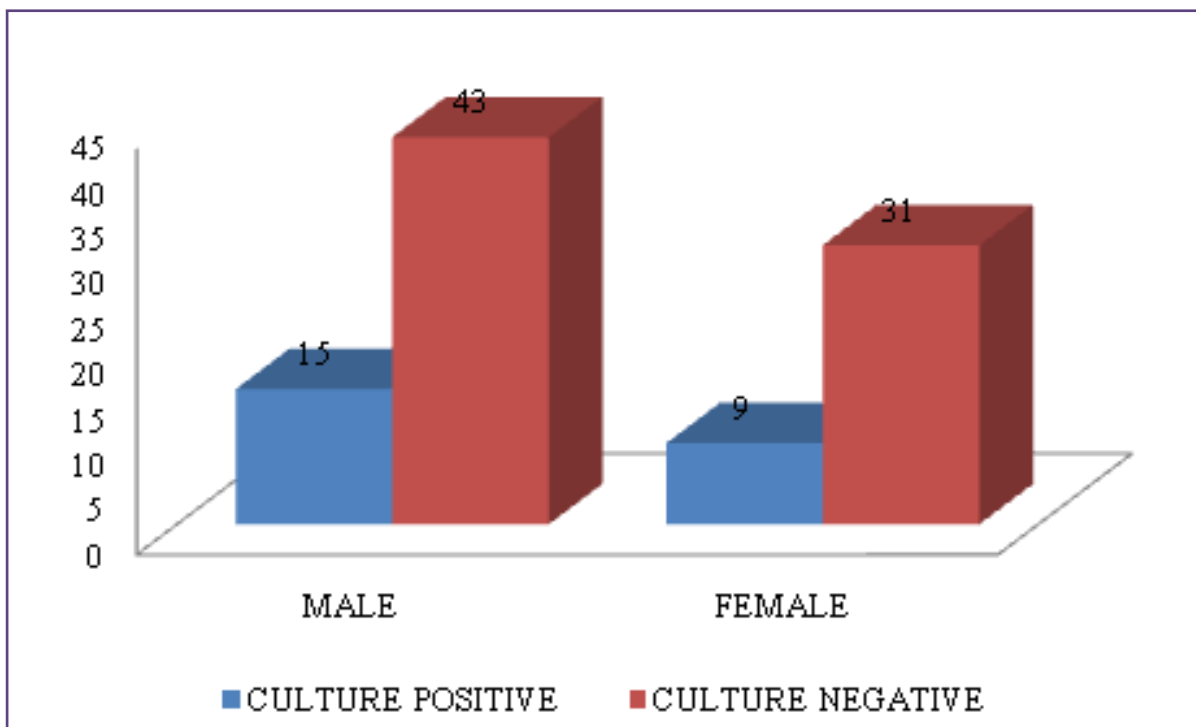**Fig. 3: Histogram showing KOH test results (n=98)..**

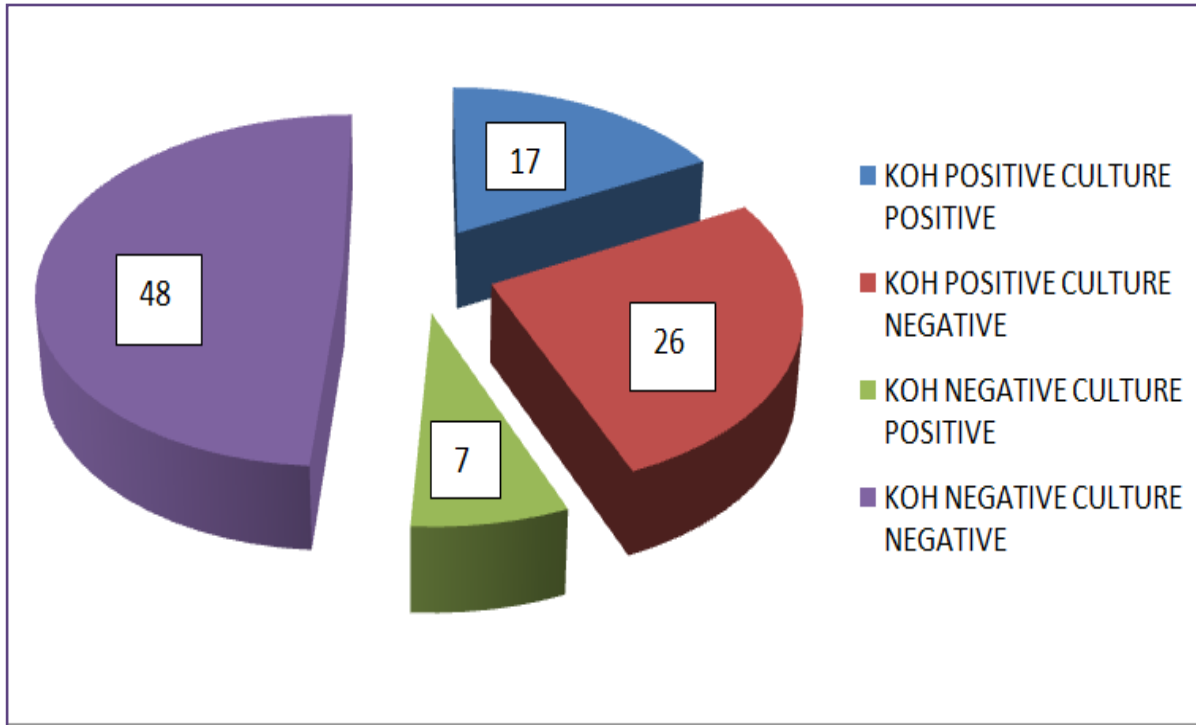**Fig. 4: Histogram showing gender wise incidences in total of 98 patients under study.**

Fig. 5: Pie chart showing sensitivity of KOH test verses culture test of 98 case study.
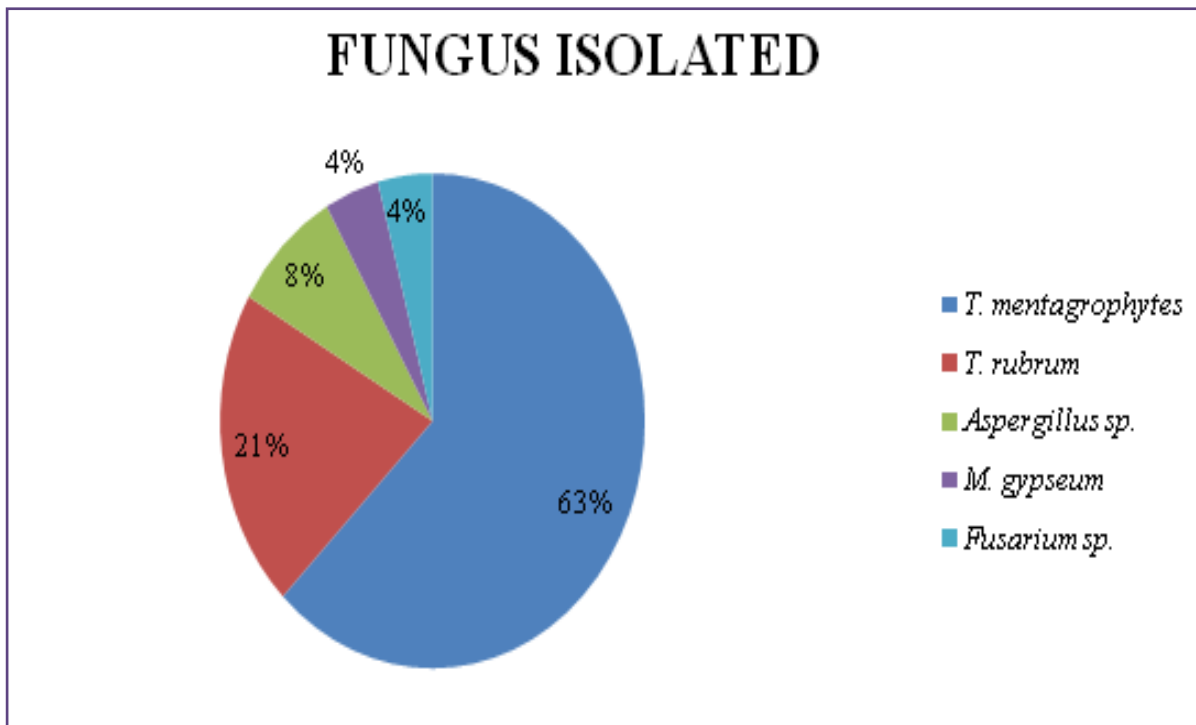

Fig. 6: Various fungus isolated on culture (n=24).

Fig. 7: shows erythematous patches with active borders near shoulder and groins.
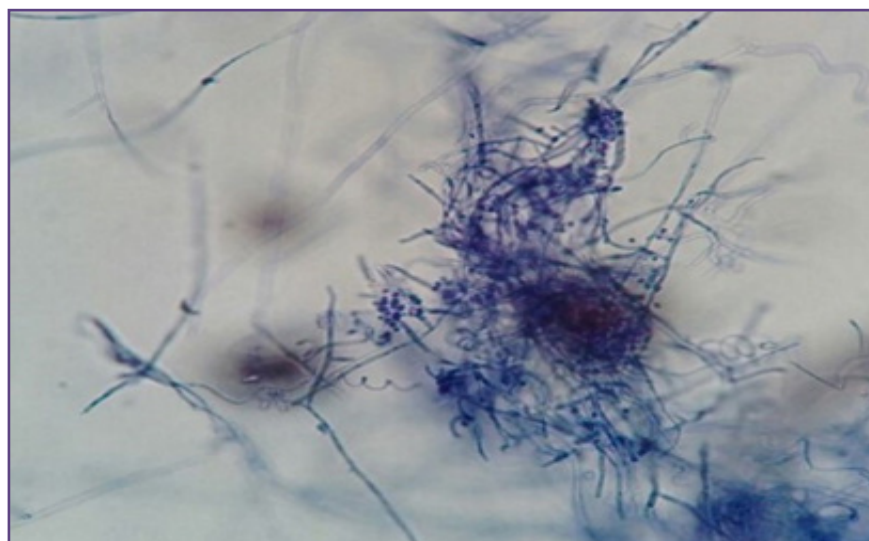

Fig. 8: Trichophyton mentagrophytes showing spiral hyphae and clusters of microconidia. (LCB * 400).

Discussion

Although any outbreak or pandemic has not been reported due to dermatophytes but incidence of severe systemic fungal infections had increased significantly. The cause of this could be excessive or irrational use of antibiotics, steroids and many other clinical disorders that makes the individual immunocompromised. The present study was done to determine the prevalence of skin mycosis caused by dermatophytic and other non-dermatophytic fungi.

The prevalence of dermatophytes among fungal skin infections was found to be 87.5%. Nasimuddin et al has shown slightly higher prevalence and Bitew et al has shown lower prevalence (69.8%).[6,7] In our study, *T. corporis* (40.8%) was the most common clinical presentation followed by *T. cruris* (17.35%), *T. pedis* (15.31%), *T. capitis* (8.16%) (Figure1). This is in concordance with the various studies by Bhatia VC et al, Sowmya N et al and Gupta S et al. [1,2,3] While the study conducted by Elmegeed et al showed that the *T. capitis* (28.6%) was the most common, followed by *T. pedis* (21.21%) and *T. corporis* (14.8%).[4] A study conducted on 67 Iraq patients (2020) with various dermatophytosis infections, *T. pedis* (45.1%) was the most common followed by *T. mannum* (22.2%), T. *capitis* (11.8%), *T. corporis* (7.8%), *T. ungium* (5.9%).[8]

Among the suspected patients there were more infections in males as compared to females (Figure 2). This correlates with other studies. [9,10,11] This may be due to the differences in occupational exposure as males are more involved in construction and other works. Among the patient data collected, out of 58 males, 25 were KOH positive and 18 out of 40 females were KOH positive (Figure 3). Similar observations were recorded at the National Skin Centre, a tertiary referral centre for dermatological diseases in Singapore where majority of patients (n=9335) (72.3%) were males. This was also seen in anthropophilic species, which is the main cause of dermatophytosis in adults.[5]

Among the patient data collected, the total culture positivity was 24.5% (24/98) (Figure 4). Similar findings were observed by Gupta et al while Sowmya et al reported much higher (49%) culture positivity. [2,3]

Out of the 98 specimens, KOH positive samples were 43 (43.87%) while culture positive were 24 (24.49%). The samples which were positive in both KOH and culture were 17.35% (17/98), those positive in KOH preparation and culture negative were 26.5% (26/98) (false positive) and KOH negative and culture positive were 7.14% (7/98) (false negative) (Figure 5). Similar results were observed in the study conducted by Gupta et al. [3]

The most common age group among males was 21-30 years and 41-50 years among females in relation to culture (Table 1). The probable reason for higher prevalence in this group could be that the individuals in this group are often most active because of their involvement in the outdoor activities such as studies, jobs etc. While the study conducted by Elmegeed et al showed that the most common age group was 0-10years followed by 11-20 years.[4] This might be because of more population of low economic status which maintain poor personal hygiene.

Out of 24 positive cultures, 21 (87.5%) were dermatophytes and 3 (12.5%) were non-dermatophytes, while that of Appalaraju B et al dermatophytes 43% isolated and non-dermatophytes were 6%.[2] The most common dermatophytes were *T. mentagrophytes* (62.5%) followed by *T. rubrum* (20.8%) and *M. gypseum* (4.33%) which is in concordance with the results from other studies (Figure 6). [1,12] In Europe there was higher incidence of *T. rubrum* infections was recorded while in Asia *T. mentagrophytes* was the commonest isolate.[8]

The most common isolate from the clinical samples in our study was *T. mentagrophytes*, followed by *T. rubrum* and *Fusarium* sp (Table 2). The reason for more prevalence of *T. mentagrophytes* in our study could be due to more labour

class population with increased migration and climatic changes.

The biochemical tests were done to differentiate among dermatophytes. *T. mentagrophytes* and *M. gypseum* were both hair perforation and urease test positive but both differ on bromo cresol agar. *T. rubrum* both hair perforation and urease test negative (Table 3).

Culture is gold standard test done for fungus but other biochemical tests are helpful in identification of fungus. Sensitivity, specificity, PPV and NPV of KOH in comparison to Culture were 70.83%, 62.16%, 37.78% and 86.8% (*P*=0.0017 test is significant). While study conducted in Jaipur showed that the sensitivity of KOH was slightly higher (84%) as compared to reference standard culture test than our study.[3]

The clinical presentation though typical of ringworm infection is very often confused with other skin disorders particularly due to topical application of steroid ointments and creams, leading to further misdiagnosis and mismanagement.[13] Hence there arises a need for correct, efficient and rapid laboratory diagnosis of dermatophytes. With the proper techniques various species can be identified. And it's the right time to look for the molecular approaches for rapid dermatophytes identification.

Conclusion

Skin mycoses is caused mainly by dermatophytes (*T. mentagrophytes* followed by *T. rubrum*) but non-dermatophytic infections do occur. Therefore, fungal culture is imperative for correct diagnosis and proper treatment. The data revealed the KOH test was false positive in 26 cases and false negative in 07 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.

Abbreviations and Symbols

SDA- Sabouraud's dextrose agar medium, *Tinea corporis- T. corporis, Tinea cruris- T. cruris, Tinea pedis- T. pedis, Tinea capitis- T. capitis, Tinea cruris et corporis- T. cruris et corporis, Tinea facei- T. facei and Tinea mannum- T. mannum, Tinea cruris et pedis- T. cruris et pedis, Tinea incognita-T. incognita, Tinea mannum et pedis- T. mannum et pedis, Trichophyton mentagrophytes- T. mentagrophytes, Trichophyton rubrum- T. rubrum and Microsporum gypseum- M. gypseum.*

Acknowledgement

Funding

Competing Interest

None

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