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RESEARCH ARTICLE
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"EVALUATION OF PERIODIC ACID SCHIFF STAIN VERSUS POTASSIUM HYDROXIDE MOUNT FOR DIAGNOSIS OF FUNGAL INFECTIONS."

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Abstract

Introduction: Fungal infections impact over 57 million people in India, highlighting the need for early and accurate diagnosis, particularly in immunocompromised individuals. This study compares the diagnostic efficacy of KOH mount, PAS staining, and fungal culture—assessing their sensitivity and specificity—to guide optimal approaches for timely and effective management.

Aim : To compare diagnostic accuracy of KOH mount versus PAS stain for fungal diagnosis.

Materials and Methods : A cross-sectional study was conducted over a period of six months on 40 clinical samples at a tertiary care centre, with each sample undergoing KOH mount, PAS staining, and fungal culture. Sensitivity and specificity for KOH mount and PAS stain were evaluated in comparison with the gold standard method, fungal culture.

Results : Out of 40 cases, 15% tested positive by all three methods. Isolated positivity was 7.5% each for PAS and KOH, and 5% for culture. PAS showed higher sensitivity (75%) and accuracy (82.5%) compared to KOH (58.33% and 77.5%, respectively). Both tests had equal specificity (85.71%).

Conclusion: PAS staining showed higher sensitivity, while KOH mount demonstrated good specificity compared to fungal culture, which remains the gold standard. A combined approach using PAS, KOH, and culture enhances diagnostic accuracy for fungal infections. Larger studies with molecular methods are recommended to further optimize diagnostic strategies.

Key Words: KOH Mount, Fungus, PAS (Periodic Acid Schiff)

Introduction

Fungal infections are a significant public health concern in India, affecting a substantial portion of the population. A comprehensive study estimated that approximately 57 million individuals in India, accounting for about 4.1% of the population, suffer from serious fungal diseases.

Early identification of fungal infections is crucial, especially in immunocompromised patients or those with systemic involvement, as timely treatment significantly reduces morbidity and mortality. Diagnosis typically involves a combination of clinical evaluation and laboratory testing.[1]

Laboratory-based diagnostic methods are central to the accurate identification of fungal pathogens.They help confirm suspected infections, identify the causative organism, and guide appropriate treatment.

Laboratory diagnostics for fungal infections include a combination of traditional techniques (e.g., microscopy and culture) and advanced methods (e.g., PCR, antigen detection). The choice of method depends on the type of infection, the fungal species, and the clinical setting.[2]

Various samples can be used for fungal infection diagnosis, such as skin scrapings, blood, urine, sputum, tissue biopsies, Fine Needle Aspiration Cytology (FNAC) aspirates and bronchoalveolar lavage (BAL) fluid. The choice of sample depends on the suspected site of infection.[3]

One of the simplest and most rapid diagnostic methods, direct microscopy (often with potassium hydroxide (KOH) mount) allows for visualization of fungal elements such as hyphae or spores directly from clinical specimens.The Periodic Acid-Schiff (PAS) stain is a widely used histochemical technique that highlights fungal elements in tissue samples. It stains the polysaccharides in the fungal cell walls, making fungi easily identifiable under a microscope. This staining method is particularly valuable for detecting fungi in tissue biopsies and cytological samples.Fungal culture is considered the gold standard for identifying the causative organism. It involves growing fungal pathogens from clinical samples in specialized media under controlled conditions. Although this method is highly reliable, it may take several days to weeks for results, which may delay treatment.[4,5]

This study was conducted to compare the diagnostic accuracy of KOH mount and PAS stain with fungal culture in FNAC, sputum, and BAL fluid samples, and to evaluate the sensitivity and specificity of each method.

Aim and objectives :

To compare diagnostic accuracy of KOH mount and PAS stain with fungal culture in FNAC, Sputum and Bal fluid samples.

Materials and Methods:

This was a cross-sectional study conducted in the department of pathology and microbiology at medical College in Andhra Pradesh from December 2024 to April 2025 with a total number of 40 cases.

Inclusion criteria:

All FNAC swellings with pus aspiration, along with sputum and BAL fluid specimens received in the microbiology department for KOH mount and fungal culture during the study period were included in this study.

Exclusion criteria:

Samples deemed inadequate and cases with previously diagnosed fungal infections were excluded from the study.Patient clinical data were collected after obtaining written informed consent and analyzed concerning age and gender.FNAC was performed using a 23G needle and a 10 mL syringe. From the aspirated pus, an air-dried smear was prepared for each case and stained with PAS, while the remaining material was sent to the microbiology department for KOH mount and fungal culture.

Similarly, sputum and BAL fluid samples received in the microbiology department for KOH mount and fungal culture were partially processed to the pathology department, where air-dried smears were prepared and stained with PAS.

PAS staining was performed by immersing the slides in 1% periodic acid for 10 minutes, followed by treatment with Schiff's reagent for 15–30 minutes.

The PAS stain was considered positive if magenta-pink branching thread-like structures (hyphae) and/or beaded spherical structures (spores) were observed under the microscope.
For the KOH mount, the standard procedure was followed, wherein 10% KOH was applied to the slides, and direct microscopic examination was performed. The slides were evaluated for the presence of hyphae or spores, and the test was considered positive if either structure was observed.
For fungal culture, samples were inoculated onto blood agar, MacConkey agar, and two tubes of Sabouraud dextrose agar with antibiotics. The cultures were incubated at 37°C and 25°C, respectively. Growth in blood agar and MacConkey agar was examined after 24-48 hours and identified using conventional methods. Growth on Sabouraud dextrose agar (SDA) was monitored every alternate day during the first week and then twice weekly for up to six weeks. Fungal identification was based on the time to growth, colony morphology, and microscopic characteristics.

Statistical analyses:
Data analysis: Data entry was performed using Microsoft Excel. Sensitivity, specificity, and diagnostic accuracy of KOH mount and PAS stain were calculated using fungal culture as the gold standard. Categorical variables such as test results were expressed as frequencies and percentages. Comparative interpretation of diagnostic values was done descriptively without inferential statistical tests.

IEC consideration and permission: IEC Consideration and Permission: The study was approved by the Institutional Ethics Committee (IEC) in accordance with ethical research practices on 27/12/2024, Lr.No.06/2025.

Results
Demographic Distribution
A total of 40 cases were included in this study, with patient ages ranging from below 20 years to over 60 years. The majority of cases (67.5%) were in the 20–60 years age group, followed by 25% in those above 60 years, and 7.5% in individuals under 20 years. This suggests that middle-aged and elderly individuals accounted for the majority of suspected fungal infection cases [Table 1].
In terms of gender distribution, males accounted for 60% (24 cases) of the study population, while females comprised 40% (16 cases) [Table 2].

Table 1. Age - wise distribution of study subjects.

Age (in years)	Number of cases	Percentage of cases
< 20 years	03	7.5%
20-60 years	27	67.5%
> 60 years	10	25%

Table 2. Gender wise distribution of study subjects.

Gender	Number of cases	Percentage of cases
Male	24	60%
Female	16	40%
Total	40	100%

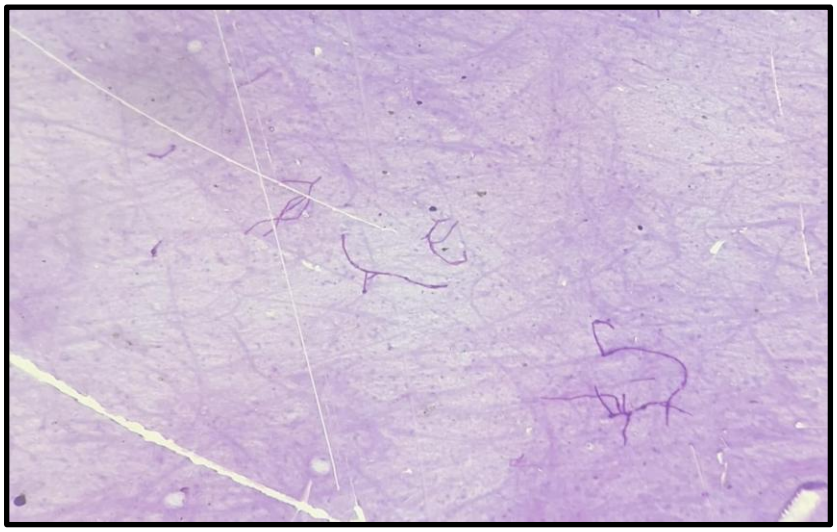

Figure 2 : Periodic acid Schiff stain shows magenta pink branching septate filamentous hyphae (PAS 100x).

Figure 2 : Periodic acid Schiff stain shows magenta pink branching septate filamentous hyphae (PAS 400x).

Table 3. Summary of test results

Test Combination	Number	Percentage (%)
All 3 test positive	6	15%
Only PAS positive	3	7.5%
Only KOH positive	3	7.5%
Only Culture positive	2	5%
Both PAS and Culture positive	3	7.5%
Both KOH and culture positive	1	2.5%
Both PAS and KOH positive	1	2.5%
All 3 test negative	21	52.5%

Diagnostic Test Results:

Out of the 40 cases included in the study, 6 cases (15%) were positive by all three diagnostic methods—KOH mount, PAS stain, and fungal culture. Three cases (7.5%) were positive only by PAS, and a similar proportion (7.5%) were positive only by KOH. Two cases (5%) were positive exclusively on fungal culture.

Combined positivity of PAS and culture without KOH was observed in 3 cases (7.5%), while only 1 case (2.5%) showed combined positivity of KOH and culture. Similarly, 1 case (2.5%) was positive on both PAS and KOH but negative on culture. The remaining 21 cases (52.5%) were negative across all three diagnostic modalities [Table 3].

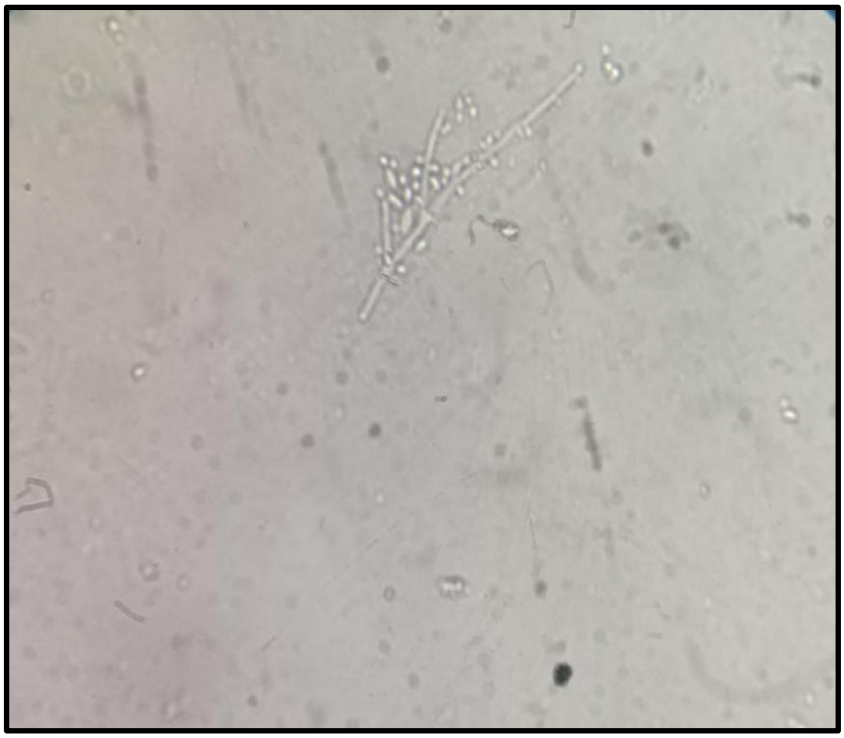

Figure 3: KOH mount showing branching fungal hyphae and spores (KOH 400×).

Table 4. Comparison of different tests with culture as the gold standard

Measure	**PAS (%)**	**KOH (%)**
Sensitivity	75%	58.33%
Specificity	85.71%	85.71%
Positive predictive value (PPV)	69.23%	63.64%
Negative predictive value (NPV)	88.87%	82.76%
Accuracy	82.50%	77.50%

The diagnostic performances of PAS staining and KOH mount were assessed against fungal culture, which is considered the gold standard for the identification of fungal pathogens. The evaluation was based on key statistical parameters: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy.

Sensitivity, which reflects the ability of a test to correctly identify those with the disease (true positives), was higher for PAS staining (75%) compared to KOH mount (58.33%). This suggests that PAS staining was more effective in detecting true positive cases of fungal infection.

Specificity, indicating the ability to correctly identify those without the disease (true negatives), was identical for both PAS and KOH, measured at 85.71%. This implies that both tests were equally reliable in ruling out non-fungal infections.

Positive Predictive Value (PPV), which represents the probability that a person with a positive test result truly has the disease, was 69.23% for PAS and 63.64% for KOH. This means that a positive PAS result was slightly more likely to reflect a true fungal infection compared to KOH.

Negative Predictive Value (NPV), the probability that a person with a negative result truly does not have the disease, was significantly higher for PAS (88.87%) than for KOH (82.76%). This indicates that a negative PAS result was more reliable in excluding fungal infections.

Overall diagnostic accuracy, which denotes the proportion of true results (both true positives and true negatives) among the total cases examined, was 82.5% for PAS and 77.5% for KOH. This reinforces that PAS staining offered better overall diagnostic reliability than KOH mount in the studied specimens.

DISCUSSION

Fungal infections were observed across all age groups in our study, with the highest prevalence (67.5%) seen in the 20–60 year age group, followed by 25% in individuals above 60 years and 7.5% in those under 20 years. This age distribution aligns with the findings of Kalyani Prava Gouda et al., where 30% of cases were in the 20–60 year age group and 8% were above 60 years.

A male predominance was noted in our study, with 60% of cases occurring in males and 40% in females. This trend is consistent with Kalyani Prava Gouda's study, which reported 80% male cases.[6] The higher incidence in males may be attributed to increased occupational or environmental exposure, although further studies are required to confirm this association.

Table 5. Comparison of sensitivity of test with other studies.

Sensitivity of test	Ujjawal Khurana et al. (2022)	Manjyot gautam et al. (2021)	Begari et al. (2019)	Hussein et al. (2011)	Present study
PAS sensitivity	98%	92%	84.56%	57.5%	75%
KOH sensitivity	90%	80%	81.82%	75%	58.33%

The PAS sensitivity in the present study (75%) is lower than that reported by Ujjawal Khurana et al. (98%)[7], Manjyot Gautam et al. (92%) [8], and Begari et al. (84.56%) [9], but higher than the value observed by Hussein et al. (57.5%) [10].

In contrast, the sensitivity of KOH mount in our study (58.33%) is lower than in all the compared studies, which reported sensitivities ranging from 75% to 90%. This suggests that in the current study population, PAS staining (75%) demonstrated greater sensitivity than the KOH mount.

Weinberger et al.[11] in their study of 105 patients, reported sensitivities of 80% for KOH mount, and 92% for PAS staining. Haiso et al.[12] reported higher sensitivities for both KOH (87%) and PAS (81%).

Shenoy et al.[13] found KOH positivity in 50% of cases and PAS positivity in 76%. Similarly, Blake et al. (2015)[14] reported KOH sensitivity of 64% and PAS sensitivity of 79%. Agha et al. (2018)[15],

however, reported a much lower KOH sensitivity of 29.4%, while their PAS sensitivity remained high at 80.3%.

These findings consistently support the superior sensitivity of PAS staining compared to KOH mount in the diagnosis of fungal infections.

Table 6. Comparison of specificity of test with other studies.

Specificity of test	Manjyot gautam et al. (2021)	Begari et al. (2019)	Present study
PAS Specificity	54%	57.14%	85.71%
KOH Specificity	79%	92.86%	85.71%

Table 6 presents a comparative analysis of the specificity of PAS staining and KOH mount in diagnosing fungal infections, juxtaposing the findings of the present study with those of Manjyot Gautam et al. (2021) and Begari et al. (2019).

In the present study, PAS staining demonstrated a high specificity of 85.71%, significantly higher than the values reported by Manjyot Gautam et al. (54%) and Begari et al. (57.14%). This elevated specificity suggests that PAS staining in our study more effectively identified individuals without fungal infections, thereby minimizing false positives. The improved specificity may be attributed to standardized sample processing and better interpretive criteria.
Similarly, the KOH mount showed a specificity of 85.71% in the present study, which is lower than the 92.86% reported by Begari et al. (2019), but higher than the 79% reported by Manjyot Gautam et al. (2021). Despite being a simple and economical method, KOH mount demonstrated commendable specificity, reinforcing its value, especially in resource-limited settings.
While PAS staining had a moderate sensitivity (75%) and high specificity (85.71%), KOH mount showed lower sensitivity (58.33%) but comparably high specificity (85.71%). This suggests that PAS is more reliable in detecting true positives, while KOH is more efficient in ruling out false positives when positive.

In terms of diagnostic accuracy, PAS staining demonstrated a high overall value of 82.5%, reinforcing its reliability in consistently identifying true fungal infections. In comparison, KOH mount showed a slightly lower accuracy of 77.5%. Although KOH remains advantageous due to its simplicity and cost-effectiveness, the relatively higher accuracy of PAS highlights its superior diagnostic performance.

Diagnostic Utility of PAS and KOH Mounts
PAS is useful for detecting fungal elements in tissue and cytological samples, as it stains fungal cell walls vividly.
KOH mount, being a rapid test, is beneficial for bedside and immediate diagnosis, but its lower sensitivity can lead to false negatives.
Combining PAS and KOH significantly improves the chances of detecting fungi early, making them essential screening tools before culture results are available.

Clinical Implications
1. PAS staining should be used routinely in cytopathological evaluations of suspected fungal infections, especially in FNAC, sputum, and BAL samples.[16]
2. KOH mount remains a quick and simple diagnostic method, but its lower sensitivity necessitates additional confirmatory testing.[17,18]

Limitations of the Study

Small sample size (40 cases) may limit the generalizability of results.

Culture, though considered the gold standard, is not 100% sensitive, and there is a possibility of some fungal cases being missed due to non-viability or poor growth conditions.

This study did not assess molecular or serological methods, which could provide additional insights into fungal identification.

CONCLUSION

This study demonstrates that PAS staining offers higher sensitivity and diagnostic accuracy compared to KOH mount, while both methods show comparable specificity. Although fungal culture remains the gold standard, combining PAS, KOH, and culture enhances diagnostic reliability for fungal infections in FNAC, sputum, and BAL fluid samples.

Larger studies incorporating molecular diagnostics are recommended to further refine and optimize fungal infection detection strategies.

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