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ORIGINAL RESEARCH

Evaluation of KOH microscopy and culture for diagnosis of dermatophytes in tertiary care centre

[1]Dr. Ananya Verma, [2]Dr. Suneel Kumar Ahirwar, [3]Dr. Sanjay Khare, [4]Dr. Anju Mahor, [5]Dr. Sadhna Sodani

[1]Post Graduate Student, [2,5]Associate Professor, [4]Assistant Professor, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, India
[3]Professor & Head, Department of Dermatology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, India

Corresponding author
Dr. Anju Mahor
Assistant Professor, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, India
Email: drsk2311@gmail.com

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ABSTRACT

Background and objective: Dermatophytes, fungi causing superficial skin diseases, cause millions of global infections, with a 10--20% lifetime risk of contracting fungus.The study aimed to identify and isolate dermatophytes from suspected cases of dermatophytosis, evaluate KOH microscopy and culture results, and diagnose dermatophyte infection.**Material and method:**A cross-sectional study involved 404 patients suspected of having dermatophytosis at Maharaja Yashwantrao Hospital in Indore, Madhya Pradesh. Samples were collected from December 2022 to January 2023, and the study was approved by scientific and ethical committees.Samples of skin, hair, and nails from 404 clinically suspected cases of dermatophytosis in both sexes and all age groups were collected, processed with KOH, and cultured on Sabouraud dextrose agar and Dermatophyte test media. **Result:** The study analyzeddermatophytosis in patients, with a slightly female preponderance. The most common clinical presentation was Tinea corporis (53.9%), followed by Tinea cruris (24.5%). Direct microscopy results showed 232 (57% of cases) were KOH positive, with a significant association between KOH mount and culture findings. The sensitivity of KOH positivity was 84.7%, and the specificity was 83.6%. The positive predictive value and negative predictive value were 87.14% and 80.70%, respectively.**Conclusion:** In conclusion, combining KOH microscopy with fungal culture in diagnostic strategies enhances the precision and speed of detecting dermatophytes in medical settings. Future studies could explore new diagnostic approaches or techniques for continuous improvement.

Key words: Dermatophytosis, *Trichophyton*,KOH, SDA

INTRODUCTION

The homogenous group of fungi known as dermatophytes is responsible for superficial skin diseases in both humans and animals. They derive their nutrients from keratin found in the stratum corneum, hair, and nails. Millions of people worldwide suffer from dermatophytosis, a serious form of superficial infection, with a 10- to 20-percent lifetime risk of contracting the fungus [1]There are over 40 species and three significant genera that are known to cause dermatophytosis in humans: *Trichophyton*, *Microsporum*, and *Epidermophyton*. Dermatophyte infections can spread either directly through contact with infected people or animals or through contaminated soil [2, 3, 4].In tropical and subtropical nations like India, superficial mycosis is more common. The clinical lesions produced by the fungi are incredibly diverse and resemble various skin conditions. Consequently, a confirmed laboratory diagnosis of a superficial skin infection must be established [5].Phenotypic diagnosis of dermatophyte infections requires both light microscopic mycological examination and fungal culture [6]. Although microscopic examination is a quick, simple, and affordable diagnostic method, up to 15% of cases may result in falsely negative results. Although the growth of the fungus in a cultured species takes about four weeks, culture methods are specific diagnostic tests for dermatophyte identification [7].The objective of the current study was to isolate and identify dermatophytes from various clinically suspected cases of dermatophytosis and evaluate the results of KOH

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microscopy and culture in order to diagnose dermatophyte infection.

MATERIAL AND METHODS

This cross-sectional study was carried out on dermatophytosis cases that were clinically suspected. The study involved 404 patients who visited the outpatient department of the department of dermatology, venereology, and leprosy at Maharaja Yashwantrao (M.Y.) Hospital, which is affiliated with Mahatma Gandhi Memorial Medical College (MGMMC), in Indore, Madhya Pradesh, and had a suspected dermatophyte infection. The Department of Microbiology received samples between December 2022 and January 2023. The scientific and ethical committees gave their clearance before the study could begin.The study was conducted after approval from the scientific and ethical committees.

Inclusion Criteria

All age groups of either sex have clinical features of dermatophytosis.

Exclusion Criteria

Nil

Sample Collection: As per standard protocols [8].The affected area was thoroughly cleaned with 70% ethanol prior to sample collection. The samples were sufficiently collected and captured from the lesion's active zone, which is the boundary of the affected area. The following materials were used to isolate the dermatophytes from the hair, nails, and skin.**Skin:**Glabrous skin lesions that exhibit an active inflammatory border were scraped from the periphery; if not, they were scraped completely. The most effective method for collecting was to use a sterile scalpel blade to collect epidermal scales from the area close to the advancing edges of the rings. **Nails:** Samples were taken from the nail bed and lower nail plate, especially around the lesion's edge, using a small curette or scalpel blade after the nails were clipped in the case of distal subungual onychomycosis. The healthy top plate of the nail was pared when proximal subungual onychomycosis was suspected, and material from the infected lower plate of the nail was then collected. By scratching the white areas, infected material for white superficial onychomycosis was gathered. **Hairs:** Hair roots and crusts were plucked from the infected area or its edge for large lesions, so that the root was included and suppurating lesions were swabbed. If hair fragility prevented this, as in "black dot" tinea capitis, a scalpel was used to scrape scales and excavate small parts of the hair root [9].

Microbiological methods

Direct microscopy of the specimen The collected specimens were divided into two portions. The first portion of the specimens was examined microscopically using a 10% potassium hydroxide (KOH) solution placed on a glass microscope slide, followed by the addition of a small specimen piece and a cover slip. Skin and hair samples were left in a petri dish for 20–30 minutes, while nail samples were soaked in a 20% KOH solution overnight. Dermatophytes were identified under the microscope by observing branching and septate hyphae with angular or spherical arthroconidia (arthrospores), usually in chains. All skin and nail specimens of ringworm fungi displayed similar characteristics.

Culture of the specimen The fungus was isolated using Sabouraud dextrose agar containing chloramphenicol (0.05%) without cycloheximide or Dermatophyte Test Media (DTM). The second portion of samples of skin, hair, and nails were directly inoculated onto the medium and then examined every alternate day for evidence of growth, with SDA and DTM plates being observed for 4 weeks and 2 weeks, respectively. Further evaluation was performed based on colony morphology, microscopic characteristics, and relevant biochemical tests for the detection of dermatophyte species.

Identification of culture growth

Tease mount preparation Place a small drop of lactophenol cotton blue (mounting medium) on a clean, grease-free microscopic slide. Remove aseptically a small portion of growth midway between the colony centre and its edge. Place that colony on a drop of lactophenol cotton blue. Tease the fungus using a pair of dissecting needles to have a thin spread out. Gently place a cover slip at the edge of the drop of mounting fluid. Excess lactophenol can be wiped out using a tissue or blotting paper. To preserve the mount, seal the edges of the coverslip with nail polish or varnish.

Slide culture Cut approximately 1 cm square from CMA or PDA agar blocks using aseptic techniques, then transfer the agar block onto a slide. Apply a small amount of colony to the four sides of the agar block. Using sterile forceps, place a coverslip on the inoculated agar block. Add 1 – 1.5 mL of sterile water to the petri dish to create a humid atmosphere that prevents the agar block from drying out. Optionally, add 5-20% glycerine to the sterile water to prevent moisture condensation on the slide. Place the slide culture in a petri dish and incubate it in the dark. The slide culture is ready to be observed when mature conidia or spores are visible. Apply a small drop of mounting medium (LPCB) to a microscopic slide. Using forceps, carefully remove the coverslip from the agar block without pushing or pulling. Pass the coverslip quickly through a flame's blue portion to heat-fix the fungus and its spores (overheating can cause hyphae collapse). Gently place the coverslip on the mounting medium to avoid trapping air bubbles, wipe off excess mounting medium, and seal the coverslip edges with nail polish. A second mount can be prepared by removing the agar block from the

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microscope slide of the slide culture setup, placing a drop of lactophenol and a coverslip, and sealing the coverslip with nail polish. To avoid air bubbles, soak the colony in 95% alcohol or ethyl acetate before using lactophenol blue.

Urease test To test the urease activity of the isolated dermatophyte species, we inoculated the slant surface of Christensen's urea agar with a pure culture of the test fungi in a test tube and then incubated it at room temperature (27°C) for 7 days. A change in color of the media from straw to pink indicated a positive result, while no color change was taken as a negative result.

Statistical analysis

The data were analysed using SSPS version 22. Frequencies and percentages were used to describe the categorical variables in this study. The results were presented as proportion ratios with a 95% confidence interval. Statistical significance was set if p-value <0.05.

RESULT

Table 1 shows the distribution of patients according to sex. There were 204 females (50.5%) and 200 (49.5%) males, showing a slightly female preponderance in the study patients. The overall male-to-female ratio was 0.98:1. Table 2 shows the most common clinical presentation of dermatophytosis was Tinea corporis, i.e., 218 cases (53.9%), followed by Tinea cruris, i.e., 99 (24.5%). Table 3 shows direct microscopy results, where 232 (57% of cases) are KOH positive and 172 (42.5%) are KOH negative. Graph 1 shows the maximum number of KOH-positive cases found in T. corporis (62.9%), followed by T. cruris (26.2%). Table 4 shows A chi-square test was fitted to this 2 x 2 contingency table, and chi square ($\chi 2$) = 144.18, with 1 degree of freedom. This proves that the test is highly statistically significant at a 95% confidence interval with a p-value <0.0001. Out of 232 KOH-positive samples, 183 (85%) were culture-positive. 172 samples were negative by KOH mount, among which 32 (15%) were culture-positive. Total culture positivity was found in 215 cases (53%). A significant association between the KOH mount and culture findings was observed. The sensitivity of KOH positivity with respect to culture was 84.7%, and the specificity was 83.6%. The positive predictive value and negative predictive value were 87.14% and 80.70%, respectively.

Table 1: Distribution of patients according to sex

	Male	Female	Total
Cases	200	204	404
Percentage	49.5	50.5	100

Table 2: **Clinical type of Dermatophytosis in the present study (n = 404)**

S.N.	Clinical type	No. of cases	Percentage
1	Tinea cruris	99	24.50
2	Tinea corporis	218	53.96
3	Tineafaciei	13	3.22
4	Tinea pedis	8	1.98
6	Tineaunguium	24	5.94
7	Tinea capitis	17	4.21
8	Tinea barbae	5	1.24
9	Combination pattern	20	4.95
	Total	404	100

Table 3: Result of KOH mount

	KOH (+)	KOH (-)
No. of cases	232	172
Percentage	57.4	42.5

Table 4: **Correlation between KOH mount and culture**

KOH mount	Culture positive for dermatophytes (%)	Culture-negative for dermatophytes (%)
KOH positive (n = 232)	183 (85%)	49(26%)
KOH negative (n = 172)	32(15%)	140 (74%)
Total (404)	215	189

Chi-square=144.18 Degree of freedom = 1 p-value<0.0001

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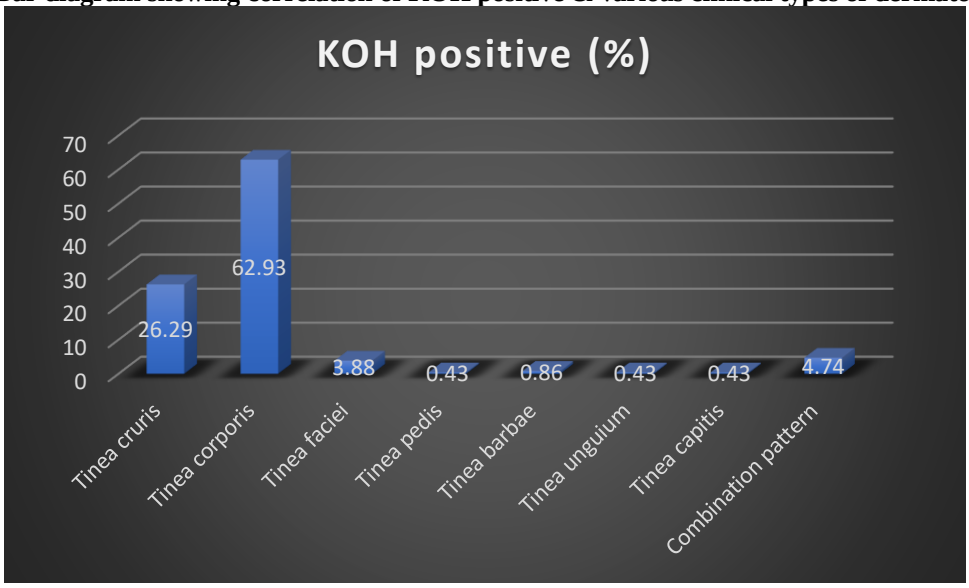
Graph 1: Bar diagram showing correlation of KOH positive & various clinical types of dermatophytosis



DISCUSSION

A divergence in culture isolation ranging from 33% to 77% has been found in the Indian subcontinent [10]. In our study, direct microscopy with KOH was positive in 57.4% of cases, while a range of 27.7% to 77% was reported by others [11, 12]. In studies by Vijay Nanoty et al. [10] (2023 and Kumar et al. [13] (2019), KOH positivity was seen in 76.67% and 71.1%, respectively. However, a study done by Farooq et al. [12] in Moradabad in 2022 showed a KOH positivity of 27.7%, which is very low compared to other studies. Some studies, like Poluri et al. [14] in Telangana, 2015, and Das et al. [15] in Kolkata, 2020, showed KOH positivity of 58% and 52.4%, respectively, which shows similarity with our study. The wide variation in KOH positivity can be due to the selection criteria of cases and the skill involved in the sampling technique. Hence, all KOH-negative samples should be cultured. In the present study, 51% of cases were culture-positive, which is similar (47.5%) to the study conducted by Singh S et al. [16]. However, a study by Das et al. [15] done in Kolkata showed a culture positivity of 81.55%, which was much higher, and a study done in Bihar showed a low rate of culture positivity of 20.6% [17]. There is a difference between the KOH positivity rate and culture positivity rate, i.e., fungal elements were seen under direct microscopy but samples failed to grow on culture, which might be due to various factors like the usage of topical corticosteroids and the unsatisfactory collection of samples containing dead fungal hyphae [18, 19]. The authors also came across instances where no fungal elements were seen under direct microscopy but showed growth on culture. This might be due to the presence of scanty fungal elements that were missed during direct microscopic examination or the presence of fungal elements in inactive sporulating form that could not be visualised under direct microscopy [18, 20]. KOH microscopy is a quick and straightforward screening method for dermatophyte infections, examining skin scrapings with potassium hydroxide. It provides results within minutes, but sensitivity may vary. Fungal culture is considered the gold standard for diagnosing dermatophyte infections, allowing identification of the exact species for targeted treatment. Additionally, even though KOH microscopy is quick, it may give false negative results if the fungal elements are few or covered in debris. This emphasises how crucial it is to prepare and collect samples correctly. Fungal culture is time-consuming and requires specialised handling and interpretation skills, despite being more specific and sensitive. Delays in culture findings may also cause a delay in the start of definitive treatment.

CONCLUSION

KOH microscopy is a quick, affordable, and effective method for detecting dermatophyte infections, while fungal culture helps identify specific dermatophyte types for precise treatment choices. Furthermore, although KOH microscopy provides quick results, its ability to detect can differ, requiring skilled individuals to interpret the findings accurately. Conversely, fungal culture, despite taking longer to produce results, is still essential for identifying rare species and determining how well antifungal drugs work. In conclusion, combining KOH microscopy with fungal culture in diagnostic strategies enhances the precision and speed of detecting dermatophytes in medical settings. Future studies could investigate new diagnostic approaches or improvements to current techniques to continuously enhance the diagnosis and care of patients with dermatophyte infections in specialized medical facilities.

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