

ORIGINAL ARTICLE

Utility of MGG and Papanicolaou stained smears in the detection of Mucormycosis in nasal swab/scraping/biopsy samples of COVID 19 patients

Ashish C. Philip MD¹  | Prarthna Madan MBBS¹ | Sonal Sharma MD¹ | Shukla Das MD²

¹Department of Pathology, University College of Medical Sciences, University of Delhi, Delhi, India

²Department of Microbiology, University College of Medical Sciences, University of Delhi, Delhi, India

Correspondence

Sonal Sharma, Department of Pathology
University College of Medical Sciences,
University of Delhi, Shahdara, Delhi 110095,
India.

Email: sonald76@gmail.com

Abstract

Background: COVID 19 has been rapidly spreading across the globe. As a result of alteration of the immune milieu by COVID 19 and its treatment, there has been a rise in opportunistic fungal infections particularly Mucormycosis in these patients. Delay in diagnosis of these fungal infections can be fatal. The usual diagnostic modalities used to detect Mucor include potassium hydroxide (KOH) mount, fungal culture, and histopathology. Since histopathology and fungal culture have a long turnaround time we are dependent on KOH mount for rapid results. Here we investigate the role of stained cytology smears in the rapid diagnosis of Mucormycosis.

Methods: A prospective observational study was conducted in a tertiary health care hospital on samples of patients clinically suspected to have Mucormycosis. We performed May Grunwald Giemsa (MGG) and Papanicolaou (PAP) stains on the remnant samples of nasal swabs/scrapings/biopsies after KOH test and fungal culture. We took 16 KOH positive and 16 KOH negative samples. We also examined 16 fresh samples from patients whose earlier samples were reported to be negative on KOH test.

Results: The 6/16 KOH positive samples were found to be positive on stained cytology smears and 2 were mixed infections wherein both Mucor and Aspergillus were seen. The 4/16 KOH negative samples were positive for Mucor with one sample having both Mucor and Aspergillus. The 3/16 repeat samples which were earlier negative on KOH test were positive for Mucor.

Conclusion: Stained cytology smears if used in conjunction with KOH test can increase the overall sensitivity of detection of Mucormycosis and mixed infections.

KEYWORDS

COVID 19, crush cytology, MGG and PAP, Mucormycosis

1 | INTRODUCTION

Since its outbreak in Wuhan, China in December 2019, Corona virus Disease (COVID 19) caused by Severe Acute Respiratory Syndrome Virus 2 (SARS CoV 2) has spread rapidly across the globe and evolved to become a major pandemic. As of August 2021, there are more than

200 million infected cases with more than 4 million deaths reported worldwide.¹ The incidence of fungal co-infections in COVID 19 patients has been on the rise. These include Aspergillosis, Mucormycosis, Candidiasis, Cryptococcosis with Mucormycosis being the commonest. Mucormycosis, a rare but potentially dangerous infection is caused by filamentous fungi Mucorales.^{2,3} By May 28, 2021, almost 14,872 cases

of Mucormycosis have been reported in India.⁴ These infections are associated with rapid progression and a poor clinical outcome. Thus, early diagnosis and appropriate quick management are of utmost importance in reducing fatality due to severe fungal co infections in COVID 19 patients.

For diagnosis, specimens are usually obtained from nasal and paranasal sinuses and include nasal swabs, scrapings, or nasal crust biopsies. Swabs can directly be taken from the fungal lesions present externally on the skin of face (eyes, nose etc.).⁵ The usual diagnostic modalities used to examine these specimens include potassium hydroxide (KOH) mount, fungal culture and histopathology. While KOH has a rapid turnaround time, results from histopathology and culture are often delayed. Although KOH has good sensitivity, it may be supplemented with a diagnostic method which can examine these specimens and give fast and reliable results.⁶⁻⁸

Traditionally cytology has been used in aspirated materials for fungal diagnosis. Cytological examination of aspirates helps identify most fungi by their morphological characteristics. Zygomycetes including *Mucor* are characterized by wide (3–25 µm) ribbon like, irregular branching pauciseptate hyphae.⁹⁻¹¹ In the present study we examined the remnants of samples including nasal swabs/scrapings/biopsies after KOH test and fungal culture were done. We performed May Grunwald Giemsa (MGG) and Papanicolaou (PAP) staining on smears prepared from the crushed material. We also performed cytospin on the saline solution in which the sample was sent. Further, we did cytological examination on fresh samples from patients who were previously reported as negative for fungus on KOH wet mount (on an earlier sample) but were clinically suspected to have Mucormycosis. Here, we report our findings and discuss the utility of cytological examination using MGG and PAP staining for diagnosis as well as species identification.

2 | MATERIAL AND METHODS

2.1 | Study design

It was a prospective observational study with duration of 2 months conducted in May–June 2021, in the Department of Pathology and Department of Microbiology of our Institute. Consent and Institutional ethical clearance have been obtained.

2.1.1 | Participants and sample size

Total sample size was 48. All the patients were Covid 19 positive (39 were RT-PCR positive, 9 patients were diagnosed on clinical and radiological findings) and now presented with clinically suspected Mucormycosis. Our study included two kinds of samples

1. Remnants of nasal swabs/scrapings/biopsies of COVID 19 patients with clinically suspected orbital/sino-nasal Mucormycosis which were sent to Department of Microbiology for KOH test. Samples included

Those testing positive for fungus on KOH = 16.

Those testing negative for fungus on KOH = 16.

2. Repeat samples from patients who tested negative for fungus on KOH test done on an earlier sample, now sent to the Cytology Laboratory, Department of Pathology, for MGG and PAP staining = 16.

2.2 | Inclusion criteria

Hospital admitted COVID 19 patients with suspected Mucormycosis, of all ages and either sex, whose samples were sent to Department of Microbiology for KOH test, and to Department of Pathology for MGG and PAP staining were included.

2.3 | Exclusion criteria

Samples having scant material were excluded.

2.4 | Methodology

Remnants from samples sent to Department of Microbiology for KOH test along with samples sent directly to Department of Pathology for cytological examination, were taken.

For swabs, the material suspended in the saline was collected using a wire loop followed by spreading it on two separate glass slides in circular motion. The smears prepared were stained with MGG and PAP stain. A 3 mL of saline in which the swab was suspended was collected in a plain test tube and was cyto-centrifuged following which MGG and PAP was done on the smears prepared. Sample collection and smear preparation were done while taking all precautions to prevent cross contamination.

For scrapings and biopsies, a small piece was taken using a loop and kept on the slide and another slide was used to spread it with uniform pressure, following which the same procedure as above was repeated.

The same procedure was followed for slides which came directly from wards to the Cytology Laboratory, Department of Pathology.

After all the smears were stained, the slides were examined for presence of fungus using Dewinters Opticals Microscope at 4×, 10×, 20×, and 40× objectives. The observations were noted as positive or negative for fungus on MGG and/or PAP stain.

3 | RESULTS

Percentage positivity for *Mucor* in MGG and/or PAP stain in all three categories of samples were calculated. We compared results of KOH test with MGG and/or PAP stain (Figure 1).

KOH status of the samples was known and result on MGG and/or PAP stain was the variable under study.

FIGURE 1 Basic structure of study conducted

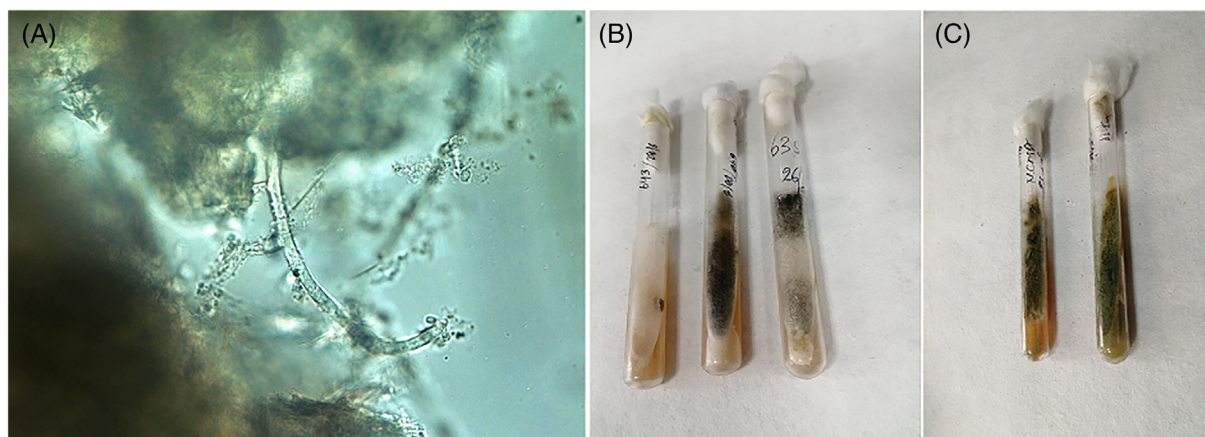
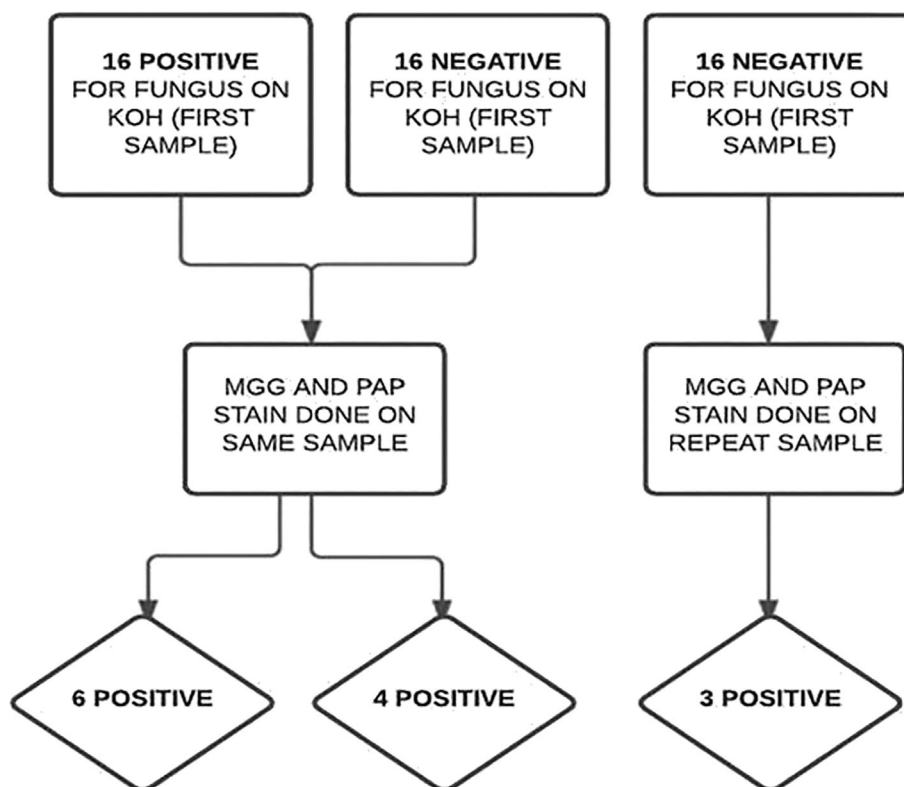


FIGURE 2 (A) KOH mount showing hyphae of Mucor. (B) Culture showing growth of Mucor. (C) Culture showing growth of Aspergillus [Color figure can be viewed at wileyonlinelibrary.com]

Out of 16 samples that were reported as positive for Mucor on KOH test (Figure 2A), 6 samples were positive for Mucor on MGG and PAP staining. Out of these six cases, two were mixed infections having both Mucor and Aspergillus (Figure 3). Four out of these six cases were culture positive (Figure 3B and C).

Out of 16 patients who were reported as negative for fungus on KOH test, 4 were positive on MGG and/or PAP stain. Out of these, one was found to have both Mucor and Aspergillus. Two out of these four samples were culture positive (Table 1).

Out of 16 repeat samples from patients who were earlier reported as negative for fungus on KOH test done on a previous

sample, 3 were positive for Mucor on MGG and/or PAP stain done on the repeat sample (Figure 4). Two out of these three were culture positive on the earlier sample on which KOH was done (Table 1).

4 | DISCUSSION

Coronavirus Disease 19 (COVID 19) caused by SARS CoV 2 has been sweeping across the globe at a rapid pace. A major cause of concern in patients with severe COVID 19 illness is susceptibility to opportunistic bacterial and fungal infections.¹² Increased susceptibility to

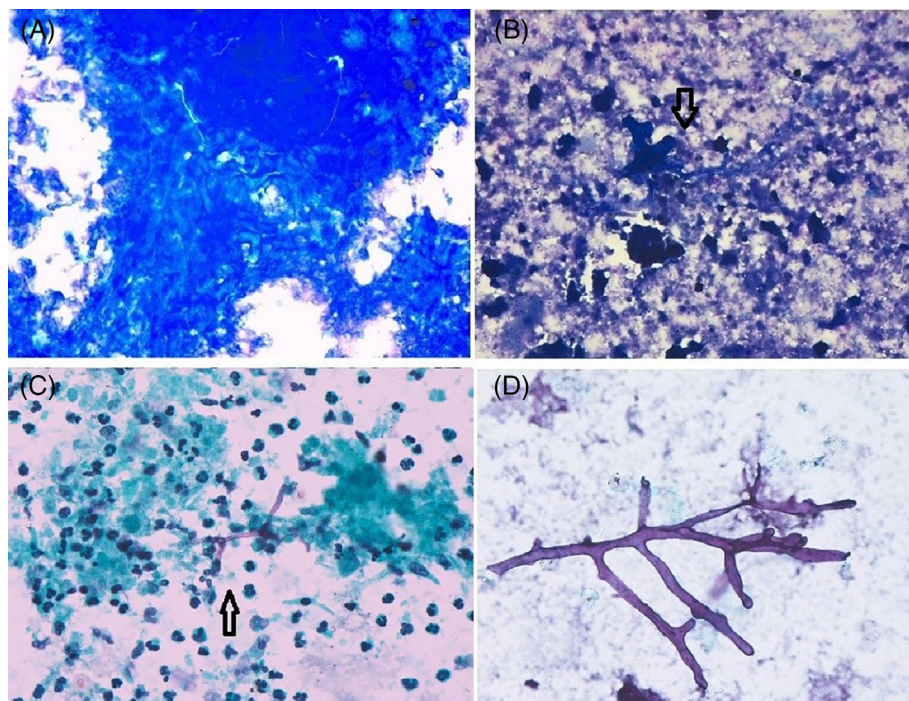


FIGURE 3 (A) Tangles of *Mucor* hyphae seen with intense background staining (MGG 400 \times). (B) *Mucor* hyphae present in necrotic background (MGG 400 \times). (C) Acute angle branching of *Aspergillus* surrounded by inflammatory cells (PAP 400 \times). (D) Thick, non septate hyphae of *Mucor* (PAP 400 \times) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Results of MGG and PAP staining and fungal culture

	Total samples	Positive on culture	Positive on MGG and/or PAP	% Positivity on MGG and/or PAP	Culture results of positive MGG and PAP samples
KOH positive samples	16	8	6	37.5%	4
KOH negative samples	16	7	4	25%	2
KOH negative on an earlier sample	16	5	3	18.75%	2

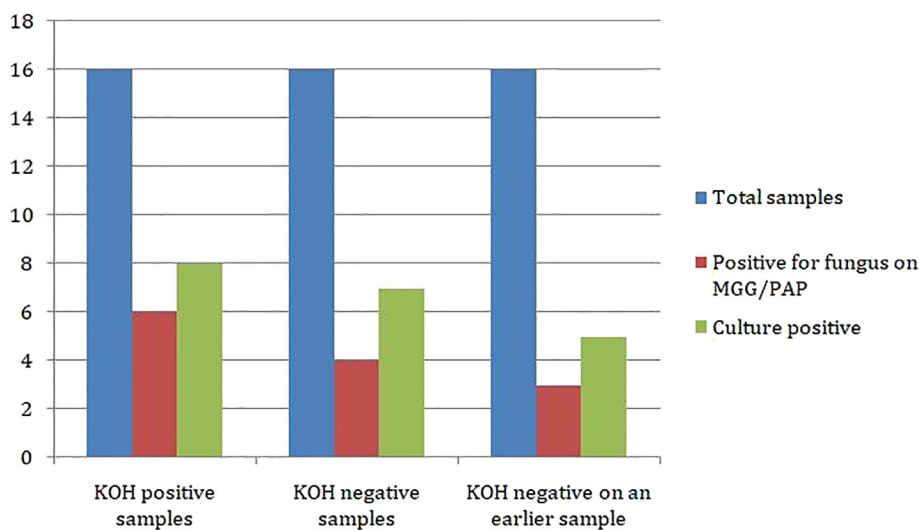


FIGURE 4 Bar graph showing total samples taken, number of samples positive for fungus on MGG/PAP staining and number of samples positive on culture [Color figure can be viewed at wileyonlinelibrary.com]

these infections is attributed to a rise in proinflammatory markers such as IL-1, IL-6, and TNF- α , decreased CD4 IFN- γ expression and reduced CD4+ and CD8+ T cells.¹³ The common opportunistic fungal infections encountered in COVID patients include Mucormycosis, Pulmonary Aspergillosis, Oropharyngeal Candidiasis, Pneumocystis

jiroveci pneumonia, Cryptococcosis, Histoplasmosis, and so on, with Mucormycosis being the commonest.^{2,3} Mucormycosis is an uncommon, acute, fulminating fungal infection caused by filamentous fungi of the family Mucoraceae (Class: Zygomycetes, Order: Mucorales).^{14,15} Factors that predispose a COVID 19 patient to

develop this fungal infection include poorly controlled diabetes, prolonged (>3 weeks) use of high dose systemic corticosteroids, zinc supplementation, longer ICU stay with oxygen support or mechanical ventilation and conditions like malignancy, organ transplantation, and so on.^{14,16}

Primary site of inoculation of the fungal spores is nose and paranasal sinuses. The clinical presentation can be rhino orbital, cerebral, pulmonary, cutaneous, gastrointestinal, or disseminated, based on the organs involved.^{14,17} Patients commonly present with unilateral facial swelling, headache, fever, visual impairment, nasal congestion and black lesions over nasal bridge and inside mouth, and so on.¹⁸ In clinical settings, fungal co-infections in COVID 19 are often neglected and thus the diagnosis of Mucormycosis is delayed.¹⁹ Fungal hyphae grow to invade the blood vessels and result in tissue infarction which leads to tissue necrosis and vessel thrombosis.⁵ Early diagnosis is important as the lesions may be rapidly progressive and destructive and may even turn out to be fatal.^{19,20}

Morphologically, under light microscopy Mucorales are identified as thin walled, ribbon like, irregularly branching, pauciseptate hyphae measuring 10–20 μm in width.¹⁷

Various techniques have been employed for diagnosing Mucorales. Direct microscopy of KOH wet mounts can be used for a quick presumptive diagnosis of Mucormycosis. This method is simple to perform, quick, inexpensive, minimally invasive and carries a good sensitivity.²¹ A definitive diagnosis is based on demonstration of fungal hyphae in biopsies from affected tissues.²² Histopathology as a diagnostic tool helps to differentiate between the presence of fungus as a pathogen and a culture contaminant.²³ Histopathology with Grocott–Gomori Methenamine Silver (GMS) stain, Periodic Acid-Schiff (PAS) can help highlight the fungal wall.^{9,24}

Mucorales can also be cultured on Sabouraud Dextrose, brain heart infusion or potato dextrose agar at 25–30°C.⁹ However, the utility of fungal culture as a method for diagnosis is limited as it does not conclusively indicate infection with the organism identified.⁹ It is relatively expensive and takes at least 3 weeks to give reliable results.²¹ Reported sensitivity of fungal culture (57%) is lower than that of KOH mount while the specificity is comparable (92.8%).¹⁵

Traditionally, nasal swab specimens have been tested by KOH wet mount for quick diagnosis of fungal infections.¹⁰ However with the advent of fungal infections post COVID there is a need to develop diagnostic modalities that can work in conjunction with KOH wet mount and increase overall diagnostic sensitivity and specificity. Delay in diagnosis may postpone treatment which may be fatal.^{11,13} In a study by Singhal et al. accuracy of fungal identification on microscopy in cytological specimens was reported to be 79%.¹¹

Our study highlights the utility of using MGG and PAP staining as an adjunct to the KOH test for fungal detection. MGG and PAP staining showed positive results in 37.5% of cases which were reported as positive for Mucor on KOH test. They showed broad, ribbon like fungal hyphae which were aseptate and showed right angled branching. This low rate of detection on MGG and PAP staining may be explained by the fact that representative samples were already used for KOH test and fungal culture. While all the samples were

reported to be positive for Mucor on KOH mount, on stained cytology smears we found two cases to have mixed infection i.e. in addition to the broad aseptate hyphae with right angled branching we found hyphae which were narrow, septate and showed acute angled branching. These were consistent with *Aspergillus*. This highlights that morphology is better appreciated on stained smears. While the organisms were identified both on MGG and PAP stains, the morphology was easily identifiable on PAP stained smears since the MGG stained smears showed intense background staining. The smears examined were cellular with some cases showing brisk neutrophilic infiltrate intermixed with ciliated columnar cells, background necrosis, and other chronic inflammatory cells. In most cases which were positive for fungus, the hyphae were lying in close association with the necrosis and neutrophilic infiltrate. However no eosinophils were identified in the smears examined. The morphology of Mucor as well as *Aspergillus* seen in our cases was consistent with that described on smears prepared from fungal lesions. Classically, *Aspergillus* has been identified by the presence of slender, septate hyphae which branch at acute angles.^{11,17} In contrast to this, Mucor has broad, aseptate hyphae that branch at right angles.²⁴

MGG and PAP stains showed a positivity rate of 25% among the samples that were reported as negative on KOH test. One of the samples showed a mixed infection with both Mucor and *Aspergillus*. This highlights the fact that if we use MGG and PAP stained smears in addition to KOH test, detection rate will increase drastically and results can be dispatched on the same day. This will lead to prompt treatment and decreased fatality in Mucormycosis patients.

Further, detection of Mucor in 3 out of 16 repeat samples from patients previously reported as negative, highlights the need for a repeat test after an initial KOH negative test. The sensitivity and specificity of KOH mount have been studied in various fungal infections and have been reported to be 64.5% and 72.5%, respectively, for oral Candidiasis,⁶ 81.8% and 92.8%, respectively, for Onychomycosis⁷ and 71% and 32%, respectively, for Dermatophytoses.⁸ Though the method can give immediate results with minimal infrastructure requirement, it fails to identify the fungus to its genus or species level.²² The samples which are reported as KOH negative are next used for a fungal culture test using culture media like Sabouraud Dextrose Agar at 25–30°C. Results for the fungal culture test take a longer period which is at least 3 weeks²¹ thus delaying quick patient management. Using the two methods (KOH test and MGG and PAP staining) in conjunction can overcome this delay and allow for quicker detection with a higher sensitivity. In addition to the increased sensitivity, MGG and PAP testing proves to be more specific as it also allows for morphological examination, thus making species identification easier.

The goal of treating fungal co infections in COVID 19 includes treating the COVID 19 infection, underlying co-morbid illnesses, and controlling the blood sugar levels. The European Conference on Infections in Leukemia (ECIL-6) recommends treating mucormycosis with a combination of lipid amphotericin B and caspofungin, or lipid amphotericin B and posaconazole. For invasive aspergillosis, ECIL-6 recommends voriconazole, and isavuconazole.²⁵ Thus, species

identification of the infective fungal organism is equally important so that appropriate treatment can be initiated, failing which the management might be insufficient. Early surgical debridement must be planned when the systemic illness is stabilized and when facilities for postsurgical care are available.²⁶

5 | CONCLUSION

With the advent of COVID 19 the occurrence of Mucormycosis has increased manifold. There is an urgent requirement of rapid and cheap diagnostic modalities as delayed diagnosis may lead to increased mortality. Traditionally stained cytology smears have not been used for evaluation of nasal swabs, nasal crust scrapings, or nasal crust biopsies. In this study we have evaluated the role of stained cytology smears on remnants of samples which were sent for KOH and culture for the detection of mucormycosis. Based on our results we conclude that stained cytology smears cannot replace KOH test but if used in conjunction, will definitely increase the overall sensitivity and specificity (species identification) of diagnosis on the same specimen.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Ashish C. Philip  <https://orcid.org/0000-0003-1077-2576>

REFERENCES

- COVID Live Update. 200,264,356 cases and 4,258,693 deaths from the coronavirus: Worldometer, Worldometersinfo; 2021. <https://www.worldometers.info/coronavirus/>
- Salehi M, Ahmadikia K, Badali H, Khodavaisy S. Opportunistic fungal infections in the epidemic area of COVID-19: a clinical and diagnostic perspective from Iran. *Mycopathologia*. 2020;185(4):607-611.
- Cafardi J, Haas D, Lamarre T, Feinberg J. Opportunistic fungal infection associated with COVID-19. *Open forum Infect Dis*. 2021;8:8-10.
- Raut A, Huy NT. Rising incidence of mucormycosis in patients with COVID-19: another challenge for India amidst the second wave? *Lancet Respir Med*. 2021;9(8):e77.
- Frater JL, Hall GS, Procop GW. Histologic features of zygomycosis: emphasis on perineural invasion and fungal morphology. *Arch Pathol Lab Med*. 2001;125(3):375-378.
- Hu L, Zhou P, Zhao W, Hua H, Yan Z. Fluorescence staining vs. routine KOH smear for rapid diagnosis of oral candidiasis—a diagnostic test. *Oral Dis*. 2020;26(5):941-947.
- Begari V, Pathakumari P, Takalkar AA. Comparative evaluation of KOH mount, fungal culture and PAS staining in onychomycosis. *Int J Res Dermatol*. 2019;5(3):554-558.
- Baddireddy K, Poojary S. A novel contrast stain for the rapid diagnosis of Dermatophytoses: a cross-sectional comparative study of Chicago sky blue 6B stain, potassium hydroxide mount and culture. *Indian J Dermatol*. 2019;64(5):420.
- Powers CN. Diagnosis of infectious diseases: a cytopathologist's perspective. *Clin Microbiol Rev*. 1998;11(2):341-365.
- Jiang X, Yang T, Li Q, et al. Liquid-based cytopathology test: a novel method for diagnosing pulmonary mucormycosis in bronchial brushing samples. *Front Microbiol*. 2018;9:1-8.
- Singhal N, Raghubanshi G, Handa U, Punia RPS, Singhal S. Fine needle aspiration cytology: a useful technique for diagnosis of invasive fungal rhinosinusitis. *Head Neck Pathol*. 2013;7(3):236-240.
- John TM, Jacob CN, Kontoyiannis DP. When uncontrolled diabetes mellitus and severe covid-19 converge: the perfect storm for mucormycosis. *J Fungi*. 2021;7(4):298.
- Bhatt K, Agolli AH, Patel M, et al. High mortality co-infections of COVID-19 patients: mucormycosis and other fungal infections. *Discoveries*. 2021;9(1):e126.
- Gupta S, Goyal R, Kaore NM. Rhino-orbital-cerebral Mucormycosis: Battle with the deadly enemy. *Indian J Otolaryngol Head Neck Surg*. 2020;72(1):104-111.
- Chandra S, Rawal R. The surge in Covid related mucormycosis. *J Infect*. 2021;S0163-4453(21):288-287.
- Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8(5):475-481.
- Greene J, Pak J, Tucci V, Vincent A, Sandin R. Mucormycosis in immunochallenged patients. *J Emerg Trauma Shock*. 2008;1(2):106-113.
- Kameshwaran S, Sriram N, Darla R, Manimekalai P, Dhanalakshmi M. Symptoms and treatment strategy of black fungus in Covid-19 patients. *Int J Pharmacol Clin Res*. 2021;5(2):24-27.
- Hoeningl M. Invasive fungal disease complicating COVID-19: when it rains, it spores. *Clin Infect Dis*. 2020;92103:1-4.
- Prabhu RM, Patel R. Mucormycosis and entomophthoromycosis: a review of the clinical manifestations, diagnosis and treatment. *Clin Microbiol Infect*. 2004;10(1):31-47.
- Gautam M, Bhatia S. Mount the menace: potassium hydroxide in superficial fungal infections. *Indian J Paediatr Dermatol*. 2020;21(4):343-346.
- Skiada A, Pavleas I, Drogari-Apiranthitou M. Epidemiology and diagnosis of Mucormycosis: an update. *J Fungi*. 2020;6(4):265.
- Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev*. 2011;24(2):247-280.
- Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev*. 2000;13(2):236-301.
- Tissot F, Agrawal S, Pagano L, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica*. 2017;102(3):433-444.
- Mucormycosis in COVID-19 | AIIMS Covid Information Portal. Covidaiimsedu; 2021. <https://covid.aiims.edu/mucormycosis-incovid-19/>

How to cite this article: Philip AC, Madan P, Sharma S, Das S. Utility of MGG and Papanicolaou stained smears in the detection of Mucormycosis in nasal swab/scraping/biopsy samples of COVID 19 patients. *Diagnostic Cytopathology*. 2022;50(3):93-98. doi:10.1002/dc.24924