

Evaluation of Sensitivity and Specificity of Direct Microscopical Examination of Suspected Mucor Mycosis Samples by Potassium Hydroxide (KOH) during Covid-19 Pandemic Era

Ullas Bhabhor, Yogita Mistry*, Summaiya Mullan

Department of Microbiology, Government Medical College, Surat, Gujarat, India

Email: *dryogitamistry@gmail.com

How to cite this paper: Bhabhor, U., Mistry, Y. and Mullan, S. (2022) Evaluation of Sensitivity and Specificity of Direct Microscopical Examination of Suspected Mucor Mycosis Samples by Potassium Hydroxide (KOH) during Covid-19 Pandemic Era. *Advances in Infectious Diseases*, 12, 776-780. <https://doi.org/10.4236/aid.2022.124054>

Received: October 7, 2022

Accepted: December 2, 2022

Published: December 5, 2022

Copyright © 2022 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Introduction: Mucormycosis is a common fungal infection in India even before the COVID-19 pandemic. The incidence of Mucor mycosis has entered public consciousness in response to an outbreak of cases in India during COVID-19 pandemic. Mortality is unacceptably high, reaching 80% in some case series. Prompt diagnosis is crucial because treatment initiation is time-critical due to the rapid progression of the infection. Direct KOH mount is a simple, rapid, inexpensive point-of-care test, which requires minimum technical aids. Interpretation of KOH smears requires some amount of experience. The present study was to evaluate the sensitivity and specificity of Direct Microscopical examination by KOH preparation of suspected Mucor mycosis samples taking the fungal culture as gold standard. **Material and Methods:** 230 samples were received in Microbiology Department from May 2021 to August 2021 from the patients suspected of Mucor mycosis. Samples were processed for KOH preparation using different concentrations of KOH like 20% and 40% till all tissues get digested. Samples were cultured also in Sabourauds dextrose agar (SDA) medium. Results of microscopy and culture were statistically analyzed using SPSS software version 23. **Result:** Sensitivity of KOH preparation was 67%, specificity was 87%. Positive predictive value was 76% and negative predictive value was 81% and accuracy was 80% when compared with the fungal culture. **Discussion:** Direct microscopic examinations using KOH preparation are the simplest, cheapest method used for the diagnosis of mycotic infections. This study is valuable in establishing a reliable method for early information on diagnosis of Mucor mycosis that may be crucial for determining appropriate therapy for the successful treatment of

Mucor mycosis patients. **Conclusion:** KOH examination plays an important role in the diagnosis of Mucor mycosis suspected patients during COVID-19 pandemic era when compared with fungal culture which is a gold standard test in diagnosis of Fungal infection. It is the simplest and cheapest method that can be developed easily with minimal infrastructure or human resources in resource-limited countries during the COVID-19 era.

Keywords

Mucormycosis, COVID-19 Pandemic Era, KOH Preparation

1. Introduction

Mucormycosis is caused by ubiquitous environmental molds with global distribution, including the *Rhizopus*, *Apophysomyces*, *Mucor*, and *Lichtheimia* species. Infection manifests as a rapidly developing, angioinvasive infection, with rhino-orbital-cerebral and pulmonary manifestations as the most common forms of disease [1]. Although prevalent worldwide, Mucor mycosis is much more common in India even before the COVID-19 pandemic. The incidence of Mucor mycosis has entered public consciousness in response to an outbreak of cases in India during COVID-19 pandemic. According to an Indian Government minister, on May 25, 2021, there were over 11,700 patients receiving care for Mucormycosis [2].

Mortality is unacceptably high, reaching 80% in some case series [3]. Prompt diagnosis is crucial because treatment initiation is time-critical due to the rapid progression of the infection [4]. Diagnosis is based on histology and tissue culture, which can be invasive, slow, and insensitive. There is no serology test or serum biomarker available to enable early diagnosis. Molecular methods are in development, but are not generally available [5] [6]. So, it becomes very important to diagnose the Mucormycosis suspected cases at the earliest so that proper therapeutic or surgical intervention can be started in a patient, which can reduce the cost of treatment by antifungal for a longer duration, hospital stay, etc.

Direct KOH mount is a simple, rapid, inexpensive point-of-care test, which requires minimum technical aids. Interpretation of KOH smears requires some amount of experience [7]. When compared to mycological culture, KOH mount had the ability to detect more fungal agents from clinical specimens.

The present study is to evaluate the sensitivity and specificity of Direct Microscopical examination by KOH preparation taking the fungal culture as gold standard during COVID-19 pandemic era.

2. Material and Method

It was an experimental study started after ethical approval for the same. Samples which were received in Microbiology Department during second wave of COVID-19 pandemic (from May 2021 to August 2021) from the patients sus-

pected for Mucormycosis were included. Samples like tissue biopsy, scrapping material were received from suspected patients of mucormycosis. Samples were processed for KOH preparation and fungal culture. For KOH preparation, part of sample was incubated with KOH either in test tube or on the slide kept in wet chamber for minimum of 2 hours to maximum of 12 hours till tissue cells get digested and clear fungal elements became visible. Different Concentration of 20% and 40% were used depending upon type of tissue, harder the tissue to digest the keratine tissue, higher concentration of KOH for longer duration is needed to make fungal element visible by microscopy. In KOH preparation, fungal elements of *Mucor* species were seen as aseptate hyaline hyphae. Sometimes nodal and internodal rhizoids were also visible. Other part of sample was processed for culture in Sabourauds dextrose agar (SDA) medium. Growth on culture medium was observed daily till 7 days and then weekly till 21 days. Visible growth in culture medium is confirmed by macroscopic observation and microscopic observation by LPCB preparation to see aseptate hyphae, rhizoids. Results of microscopy and culture were entered in excel sheet and statistical analysis was done using SPSS software version 23.

3. Result

230 samples were received for suspected *Mucor* mycosis during 4 month period from May 2021 to August 2021. Out of which 27% were positive by KOH Microscopy and 23% were positive by culture as per **Table 1**. **Table 2** is showing sensitivity, specificity, positive predictive value, negative predictive value and accuracy of Microscopic result in comparison with fungal culture done for other fungal infection by different authors [8] [9] [10]. Present study shows KOH preparation for diagnosis of *Mucor* mycosis have 68% sensitivity, 88% specificity, 77% positive predictive Value, 82% negative predictive value, 80% accuracy at 95% confidence interval when compared with the fungal culture as a gold standard. Compared to Fungal cultures (23%), KOH preparation (27%) shows higher

Table 1. Percentage of positive and negative samples by KOH and culture.

Methods	Positive	Negative
KOH	27%	73%
Culture	23%	77%

Table 2. Comparison of KOH microscopic result with culture results.

Statistic	Value	95% Confidence Interval
Sensitivity	67.31%	57.41% to 76.19%
Specificity	87.79%	81.94% to 92.28%
Positive Predictive Value	76.92%	68.60% to 83.57%
Negative Predictive Value	81.62%	77.02% to 85.47%
Accuracy	80.07%	74.87% to 84.62%

Table 3. Comparison of present study with other study done for KOH Microscopy.

Statistic	Present study	Study by V. Begar <i>et al.</i> for fungal onychomycosis [7]	Study by S. Manickdas <i>et al.</i> for onychomycosis [8]	Study by Jacob Oren <i>et al.</i> for tenia pedis [9]
Sensitivity	67.31%	81.82%	83.02%	73.3%
Specificity	87.79%	92.86%	70.1%	42.5%
Positive predictive value	76.92%	69.23%	60.27%	46.6%
Negative predictive value	81.62%	83.33%	88.31%	69.9%

positivity rate. Although fungal culture is a gold standard test, but results are affected because of longer incubation duration, other fungal or bacterial contamination overgrowth, improper technical hand during sample inoculation etc. As limited data is accessible for mucormycosis culture and KOH comparison, we have used for onychomycosis and tenia pedis instead for comparison which is shown in **Table 3**.

4. Discussion

Laboratory diagnosis of many fungal infections relies on direct microscopic examination of fungal elements in the clinical sample and mycological culture of the particular fungal species concerned.

Direct microscopic examinations are the simplest, cheapest method used for the diagnosis of mycotic infections. Direct mycological examination of specimens in suspected cases of Mucor mycosis provides early detection when compared to culture, which can take days or weeks. This study is valuable in establishing a reliable method for early information on diagnosis of Mucormycosis that may be crucial for determining appropriate therapy for the successful treatment of Mucormycosis patient [10]. Although quality of the sample and experience of the microbiologist are the major factors that determine the successful rate of microscopic examination, still it will help in early identification of fungal infection and thereby starting proper antifungal treatment or other therapeutic intervention application in patient.

5. Conclusion

KOH examination plays an important role in diagnosis of Mucor mycosis suspected patients during COVID-19 pandemic era when compared with fungal culture, which is a gold standard test in diagnosis of Fungal infection. It is the simplest and cheapest method that can be developed easily with minimal infrastructure or human resources in resource-limited countries during COVID-19 era.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Prakash, H. and Chakrabarti, A. (2021) Epidemiology of Mucormycosis in India.

- Microorganisms*, **9**, No. 523. <https://doi.org/10.3390/microorganisms9030523>
- [2] Sharma, K., Jyoti and Kaur, R. (2021) Mucormycosis in COVID-19 Patients: A Review. *Indian Journal of Continuing Nursing Education*, **22**, 123-126. <https://www.ijcne.org/text.asp?2021/22/2/123/336899>
https://doi.org/10.4103/ijcn.ijcn_87_21
- [3] Rodrigues, M.L. and Albuquerque, P.C. (2018) Searching for a Change: The Need for Increased Support for Public Health and Research on Fungal Diseases. *PLOS Neglected Tropical Diseases*, **12**, e0006479. <https://doi.org/10.1371/journal.pntd.0006479>
- [4] Bongomin, F., Gago, S., Oladele, R. and Denning, D. (2017) Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision. *Journal of Fungi*, **3**, No. 57. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5753159/>
<https://doi.org/10.3390/jof3040057>
- [5] Roden, M.M., Zaoutis, T.E., Buchanan, W.L., Knudsen, T.A., Sarkisova, T.A., Schaufele, R.L., et al. (2005) Epidemiology and Outcome of Zygomycosis: A Review of 929 Reported Cases. *Clinical Infectious Diseases*, **41**, 634-653. <https://doi.org/10.1086/432579>
- [6] Millon, L., Scherer, E., Rocchi, S. and Bellanger, A.-P. (2019) Molecular Strategies to Diagnose Mucormycosis. *Journal of Fungi*, **5**, E24. <https://doi.org/10.3390/jof5010024>
- [7] Begari, V., Pathakumari, P. and Takalkar, A.A. (2019) Comparative Evaluation of KOH Mount, Fungal Culture and PAS Staining in Onychomycosis. *International Journal of Research in Dermatology*, **5**, 554-558. <https://doi.org/10.18203/issn.2455-4529.IntJResDermatol20192107>
- [8] Dass, S.M., Vinayaraj, E.V., Pavavni, K., Pallam, A. and Rao, M.S. (2015) Comparison of KOH, Calcofluor White and Fungal Culture for Diagnosing Fungal Onychomycosis in an Urban Teaching Hospital, Hyderabad. *Indian Journal of Microbiology Research*, **2**, 148-153. <https://doi.org/10.5958/2394-5478.2015.00004.7>
- [9] Levitt, J.O., Levitt, B.H., Akhavan, A. and Yanofsky, H. (2010) The Sensitivity and Specificity of Potassium Hydroxide Smear and Fungal Culture Relative to Clinical Assessment in the Evaluation of Tinea Pedis: A Pooled Analysis. *Dermatology Research and Practice*, **2010**, Article ID: 764843. <https://www.hindawi.com/journals/drpr/2010/764843/>
<https://doi.org/10.1155/2010/764843>
- [10] Miller, M.A. and Hodgson, Y. (1993) Sensitivity and Specificity of Potassium Hydroxide Smears of Skin Scrapings for the Diagnosis of Tinea Pedis. *Archives of Dermatology*, **129**, 510-511. <https://doi.org/10.1001/archderm.1993.01680250126023>