



A comparative study of 10% potassium hydroxide mount and histopathological examination using periodic acid-schiff stain of cutaneous dermatophytosis: A retrospective study

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Abstract

Introduction: In a clinical setup, cutaneous dermatophytosis is routinely diagnosed using 10% Potassium hydroxide mount of the skin scraping. The skin punch biopsy which is usually not done for fungal infection as they involve only stratum corneum and scar is the sequelae of skin biopsy.

Objectives: To determine the accuracy of KOH mount and PAS stained skin punch biopsy in clinically suspected tinea patients.

Methods: The present study is a retrospective cross sectional study. Data was collected based on the records of 45 patients, who attended the dermatology OPD in our hospital, presenting with clinical suspicion of fungal for

whom KOH mount and skin punch biopsy was done in the past 6 months

Inclusion criteria: All cases of clinically suspected tinea patients with data of KOH and skin punch biopsy samples are included in this study.

Exclusion criteria: Nail and scalp fungal infections were ruled out due to the impracticality of crushing and fixing nails and hair onto slides for examination.

Results: Of the 45 suspected cases of superficial cutaneous mycoses, fungal elements were detected in 39 cases through either or both diagnostic techniques. Among these, 23 cases (58.9%) tested positive through KOH preparation, while all 39 cases showed positive results with PAS-stained skin punch biopsy. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of PAS-stained biopsy were all 100%, while those for KOH preparation were 58.9%, 100%, 100%, 27.27%, and 64.44% respectively. Fungal element quantity on the smear was graded as follows: 1+ in 18 cases, 2+ in 15 cases, and 3+ in 6 cases.

Conclusion: PAS-stained skin punch biopsy is a more sensitive method than conventional KOH preparation. A Confirmatory test with PAS staining of the biopsy sample can reduce the false negative results and aids in better management.

Keywords: Dermatophytosis, Tinea, Tinea Corporis, Fungal Skin Infection, Skin Punch Biopsy, PAS Stain, Periodic Acid- Schiff Stain.

Introduction

Superficial dermatophytosis poses a significant threat and is currently viewed as an epidemic in the Indian sub-continent, primarily because of the humid climate and widespread misuse of over-the-counter topical steroids¹. It can be classified into two types: superficial mycoses and deep type².

Superficial mycoses are divided into surface and cutaneous types, while deep mycoses are categorized as subcutaneous and systemic. Among dermatophyte species, the three frequently encountered are *Microsporum*, *Trichophyton*, and *Epidermophyton*.

Candida species are capable of causing both superficial and deep mycoses.

Subcutaneous deep mycoses are often attributed to species such as *Aspergillus*,

Zygomycosis, *Rhinosporidium* spp., and chromomycosis.

Skin involvement as a secondary effect can occur in systemic infections with *Cryptococcus* and *Histoplasma*.

The stains used in direct microscopic examination of wet mounts includes KOH,

Chicago sky blue stain, Parker blue-black ink, Swartz-Lamkins stain, calcofluor white, India ink—fluorescent antibody staining³.

The KOH examination involves the direct microscopic visualization of fungal elements through the dissolution of keratinized tissues, providing a rapid and cost-effective tool for initial diagnosis. Positive results typically reveal the presence of refractive, elongated, smooth, undulating, branched, and septate hyphal filaments, along with or without arthroconidiospores⁴. On the other hand, the skin punch biopsy with Periodic Acid-Schiff (PAS) stain offers a histopathological analysis, allowing for a detailed examination of tissue morphology and identification of specific fungal structures. Skin punch biopsy are not usually done as tinea is a superficial infection only involving stratum corneum and it is an invasive procedure leading to scar formation. Periodic Acid-Schiff is a carbohydrate stain which reacts with polysaccharides and chitin present in the fungal capsules or walls of many species⁵.

Better understanding of these diagnostic techniques is crucial for refining diagnostic strategies and improving the overall management of tinea infections.

This study seeks to assess the effectiveness of PAS-stained skin punch biopsy for diagnosing suspected fungal skin infections and compare its diagnostic performance with that of KOH preparation.

Aims and Objectives

To assess the accuracy of KOH mount and PAS stained skin punch biopsy in clinically suspected tinea patients

Methods and Materials

Study design: Retrospective-Cross Sectional study

Study duration: August 2023- January 2024

Source of data: The present study is a retrospective study. Data was collected based on the records of patients presenting with clinical suspicion of tinea for whom KOH mount and skin punch biopsy was done. Informed consent from the patient will be obtained.

Sample size: 45

Inclusion criteria: All cases of clinically suspected tinea patients with data of KOH and skin punch biopsy samples are included in this study.

Exclusion criteria: Nail and scalp fungal infections were ruled out due to the impracticality of crushing and fixing nails and hair onto slides for examination.

Description of the process: Technique of obtaining the skin scrape: Following a meticulous cleaning of the affected area with an alcohol swab, scrapings were obtained from the active borders of the lesion using either a scraper or the edge of a slide⁶.

Technique of KOH mounts preparation: Position the specimen onto a clean glass slide. Apply a single drop of 10% KOH solution. Cover the slide with a cover glass and gently apply pressure to eliminate air bubbles. Dab away excess solution using gauze. Position the slide onto the

microscope stage and examine using low magnification (10x). Reduce illumination by adjusting the condenser to visualize epithelial cells. Check for fungal elements like hyphae or yeast. If any appear suspicious, switch to the 40x setting (high-dry objective) for further investigation, as the presence of hyphae or budding yeast indicates fungal activity⁶.

Technique of skin punch biopsy: The area is anesthetized and the skin is stretched in a direction perpendicular to the resting skin lines. The punch is gripped using three fingers, with the thumb and middle finger providing support and facilitating rotation while the index finger stabilizes and applies pressure. Rotational movements are employed to push the punch into the skin until a sensation of "give away" is felt, indicating penetration into the semisolid subcutaneous tissue layer. The resulting wound can be either sutured or left to heal naturally through secondary intention⁷.

Table 1: Grading of fungal hyphae:⁸

Number of fungal hyphae(per 100 fields)	Hyphal index
Absence of fungal hyphae	0
Presence of up to 4 hyphae	+1
Number of hyphae present between Hyphal Index 1+ and 3+	+2
Presence of multiple hyphae	+3

Results

The study took place in Mangalore, Karnataka, over a duration of six months, starting from August 2023 and ending in January 2024. Among the 45 suspected cases of skin fungal infections, fungal elements were observed in 39 cases using one or both techniques. In the remaining six cases where fungal elements were not detected. Of which, under skin punch biopsy, 3 were detected as seborrheic dermatitis, 2 were allergic contact dermatitis and 1 was eczema. Among the 45 patients involved in the study, there were 27 male patients (60%) and 18 female patients (40%). The male-to-female ratio is 3:2. The

distribution of genders within the study population is summarized in Table 2.

GENDER	NUMBER	PERCENTAGE(%)
Male	27	60
Female	18	40

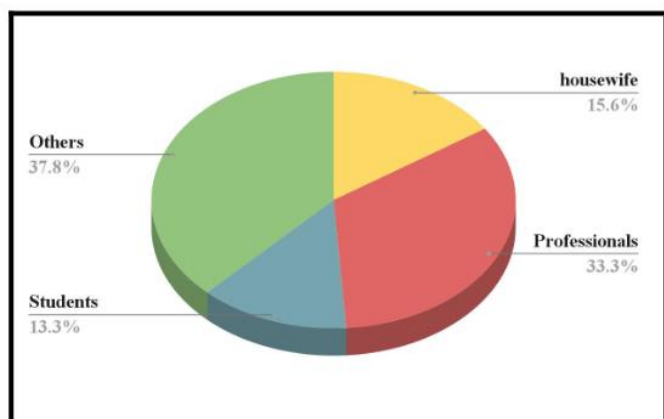
The age of the subjects ranged from 18 years to 57 years, with a mean age of 35 ± 11.2 years. The age distribution within the study population is summarized in Table 3.

Table 3: Age distribution

AGE GROUP	NUMBER	PERCENTAGE (%)
20 and below	3	6.7
21-30	16	35.6
31-40	12	26.7
41 and above	14	31.1

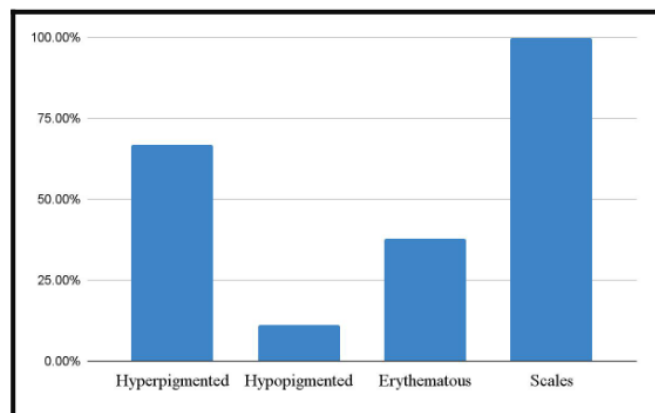
The majority of patients were professional (15= 33.3%) involved in high collar jobs, followed by housewives (7=15.6%), students (5= 13.3%) etc. Occupational distribution is summarized in Figure 1.

Figure 1: Occupational distribution



The lesions in these patients showed hyperpigmented (30 = 66.7%), hypopigmented (5 = 11.1%) and erythematous (17= 37.8%) plaques. All the patients presented with scaly lesions. It is depicted in Figure 2.

Figure 2: Lesional Characteristics



The clinical presentation were tinea cruris in 24 cases (53.3%), tinea corporis in 28 cases (62.2%), tinea faciei in 2 cases (4.4%), tinea pedis in 4 cases (8.9%) and pityriasis versicolor in 2 cases (4.4%). Also out of 39 cases, 11 patients (28.2%) were treatment naive cases and 9 (23%) cases had received anti-fungal therapy, 11 (28.2%) were on steroid treatment and 8 (20.5%) were on unknown topical therapy.

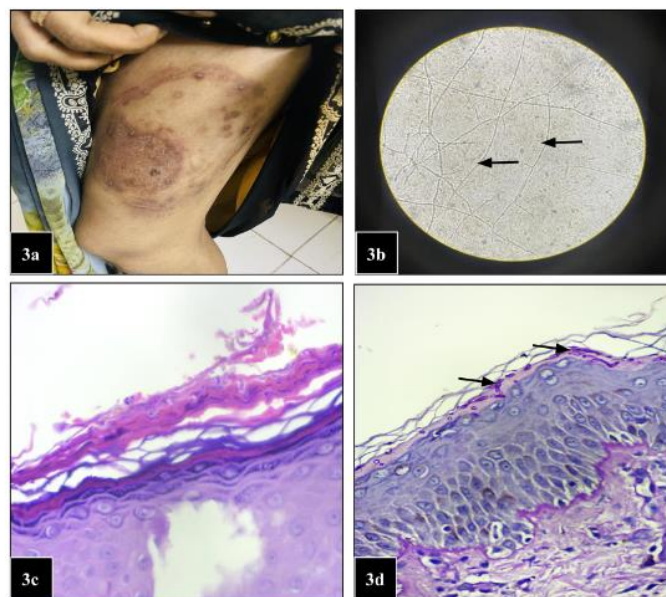


Figure 3: (a) clinical picture of a female patient presenting hyperpigmented scaly annular plaque. (b) microscopic examination of KOH preparation of the scales from the patient showing long hyphae (black arrow). (c) hematoxylin-eosin stain of skin punch biopsy and PAS

stain respectively. (d) PAS stain showing hyphae (black arrow).

The KOH preparation yielded positive results in 23 of 39 cases, accounting for 58.9%. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the KOH preparation were 58.9%, 100%, 100%, 27.27%, and 64.44% respectively. The PAS-stained skin punch biopsy showed positive results in all 39 cases (100%). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of this technique were all 100%. Fungal element quantity was graded on the smear as follows: 1+ in 18 cases, 2+ in 15 cases, and 3+ in 6 cases. Among the sixteen cases where KOH was negative, there was a predominance of low-grade positivity, with twelve cases exhibiting 1+ positivity and four cases exhibiting 2+positivity. Inflammatory components were observed in 5 out of the 39 patients (12.8%), and 3 cases (7.7%) additionally showed bacterial colonies.

Discussion

The study took place in Mangalore, Karnataka, over duration of six months, starting from August 2023 and ending in January 2024. During these six months the temperature in Mangalore ranged from a cool 22°C to a warm 34°C, and the relative humidity fluctuated between 28% and 97%. The warm and humid weather encourages the growth of superficial mycoses. In these 39 cases of tinea infections, the following clinical forms were observed: Tinea corporis, tinea cruris, tinea pedis, and tinea faciei. The age group with the highest incidence of cases was 21 to 30 years, comprising 35.6% of the total cases. Here, there was a noted predominance in males. Similar findings of higher incidence among males were reported in the study conducted by Khurana U et al⁹. This

could be attributed to increased physical activity and sweating in males.

Table 4: Comparison of the indexed study with earlier studies published in India.

Analysed variable	Khurana <i>et al</i> [9]	Suredran <i>et al</i> [10]	Bindu <i>et al</i> [11]	Present study
City	Bhopal	Mangalore	Calicut	Mangalore
No. Of patients	50	100	150	39
Sex ratio (M:F)	2.5:1	1.63:1	2.06:1	1.5:1
Age distribution	1-62 years	6-60 years	13days-75 years	18-57 years
Most common decade of distribution	21 -30 years (38%)	16-30 years (44%)	11-20 years (23.3%)	21-30 years (35.6%)
Clinical pattern	T. cruris with T. corporis (40%)	T.corporis (44.3%)	T.corporis (54.6%)	T.cruris (53.3%)
KOH positivity	90%	96%	64%	58.9%
Culture positivity	Not done	39%	45.3%	Not done
Species identified	Not done	T.rubrum (67.5%)	T.rubrum (66.2%)	Not done
PAS stained sensitivity	98%	Not done	Not done	100%

The KOH preparation yielded positive results in 23 of 39 cases, accounting for 58.9% while The PAS-stained skin punch biopsy showed positive results in all 39 cases (100%). Among the 39 patients who were PAS positive in biopsy, KOH examination was positive only for 23 of them and rest 16 were negative. This negative KOH finding can be attributed to incomplete treatment with antifungal or use of topical steroids.

During interpretation, it is important to consider that starch granules found in glove powder exhibit PAS positivity and possess a diamond-shaped refractile center, thus, they should not be confused with yeast forms. Additionally, the cytoplasm of epidermal cells, owing to its glycogen content, also demonstrates PAS positivity, serving as an internal control for assessing the quality of the PAS stain⁹.

Conclusion

This study concludes that PAS-stained skin punch biopsy is a more sensitive method than conventional KOH preparation. The results from PAS stain are not subjective

and hence give a more accurate diagnosis. Skin punch biopsy also helps in looking at the skin cellular elements and inflammatory components. Its use should be encouraged as it gives a specimen block which are a permanent record and can be archived at a later stage for further examination. Henceforth, a confirmatory test with PAS staining of the biopsy sample can reduce the false negative results and aids in better management. The limitations of this study is that the sample size was small and culture were not done as this study was a retrospective study. Also, this study had lesser number of negative controls. So a future study with larger sample and culture to identify species can be done.

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References

1. The current Indian epidemic of dermatophytosis: a study on causative agents and sensitivity patterns. Das S, De A, Saha R, et al. Indian J Dermatol. 2020;65:118–122.
2. Lim CS-H, Lim S-L Practical tip: Chicago sky blue (CSB) stain can be added to the routine potassium hydroxide (KOH) wet-mount to provide a color contrast and facilitate the diagnosis of dermatomycoses Dermatol Online J 2011 17 11.
3. Mulla AF, Shah AA, Koshy AV, Mayank M Laboratory diagnosis of fungal infection Univ Res J Dent 2015 5 49- 53
4. Kurade SM, Amladi SA, Miskeen AK. Skin scraping and a potassium hydroxide mount. Indian J Dermatol Venereol Leprol.
5. Layton C, Bancroft JD Carbohydrates Suvarna SK, Layton C, Bancroft JD Bancroft's Theory and Practice of Histological Techniques 7th ed China Churchill Livingstone Elsevier 2013 215 -386
6. Ponka D, Baddar F. Microscopic potassium hydroxide preparation. Can Fam Physician. 2014 Jan;60(1):57. PMID: 24452564; PMCID: PMC3994805.
7. Nischal U, Nischal Kc, Khopkar U. Techniques of skin biopsy and practical considerations. J Cutan Aesthet Surg. 2008 Jul;1(2):107-11. doi:10.4103/0974-2077.44174. PMID: 20300359; PMCID: PMC2840913.
8. Chandramohan A, Srinivas CR. Hyphal Index Following a Potassium Hydroxide Mount in Dermatophytosis. Cureus. 2021 Dec 21;13(12):e20576. doi:10.7759/cureus.20576. PMID: 35103155; PMCID: PMC8776321.
9. Khurana U, Marwah A, Dey V, Uniya U, Hazari R. Evaluating diagnostic utility of PAS stained skin scrape cytology smear in clinically suspected superficial cutaneous mycoses: A simple yet unpracticed technique. J Family Med Prim Care 2022;11:1089-94
10. Surendran KAK, Bhat RM, Baloor R, Nandkishore B, Sukumar D. A clinical and mycological study of dermatophytic infections. Indian J Dermatol 2014;59:2627.
11. Bindu V, Pavithran K. Clinicomycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol 2002;68:25961.