

Clinico-cytological study of dermatophytic infections and interobserver agreement of potassium hydroxide mount diagnosis: A prospective cross-sectional study at a tertiary care centre.

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Abstract

Background

Objectives: To determine potassium hydroxide mount positivity among clinically diagnosed dermatophytic infections, compare positivity between treatment-naïve and previously treated patients, describe positivity across clinical subgroups, and quantify interobserver agreement between a faculty observer and a senior resident.

Methods

This prospective, hospital-based cross-sectional study was conducted at Government Medical College/Government General Hospital, Suryapet, from January 2021 to December 2022. Among 7,435 patients clinically diagnosed with dermatophytosis during the study period, potassium hydroxide mount examination was performed in 1,182 (15.9%), who constituted the analytical cohort. Skin scrapings, nail clippings, or hair samples were processed using tissue-appropriate potassium hydroxide concentrations. The same preparations were interpreted by an Associate Professor and a senior resident, and agreement was assessed using Cohen's kappa.

Results

Of 7,435 clinically diagnosed dermatophytosis cases, 1,182 underwent potassium hydroxide mount examination (15.9%). Among the tested participants, 711 were KOH-positive (60.2%; 95% CI, 57.3%-62.9%). Positivity did not differ by sex ($\chi^2(1) = 0.053$, $p = .817$), but varied by age group ($\chi^2(6) = 26.088$, $p < .001$). Treatment-naïve patients had higher positivity than previously treated patients (74.5% vs 45.7%; $\chi^2(1) = 102.236$, $p < .001$; odds ratio = 3.47, 95% CI, 2.71-4.43). Tinea corporis contributed 193 of 711 positive results (27.1%), followed by infection at two or more sites (179/711, 25.2%); positivity differed across clinical types ($\chi^2(8) = 35.290$, $p < .001$). Overall observer agreement was 93.3%, with $\kappa = 0.86$ (95% CI, 0.83-0.89; $p < .001$).

Conclusion

Potassium hydroxide mount provided a clinically useful diagnostic yield and showed excellent reproducibility between observers. Standardized specimen collection, microscopy, and supervised observer training can strengthen its reliability in high-volume dermatology services.

Recommendations

Regular hands-on training, standardized sampling, and quality review of negative or discordant slides are recommended in teaching hospitals.

Keywords: Dermatophytosis; Potassium hydroxide mount; Tinea corporis; cytology; Interobserver agreement; Cross-sectional study

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Introduction

Dermatophytoses are among the most frequently encountered superficial fungal infections in dermatology practice and affect keratinized structures such as the stratum corneum, hair, and nails. Global epidemiological reports indicate that superficial mycoses continue to represent a substantial public health burden, especially in warm and humid regions where crowding, sweating, occlusive clothing, shared fomites, and delayed treatment contribute to transmission [1]. Dermatophytes are keratinophilic fungi traditionally grouped under Trichophyton, Microsporum, and Epidermophyton; newer taxonomy has expanded the classification to include additional genera based on molecular methods [2]. Clinically, these infections present as tinea corporis, tinea cruris, tinea faciei, tinea pedis, tinea manuum, tinea capitis, tinea barbae, and tinea unguium, depending on the anatomical site involved [3].

Although many cases are suspected clinically, laboratory confirmation is important when lesions are atypical, modified by prior topical applications, recurrent, extensive, or clinically similar to eczema, psoriasis, candidiasis, or other inflammatory dermatoses. The potassium hydroxide mount is a simple cytological technique used to demonstrate fungal hyphae and spores in clinical samples. KOH dissolves keratin and cellular debris, allowing fungal elements to be seen under microscopy. The technique requires limited infrastructure, provides rapid results, and is suitable for outpatient settings, making it particularly useful in resource-limited and high-volume centres [4,5].

Despite its simplicity, the diagnostic yield of KOH mount depends on proper lesion selection, collection from the active border, sufficient scraping, correct concentration of KOH according to tissue type, adequate clearing time, and careful microscopic interpretation. Recent reviews emphasize that conventional microscopy and culture continue to remain relevant despite the availability of molecular methods, because they are affordable and accessible for routine clinical use [6]. Indian clinicomycological studies have shown variable KOH positivity rates, reflecting differences in case selection, prior treatment exposure, sample quality, disease chronicity, and technical expertise [7,8]. Therefore, local data from tertiary care teaching hospitals provide useful insight into positivity patterns and training needs.

Teaching hospitals also have an academic responsibility to train residents in point-of-care diagnostic methods. Interobserver agreement between a trained faculty member and a resident helps assess the reproducibility of slide interpretation and the adequacy of training. The present

study was conducted to determine the positivity rate of KOH mount among clinically diagnosed dermatophytic infections, compare KOH positivity between treatment-naïve and previously treated patients, describe the distribution of KOH positivity according to age, sex, and clinical type, and assess the degree of agreement in KOH diagnosis between an Associate Professor and an under-training senior resident.

Methodology

Study design and setting

This hospital-based, prospective cross-sectional analytical study was conducted in the Department of Dermatology, Venereology and Leprosy, Government Medical College/Government General Hospital, Suryapet, Telangana, India, from January 2021 to December 2022. Government General Hospital is a 400-bed tertiary care teaching hospital attached to Government Medical College and provides multispecialty outpatient, inpatient, emergency, diagnostic, and laboratory services. The dermatology service evaluates patients with disorders of the skin, hair, and nails, in addition to venereological and leprosy-related conditions, and provides microscopy-based diagnostic support.

Sample size and study participants

A minimum sample size of 1,152 was estimated using the single-proportion formula, with an anticipated dermatophytosis prevalence of 25%, a 95% confidence level, and a relative precision of 10%. Patients of all ages and both sexes with a clinical diagnosis of dermatophytosis involving the skin, hair, or nails were eligible. Patients using topical or systemic corticosteroids and those who declined informed consent were excluded. Previous antifungal treatment was not an exclusion criterion; such participants were classified as previously treated for subgroup analysis. During the study period, 7,435 patients were clinically diagnosed with dermatophytosis. Potassium hydroxide mount examination was performed in 1,182 patients (15.9%), exceeding the estimated minimum sample size; these patients constituted the analytical cohort.

Participant selection and enrolment

All clinically diagnosed dermatophytosis cases attending the dermatology outpatient service during the study period were recorded. Of 7,435 cases, 1,182 underwent potassium hydroxide mount examination and were enrolled in the cytological and interobserver analyses. The remaining 6,253

cases did not undergo KOH examination and were not included in the analytical cohort. One study record was created for each tested participant, and the relevant skin, hair, or nail specimen was obtained from the clinically active site before microscopic interpretation.

Bias

Potential selection bias was addressed through predefined clinical and exclusion criteria for patients undergoing potassium hydroxide examination. However, KOH testing was performed in 15.9% of all clinically diagnosed cases; therefore, testing-selection bias cannot be excluded, and the analytical cohort may not fully represent every dermatophytosis patient seen during the study period. Measurement bias was limited through standardized lesion preparation, collection from the active margin, tissue-specific potassium hydroxide concentrations, predefined clearing times, and uniform microscopic criteria for positivity. Both observers assessed the same preparations using the same diagnostic definition, and their initial readings were recorded separately before agreement analysis. All participants with paired, interpretable readings were included in the analysis. Residual verification bias remained possible because fungal culture, histopathology, or molecular confirmation was not used as an external reference standard.

Clinical assessment

A detailed clinical history was recorded for each participant, including age, sex, duration of disease, type of dermatophytosis, history of similar lesions, family history, contact with infected persons or animals, and prior treatment. Treatment history included oral or topical antifungals, topical corticosteroids, fixed-dose combination preparations, and other over-the-counter applications. General and systemic examination findings were documented, and hematological investigations were performed when clinically indicated.

Sample collection and potassium hydroxide procedure

The lesion surface was cleaned with alcohol. Skin was stretched, and scrapings were collected from the active border using the blunt side of a No. 15 scalpel blade. Nail clippings and subungual scrapings were obtained for suspected tinea unguium, while affected hair shafts with roots and scalp scrapings were collected for suspected tinea capitis. Samples were placed on clean glass slides and treated with 10% potassium hydroxide for skin, 20% for

hair, and 40% for nail specimens, covered with a coverslip, and gently pressed. Skin scrapings were gently heated or allowed to clear for 30 minutes to 2 hours; hair and nail samples were retained for 24-48 hours when required. Slides were examined first under 10x and then under 40x magnification. Each preparation was interpreted independently by an Associate Professor and a senior resident, and the initial readings were documented before comparison [4,5].

Outcome measures and statistical analysis

Preparations showing fungal hyphae, with or without spores, were classified as potassium hydroxide positive; slides without visible fungal elements were classified as negative. Categorical variables were summarized as frequencies and percentages, and the overall positivity estimate was accompanied by a 95% confidence interval. Pearson's chi-square test was used to compare positivity by sex, age group, treatment status, and clinical type. The association between treatment status and positivity was expressed as an odds ratio with a 95% confidence interval. Interobserver reliability was quantified using Cohen's kappa with its 95% confidence interval, together with observed percentage agreement. All tests were two-sided, and $p < .05$ was considered statistically significant.

Ethical consideration

The study protocol was approved by the Institutional Ethics Committee, Government Medical College, Suryapet, Telangana, India. Participant confidentiality was protected by using coded study records and limiting access to identifiable information.

Informed consent

Written informed consent was obtained from all adult participants before enrolment and specimen collection. For participants younger than 18 years, written consent was obtained from a parent or legal guardian. Participation was voluntary, refusal did not affect clinical care, and participants could withdraw without penalty. Data were analysed and reported without personal identifiers.

Results

Participant flow

During the study period, 7,435 patients were clinically diagnosed with dermatophytosis in the dermatology outpatient service. Potassium hydroxide mount examination was performed in 1,182 patients (15.9%), who constituted the study cohort. The remaining 6,253 patients (84.1%) did

not undergo KOH examination and were not included in the cytological or interobserver analyses. Specimens from all 1,182 tested participants were independently interpreted by

both observers. No paired diagnostic results were missing; therefore, all 1,182 participants were included in the final statistical analysis.

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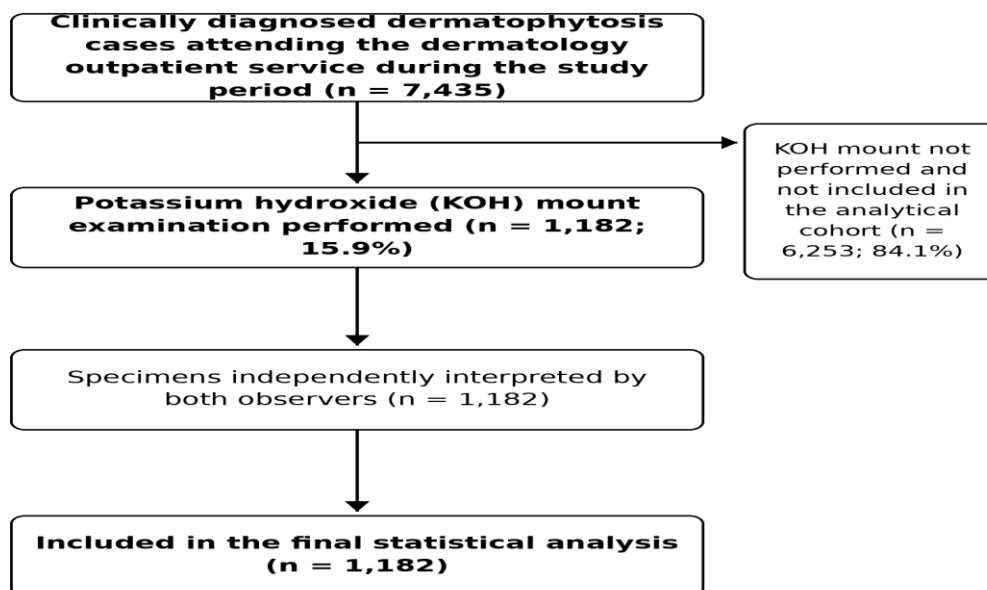


Figure 1: Flow of clinically diagnosed dermatophytosis cases through potassium hydroxide mount examination and final analysis

Among the 1,182 analysed participants, 620 (52.5%) were male, and 562 (47.5%) were female, giving a male-to-female ratio of 1.1:1. Potassium hydroxide mount was positive in 711 participants (60.2%; 95% CI, 57.3%-62.9%)

and negative in 471 (39.8%). Positivity was similar in males and females (59.8% vs 60.5%; $\chi^2(1) = 0.053$, $p = .817$) (Table 1).

Table 1: Sex distribution of potassium hydroxide mount results

Sex	KOH positive, n (%)	KOH negative, n (%)	Total, n
Male	371 (59.8)	249 (40.2)	620
Female	340 (60.5)	222 (39.5)	562
Total	711 (60.2)	471 (39.8)	1,182

Note. Values are n (% within sex category), except the total row. $\chi^2(1) = 0.053$, $p = .817$.

The age of potassium hydroxide-positive participants ranged from 1 to 79 years, with a mean of 31.39 $\pm$ 17.55 years. The largest number of positive results occurred in the

31-40-year group (n = 149), followed by the 21-30-year group (n = 131). Positivity differed significantly across age groups ($\chi^2(6) = 26.088$, $p < .001$) (Table 2).

Table 2: Age-group distribution of potassium hydroxide mount results

Age group (years)	KOH positive, n (%)	KOH negative, n (%)	Total, n
0-10	99 (50.3)	98 (49.7)	197
11-20	110 (55.0)	90 (45.0)	200
21-30	131 (55.7)	104 (44.3)	235
31-40	149 (67.1)	73 (32.9)	222
41-50	111 (67.3)	54 (32.7)	165
51-60	74 (71.8)	29 (28.2)	103
>60	37 (61.7)	23 (38.3)	60
Total	711 (60.2)	471 (39.8)	1,182

Note. Values are n (% within age group), except the total row. Row totals for the 31-40-year and 41-50-year groups were corrected from the reported cell frequencies. $\chi^2(6) = 26.088$, $p < .001$.

Treatment status was strongly associated with potassium hydroxide positivity. Among 595 treatment-naive participants, 443 were positive (74.5%), compared with 268 of 587 previously treated participants (45.7%). Treatment-

naive participants had 3.47-fold higher odds of a positive result (95% CI, 2.71-4.43; $\chi^2(1) = 102.236$, $p < .001$) (Table 3).

Table 3: Potassium hydroxide mount results according to previous treatment status

Treatment status	KOH positive, n (%)	KOH negative, n (%)	Total, n
Treatment-naive	443 (74.5)	152 (25.5)	595
Previously treated	268 (45.7)	319 (54.3)	587
Total	711 (60.2)	471 (39.8)	1,182

Note. Values are n (% within treatment category), except the total row. $\chi^2(1) = 102.236$, $p < .001$; odds ratio = 3.47 (95% CI, 2.71-4.43) for positivity in treatment-naive versus previously treated participants.

Tinea corporis accounted for the largest number of potassium hydroxide-positive results (193/711, 27.1%), followed by dermatophytosis involving two or more anatomical sites (179/711, 25.2%) and tinea cruris (128/711,

18.0%). The proportion testing positive differed significantly across clinical types ($\chi^2(8) = 35.290$, $p < .001$) (Table 4).

Table 4: Clinical types of dermatophytosis and potassium hydroxide mount results

Clinical type	KOH positive, n (%)	KOH negative, n (%)	Total, n
Tinea corporis	193 (58.7)	136 (41.3)	329
Tinea cruris	128 (48.9)	134 (51.1)	262
Tinea faciei	45 (78.9)	12 (21.1)	57
Tinea barbae	36 (62.1)	22 (37.9)	58
Tinea manuum	45 (75.0)	15 (25.0)	60
Tinea pedis	61 (71.8)	24 (28.2)	85
Tinea capitis	12 (75.0)	4 (25.0)	16
Tinea unguium	12 (70.6)	5 (29.4)	17
Two or more sites	179 (60.1)	119 (39.9)	298
Total	711 (60.2)	471 (39.8)	1,182

Note. Values are n (% within clinical type), except the total row. Positivity differed across clinical types, $\chi^2(8) = 35.290$, $p < .001$. Tinea corporis and multisite disease accounted for 27.1% and 25.2%, respectively, of all positive results.

Interobserver analysis showed 1,103 concordant interpretations: 701 preparations were positive according to both observers and 402 were negative according to both. The observed agreement was 93.3%. Cohen's kappa was 0.86

(95% CI, 0.83-0.89; $p < .001$), indicating excellent agreement between the Associate Professor and senior resident (Table 5).

Table 5: Interobserver agreement for potassium hydroxide mount interpretation

Senior resident interpretation	Associate Professor: KOH positive	Associate Professor: KOH negative	Total, n
KOH positive	701	69	770
KOH negative	10	402	412
Total	711	471	1,182

Note. Observed agreement = 93.3%; Cohen's $\kappa = 0.86$ (95% CI, 0.83-0.89), $p < .001$.

Discussion

The present study evaluated KOH mount positivity and diagnostic agreement among 1,182 patients who underwent KOH examination from a total of 7,435 clinically diagnosed dermatophytosis cases (15.9%). The overall KOH positivity rate was 60.2%, confirming that direct microscopy remains a useful diagnostic adjunct in routine dermatology practice. This finding is close to the KOH positivity reported by Poluri et al., who observed fungal filaments in 58.18% of clinically suspected cases in South India [8]. However, Singh et al. reported a much higher KOH positivity rate in

an eastern Indian tertiary care setting [7]. These differences reflect variations in sampling method, prior treatment, lesion chronicity, local epidemiology, and the threshold used for clinical inclusion.

The age distribution in the present study showed higher KOH positivity among adults aged 21-40 years, especially the 31-40-year age group. This pattern is consistent with the greater exposure of young and middle-aged adults to outdoor activity, sweating, shared clothing, occupational contact, and delayed consultation. Similar age clustering has been described in Indian clinicomycological studies [7,8]. Male and female participants showed comparable KOH

positivity, and sex-wise differences were not statistically significant. This observation suggests that environmental and behavioral exposures, rather than sex alone, influenced disease occurrence in this study population.

Treatment status showed a strong association with the potassium hydroxide result. Positivity was 74.5% among treatment-naïve patients and 45.7% among previously treated patients, corresponding to an odds ratio of 3.47 (95% CI, 2.71-4.43). A considerable proportion of treated patients nevertheless retained demonstrable fungal elements. This finding has practical relevance because partial treatment, irregular antifungal use, and over-the-counter topical preparations may alter lesion morphology and reduce diagnostic clarity. Potassium hydroxide microscopy provides rapid evidence of fungal elements and may help clinicians avoid inappropriate empirical steroid-containing combinations. Direct microscopy is also supported by diagnostic literature describing potassium hydroxide examination as more rapid and, in some clinical contexts, more sensitive than culture [10]. For nail disease, combined approaches using direct microscopy, culture, and histopathology improve confirmation, especially when fungal load is low or sampling is difficult [11].

Tinea corporis contributed the largest share of positive preparations (27.1%), followed by involvement of two or more sites (25.2%); however, positivity rates varied significantly across clinical types. This pattern mirrors the burden of extensive and recurrent glabrous-skin dermatophytosis encountered in Indian dermatology practice. Consensus recommendations from India endorse potassium hydroxide mount as a point-of-care test and emphasize culture in chronic, recurrent, relapsing, recalcitrant, or multisite disease [12]. The kappa value of 0.86 (95% CI, 0.83-0.89), together with 93.3% observed agreement, demonstrates excellent reproducibility between the faculty observer and senior resident. This supports the procedure as both a service tool and a competency-based learning method. Recent literature on pathogenesis and management also highlights that accurate diagnosis remains central to preventing chronicity, relapse, and inappropriate antifungal use [13,14].

Generalizability

The findings are most applicable to tertiary care dermatology outpatient settings in tropical and resource-limited regions where dermatophytosis is common, and KOH microscopy is routinely available. Because the study included a large sample across age groups, sexes, treatment categories, and clinical types, the results offer practical guidance for similar teaching hospitals. Still, extrapolation

to community clinics, culture-based laboratories, and populations with different antifungal exposure patterns should be done with contextual judgment.

Conclusion

This prospective cross-sectional study demonstrates that potassium hydroxide mount is a rapid, low-cost, and reproducible diagnostic method for dermatophytic infections in a tertiary care setting. Overall positivity was 60.2%, with significantly higher positivity among treatment-naïve participants and significant variation across age groups and clinical types. Sex was not associated with positivity. Excellent interobserver agreement between the faculty observer and senior resident supports the use of standardized sampling, microscopy, and supervised training. Routine potassium hydroxide examination can strengthen clinical diagnosis, reduce unnecessary empirical treatment, and support rational antifungal therapy in high-volume dermatology outpatient practice.

Limitations

The study excluded patients receiving topical or systemic corticosteroids, reducing representation of steroid-modified dermatophytosis. Fungal culture, species identification, antifungal susceptibility testing, and molecular confirmation were not performed. The study was conducted at a single tertiary care centre. Observer agreement was assessed between only two observers. Follow-up outcomes after antifungal therapy were not evaluated, limiting correlation between KOH status and clinical response. Furthermore, potassium hydroxide examination was performed in only 1,182 of 7,435 clinically diagnosed cases (15.9%), which may have introduced testing-selection bias and limits extrapolation of the analytical findings to all dermatophytosis patients attending the service.

Recommendations

KOH mount should be performed routinely for clinically suspected dermatophytosis, especially in recurrent, extensive, atypical, treated, or multisite lesions. Samples should be collected from the active border after proper cleaning, and tissue-specific KOH concentrations should be used. Dermatology departments should conduct periodic resident training sessions with slide review, quality control, and supervised reporting. Negative KOH results in strongly suspected cases should prompt repeat sampling or additional tests such as culture, PAS staining, or molecular methods where available. Public education regarding avoidance of steroid-combination creams and incomplete antifungal

therapy is also necessary for reducing recurrence and preventing diagnostic delay in practice.

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Abbreviations

KOH: Potassium hydroxide
PAS: Periodic acid-Schiff
GMS: Grocott methenamine silver
PCR: Polymerase chain reaction
SDA: Sabouraud dextrose agar
DTM: Dermatophyte test medium

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

TJ-Concept and design of the study, results interpretation, review of literature, and preparing the first draft of the manuscript. Statistical analysis and interpretation, revision of the manuscript. PK-results interpretation, review of literature, and preparing the first draft of the manuscript; revision of the manuscript.FMS-review of literature and preparing first draft of manuscript, revision of manuscript.

Data availability

Data available on request

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