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Sensitivity of liquid-based cytology in the diagnosis of mucormycosis in COVID-19 treated patients

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Abstract

Background: The coronavirus disease 2019 (COVID-19) infection caused by the novel severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is associated with a wide range of disease patterns, ranging from mild to life-threatening pneumonia. COVID-19 can be associated with a suppressed immune response and/or hyperinflammatory state due to a cytokine storm. Reduced immunity, combined with steroid usage to prevent a cytokine storm along with various pre-existing comorbidities can prove to be fertile ground for various secondary bacterial and fungal infection, including mucormycosis. Diagnosis of *Mucor* is a challenging task given the high negativity rate of various detection methods. While histopathology is considered the gold standard, the acquisition of necessary tissue biopsy specimens requires invasive procedures and is time consuming.

Method: In this study five different methods of *Mucor* detection, namely conventional cytopathology, liquid-based cytology (LBC, BD SurePath™), potassium hydroxide (KOH) preparation, culture, and histopathology were analysed. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for all five methods.

Results: LBC had values for sensitivity, specificity, PPV, and NPV of 72.4%, 100%, 100%, and 38.4%, respectively, closely matching histopathology in sensitivity (75.9%). The sensitivity of culture, conventional cytopathology, and KOH were very low compared to histopathology and LBC.

Conclusion: This study showed that LBC, can be a rapid and effective alternative to histopathology in *Mucor* diagnosis.

KEYWORDS
COVID-19, liquid-based cytology, Mucormycosis

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) infection caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) may be associated with a wide range of disease patterns, ranging from mild to life-threatening pneumonia.[1] This happens due to uncontrolled viral replication and an explosive immune response from the host. In the presence of uncontrolled viral replication, the presence of an increased number of infected epithelial cells and cell debris leads to a massive cytokine release (cytokine storm)

characterised by hyperinflammation and immune suppression with increased Th[17] and cytotoxic cell activity and reduced T helper cell activity. Important inflammatory mediators involved are IL-6, IL-1 and TNF alpha.[2,3,4] Studies have shown that any intervention which can prevent this catastrophe can also prevent the lung damage and pulmonary thromboembolism.[3,4] It is with this pathophysiology in mind that intervention with corticosteroids has been undertaken in COVID-19, which in turn increases the risk for secondary infections.[5] Besides steroid intake, the immune dysregulation associated with COVID-19 can also lead to a wide range of bacterial and fungal infections, notably mucormycosis.[1] In addition, poorly controlled diabetes mellitus (DM) and other comorbidities are risk factors for both severe COVID-19 and mucormycosis.[6] The most common comorbidities associated with mucormycosis are renal diseases, liver diseases, haematological disorders, cancer, organ transplant, and intensive care unit admission.[7] An explosive increase in mucormycosis cases in COVID-19 patients leads to the assumption that the central line of oxygen supply, with humidifiers and a possible iatrogenic seepage with any localised fungal growth, could be one of the risk factors. Other iatrogenic risk factors could be contaminated medical devices and dressings.[8]

Mucormycosis is an angioinvasive fungal infection due to fungi of the order Mucorales. Currently, Mucorales fungi are the next most common fungal pathogens after *Aspergillus* leading to invasive fungal disease in patients with malignancy or transplantation.[9]

The most common routes of fungal infection are inhalation, ingestion, or direct inoculation of wounds by sporangiospores.[8] *Mucor* infection generally occurs around 15 days after being diagnosed with COVID-19. Angioinvasion seen in advanced stages of mucormycosis can lead to spread of infection especially to the brain.[7] Fungal cells require iron for angioinvasion, which is generally bound to iron binding proteins; acidosis results in dissociation of iron from the sequestering protein and thus promotes angioinvasion.[8]

The molecular mechanism of mucormycosis is an interaction between fungal CotH3 (homologue of bacterial spore coat protein) protein and mammalian nasal glucose-regulated protein 78 (GRP78). High glucose, iron, and ketone body levels seen in various comorbidities leads to increased expression of both CotH3 and GRP78, promoting mucormycosis.[7,10]

Mucormycosis has been a common fungal infection in India in the past, with numbers of cases being almost 70 times higher than in developed countries. The disease prevalence in India is around 140 cases per million population.[11,12] Before the COVID-19 pandemic, mortality due to the mucormycosis was 50%, which increased to 85% during the current pandemic.[13]

The main reasons for this sudden jump in mortality were crowded hospitals, a scarcity of healthcare resources, overburdened healthcare workers, and poor diagnostic quality.[13] A twofold increase in mucormycosis cases was reported by a study in 2020 as compared the previous year. The prevalence of COVID-associated mucormycosis among hospitalised COVID-19 patients was reported as 0.27%.[14]

Diagnosis of mucormycosis is based on clinical suspicion, direct smear, histopathology, and culture. Newer methods of diagnosis include various polymerase chain reaction-based techniques. Direct microscopy can be used for a rapid presumptive diagnosis of mucormycosis. Culture of specimens is essential for the diagnosis of mucormycosis since it allows identification of the genus and species, and eventually antifungal susceptibility testing. Nevertheless, there are challenges in establishing a clinical diagnosis of mucormycosis due to the difficulty in obtaining a positive culture in some cases and the fact that tissue biopsy for histopathology is an invasive procedure not suitable for some cases. Cytopathology is receiving increased attention in the examination of fungal diseases because of its rapidity, accuracy, and minimal invasiveness.[15] However, conventional cytology has poor sensitivity owing to various artefacts caused by air drying, and the presence of proteins, mucous, inflammation, haemorrhage, and necrosis.[16]

Liquid-based cytology (LBC), developed in 1991, improves the quality of samples and effectiveness of cytopathological tests.[17] With the advantages of standardised and automated preparation, it has reduced the unsatisfactory rate and improved specimen adequacy and the ability to perform ancillary tests with residual specimen.[18] Accordingly, it is more sensitive, specific, and cost-effective as compared to conventional cytopathology.[19] Recently, LBC has been utilised for the diagnosis of pulmonary aspergillosis.[20] The present study attempts to evaluate the applicability of LBC to the quick and accurate diagnosis of mucormycosis as compared to other direct microscopy methods such as potassium hydroxide (KOH) examination, conventional smears, and histopathology (Table 1).

The objective of this study was to evaluate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of various available diagnostic modalities for mucormycosis detection.

2 | MATERIALS AND METHODS

A prospective study was conducted in the pathology and microbiology department of a COVID-dedicated tertiary centre between April 2021 to July 2021. Without compromising on safety protocols in place during the COVID crisis, the sample was taken to include as many cases as possible for which results were available for all five diagnostic modalities examined in the study (ie, histopathology, conventional cytopathology, LBC, KOH preparation, and culture). Patients who had received antifungal therapy were excluded from the study. A total of 34 COVID-19 treated patients suspected of having mucormycosis, whose samples were sent to the pathology and microbiology departments during April to July 2021, were included in the study. Detailed histories were taken, and physical examinations were noted (Table 1). Out of 34 patients, 31 (91.2%) had received steroid therapy for moderate to severe disease.

In the present study, a special cytobrush (BD SurePath™) was used to collect samples from the hard/soft palate, lateral nasal wall, middle/inferior turbinate, and orbital apex (post exenteration). Smears were prepared on two glass slides for each patient, which were allowed to air dry and wet fix, respectively. All of the samples

TABLE 1 Comparison of various diagnostic modalities for *Mucor* detection

Sl. No	Age/Sex	Co-morbidity	Site	Size of biopsy (cm)	LBC	Histopathology	Conventional cytology	Culture	KOH	Final
1	43/m	Diabetes	Lateral nasal wall	1×0.8 ×0.8	Neg	Neg	Neg	Neg	Neg	Neg
2	50/f	Diabetes	Inferior turbinate	0.8×0.6 ×0.6	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	Neg	*Mucor*	Neg	Pos
3	50/f	Diabetes	Orbital apex	2.8×2.6×2.6	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	*Mucor*	*Mucor*	*Mucor*	Pos
4	50/m	Diabetes	Middle turbinate	1×1×0.8	*Mucor*	*Mucor*	*Mucor*	*Mucor*	*Mucor*	Pos
5	76/f	Diabetes	Inferior turbinate	1.4×1.2×1	*Mucor*	*Mucor*	Neg	Neg	Neg	Pos
6	56/m	Leukaemia	Middle turbinate	1.2×1.2×1	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	Neg	Neg	Neg	Pos
7	51/m	Diabetes	Inferior turbinate	1.8×1.5×1.4	*Mucor*	*Mucor*	Neg	*Mucor*	Neg	Pos
8	54/m	Diabetes	Inferior turbinate	1.2×1.2×1	*Mucor*	*Mucor*	Neg	*Mucor*	Neg	Pos
9	63/m	Diabetes	Middle turbinate	2×1.8×1.8	*Mucor*	*Mucor*	Neg	Neg	Neg	Pos
10	52/m	Solid tumour	Middle turbinate	1×1×0.8	*Mucor*	*Mucor*	Neg	*Mucor*	Neg	Pos
11	75/f	Diabetes	Middle turbinate	1.3×1.2×1.2	Neg	Neg	Neg	Neg	Neg	Neg
12	47/m	Diabetes	Middle turbinate	2.2×1.8×1.8	*Mucor*	*Mucor*	Neg	*Mucor*	Neg	Pos
13	38/m	Diabetes	Inferior turbinate	2.2×2×1.8	Neg	Neg	*Mucor*	*Mucor*	*Mucor*	Pos (KOH, cul, conv)
14	43/m	Solid tumour	Inferior turbinate	2×2×2	*Mucor*	Neg	Neg	Neg	*Mucor*	Pos (LBC, KOH)
15	56/f	Diabetes	Inferior turbinate	1.2×1×1	*Mucor*	*Mucor*	*Mucor*	*Mucor*	*Mucor*	Pos
16	56/f	Tuberculosis	Palate	1.6×1.5×1.2	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	Pos
17	52/f	Diabetes	Inferior turbinate	1.8×1.6×1.5	Neg	*Mucor*	Neg	Neg	Neg	Pos
18	45/f	Diabetes	Middle turbinate	1.5×1.5×1.2	*Mucor*	*Mucor*	Neg	Neg	Neg	Pos
19	54/f	Diabetes	Middle turbinate	1.8×1.8×1.2	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	Neg	*Mucor*	Neg	Pos
20	63/m	Solid tumour	Middle turbinate	2.5×2×2	*Mucor* + *Candida*	*Mucor* + *Candida*	*Mucor*	*Mucor*	Neg	Pos
21	58/f	Solid tumour	Lateral nasal wall	1.8×1.4×1.4	Neg	Neg	Neg	Neg	Neg	Neg
22	46/m	Diabetes	Inferior turbinate	2.4×2×2	Neg	Neg	Neg	Neg	Neg	Neg
23	50/m	Leukaemia	Middle turbinate	1.8×1.6×1.6	*Mucor*	*Mucor*	*Mucor*	*Mucor*	Neg	Pos
24	34/m	Diabetes	Middle turbinate	1.6×1.6×1.5	Neg	*Mucor*	Neg	Neg	Neg	Pos
25	45/m	Diabetes	Inferior turbinate	1.5×1.5×1.4	Neg	Neg	Neg	Neg	Neg	Neg
26	43/m	Diabetes	Middle turbinate	1.4×1.2×1.2	Neg	Neg	Neg	*Mucor* + *Candida*	Neg	Pos (cul)
27	47/f	Diabetes	Inferior turbinate	0.8×0.8×0.8	*Mucor*	*Mucor*	*Mucor*	*Mucor* + *Aspergillus*	*Mucor* + *Aspergillus*	Pos

TABLE 1 (Continued)

Sl. No	Age/Sex	Co-morbidity	Site	Size of biopsy (cm)	LBC	Histopathology	Conventional cytology	Culture	KOH	Final
28	56/m	Solid tumour	Lateral nasal wall	1×0.8×0.8	Neg	Neg	*Mucor*	Neg	Neg	Pos (conv)
29	47/f	Tuberculosis	Middle turbinate	1.3×1.3×0.7	*Mucor*	*Mucor*	*Mucor*	*Mucor*	Neg	Pos
30	53/f	Diabetes	Orbitapex	3×2.8×2.8	*Mucor*	*Mucor*	*Mucor*	Negative	Negative	Pos
31	42/m	Diabetes	Middle turbinate	1.7×1.5×1.2	Neg	Neg	Neg	Neg	*Mucor*	Pos (KOH)
32	45/m	Leukaemia	Inferior turbinate	1.5×1.4×1.4	*Mucor*	*Mucor*	*Mucor*	*Mucor* + *Candida*	Neg	Pos
33	37/f	Diabetes	Middle turbinate	1.9×1.8×1.8	Neg	Neg	*Mucor*	Neg	Neg	Pos(con)
34	65/m	Diabetes	Palate	1.6×1.5×1.5	Neg	Neg	Neg	Neg	*Mucor*	Pos (KOH)

Abbreviations: conv, conventional cytology; cul, culture; KOH, potassium hydroxide preparation; LBC, liquid-based cytology; Sl.No, serial number.

were taken by one investigator (senior resident doctor) following a standard guideline for all cases. A separate brush was used for each patient. A fresh cytobrush was inserted into the various cavities and rotated to cover the maximum surface area. After making smears, the tip of the brush was detached into the BD CytoRich™ vial for LBC. Later, a biopsy was taken from an appropriate site, and part of the tissue sample was sent in sterile containers for KOH preparation and culture, while the rest of the tissue was sent in 10% buffered formalin container for histopathology.

Dry and wet fixed slides received in the cytology laboratory were stained by Giemsa and Papanicolaou (PAP) stains respectively, while LBC samples were processed using the BD Totalys™ SlidePrep slide processor (Burlington, NC, United States) and slides were prepared. Tissue samples received in the histopathology laboratory were routinely processed, formalin fixed, and paraffin embedded. Blocks were made, sections were taken on glass slides and stained with haematoxylin and eosin stain. Silver methenamine and periodic acid-Schiff staining was done wherever diagnosis was doubtful. The samples received in the mycology lab were subjected to direct microscopy by KOH mount to look for fungal hyphae. All the samples were simultaneously cultured on the fungal culture media (sabouraud dextrose agar with antibiotics) and were incubated at 37 °C and 25 °C. The growth on culture media was identified by lactophenol cotton blue mount.

All the conventional cytology and LBC slides were scanned by two independent cytopathologists. Findings of the histopathology slides, KOH preparation, and culture were not available to the cytopathologists until the time of data analysis, to avoid any bias.

On LBC, *Mucor* hyphae are broad, non-septate filaments with right angle branching, while *Aspergillus* appears as thin, septate filaments with acute angle branching, and *Candida* appears as yeasts and pseudo hyphae, with background showing desquamated epithelial cell (pseudo stratified ciliated and squamous) neutrophils, lymphocytes, macrophages, and necrotic material. These background features are more prominent on the conventional smears. On histopathology slides, *Mucor* hyphae are seen as broad, non-septate filaments with right angle branching, while *Aspergillus* appears as thin, septate filaments with acute angle branching, and *Candida* appears as yeasts and pseudo hyphae, with surrounding tissue showing areas of necrosis and inflammatory infiltrate.

Sensitivity, specificity, PPV, and NPV were calculated for the various modalities (Table 2). Statistical analysis was done by Fisher's exact test using SPSS v.26 software. Ethical approval was obtained from the ethics committee of Maulana Azad Medical College, approval number F.1/IEC/MAMC/ (84/02/2021/No 396), dated 9 June 2021.

3 | RESULTS

Samples from a total of 34 patients were evaluated. The comorbidities present were DM in 70.1% (24/34) of the cases, solid tumour

		Final diagnosis			*P* value (Fisher's exact test)
		Positive	Negative	Total	
Histopathology	Positive	22 (100%)	0	22	0.003
	Negative	7 (58.3%)	5 (41.7%)	12	
LBC	Positive	21 (100%)	0	21	0.005
	Negative	8 (61.5%)	5 (38.5%)	13	
Culture	Positive	17 (100%)	0	17	0.103
	Negative	12 (70.6%)	5 (29.4%)	17	
Conventional cytology	Positive	13 (100%)	0	13	0.132
	Negative	16 (76.2%)	5 (23.8%)	21	
KOH	Positive	9 (100%)	0	9	0.293
	Negative	20 (80%)	5 (20%)	25	

Abbreviations: KOH, potassium hydroxide preparation; LBC, liquid-based cytology.

TABLE 2 Summary of various diagnostic modalities compared to the final diagnosis

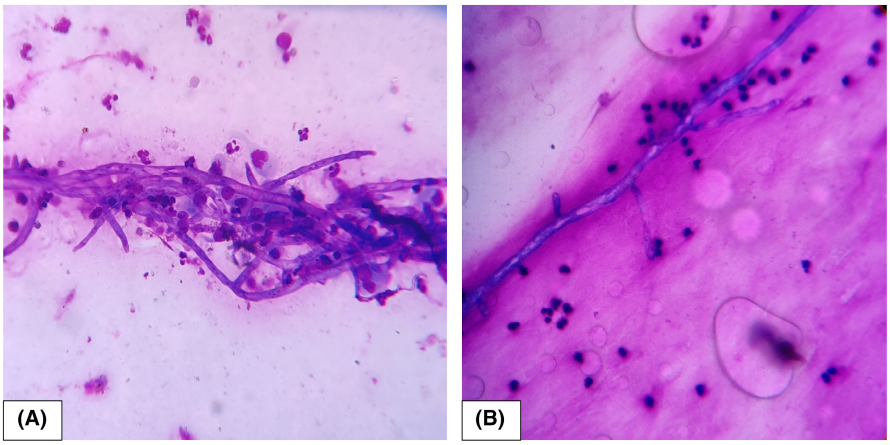

FIGURE 1 Mucor hyphae on conventional smear with inflammatory cells in the background (Giemsa, 400×)

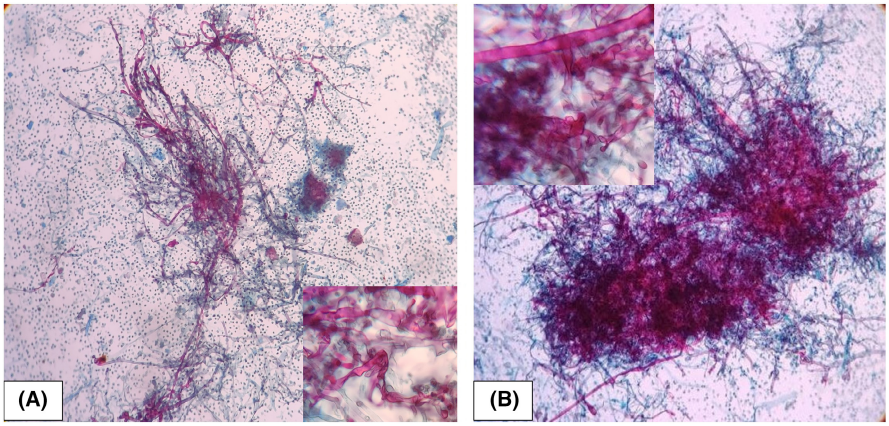

FIGURE 2 (A) *Mucor* hyphae on BD SurePath ™ LBC smear (PAP, 100×; inset: PAP, 400×). (B) *Mucor* and *Aspergillus* hyphae in a case of mixed infection on BD SurePath™ LBC smear (PAP, 100×; inset: PAP, 400×)

in 14.7% (5/34), leukaemia in 8.8% (3/34), and tuberculosis in 5.9% (2/34). Ages of the patients ranged from 34 to 76 years with a median of 50 years, and the M:F ratio was 20:14. The most common site for sampling was middle turbinate in 44.1% (15/34) of the patients, followed by inferior turbinate in 35.3% (12/34), lateral nasal wall in 8.8% (3/34), while the palate and orbital apex were each sampled in 5.9% (2/34). Biopsy size ranged from small biopsies of 0.8×0.6×0.6 cm to an eyeball measuring 3×2.8×2.8 cm. Clinical details and complete case profiles are described in Table 1.

In this study, out of 34 clinically suspected cases, 85.3% (29/34) of the patients had documented mucormycosis or mixed fungal infections. Out of these 29 patients, 75.9% (22/29) had a positive histopathology report. In the remaining 24.1% (7/29) of the patients, diagnosis was established by other diagnostic methods. Two cases (6.9%) each were detected on conventional cytology and KOH, one case (3.4%) was detected on culture, one case (3.4%) was positive for *Mucor* on conventional cytology, culture, and KOH, while one case was positive on LBC and KOH (Table 1).

All five patients who did not have *Mucor* had mild COVID-19 and recovered within 10 days as compared to cases with *Mucor*, who needed longer hospital stays (>20 days). These patients with concomitant COVID and mucor had bad prognosis, with 41.4% (12/29) of the patients succumbing to their illness.

Conventional cytopathology showed that 13/34 (38.2%) cases were positive for fungal infection with only *Mucor* in 12 cases and *Mucor* plus *Aspergillus* co-infection in one case. Background features like inflammatory infiltrate, necrosis, protein/mucus were prominent in conventional cytology smears (Figure 1).

LBC showed that 21/34 (61.8%) cases were positive for fungal infection with only *Mucor* in 15 cases, *Mucor* plus *Aspergillus* co-infection in 5 cases, and *Mucor* plus *Candida* co-infection in one case. Based on LBC results we reviewed the conventional cytology smears but there was no change in the diagnosis (Figure 2).

Nine out of 34 (26.5%) KOH specimens showed positive result for fungus with only *Mucor* in 7 cases and *Mucor* plus *Aspergillus* co-infection in 2 cases (Figure 3A). Seventeen out of 34 (50%) culture samples showed fungus with only *Mucor* in 13 cases, while *Mucor* plus *Aspergillus* co-infection was seen in two cases, and *Mucor* plus *Candida* co-infection was seen in two cases (Figure 3B).

On histopathology, 22 of 34 (64.7%) patients were positive for fungal infection with only *Mucor* in 16 cases, *Mucor* plus *Aspergillus* co-infection in 5 cases, and *Mucor* plus *Candida* co-infection in one case (Figure 4). These findings are summarised in Table 2.

Histopathology showed sensitivity, specificity, PPV, and NPV of 75.9%, 100%, 100%, and 41.7%, respectively. LBC showed sensitivity, specificity, PPV, and NPV of 72.4%, 100%, 100%, and 38.4%, respectively. Culture showed sensitivity, specificity, PPV, and NPV of 58.6%, 100%, 100%, and 29.4%, respectively. Conventional cytopathology showed sensitivity, specificity, PPV, and NPV of 44.8%, 100%, 100%, and 23.8%, respectively, and KOH showed sensitivity, specificity, PPV, and NPV of 31%, 100%, 100%, and 20%, respectively, as summarised in Table 3.

The microscopic fields in LBC slides were generally clearer than conventional cytopathology slides, showing less necrosis, mucus, inflammatory cells, and blood. But background features were better visualised on histopathology slides and conventional cytopathology smears as compared to LBC slides.

4 | DISCUSSION

COVID-19 is associated with a wide range of disease patterns, ranging from mild to life-threatening pneumonias.[1] Increased risk of *Mucor* is seen due to virus-induced immune suppression, cytokine storm, steroid use, and immunosuppressed states such as DM.[1,6] As the literature on COVID-19 continues to increase, there have been many studies on *Mucor* infection in COVID-19 patients worldwide with greatest number of cases being from India.[21] India contributed to approximately 71% of the global cases of mucormycosis in patients with COVID-19 based on published literature from December 2019 to the start of April 2021.[22] Most of these studies diagnosed *Mucor* on histopathology and/or culture.

Singh et al analysed 101 cases of mucormycosis in people with COVID-19 reported by different authors all over the world.[21] Eighty-two of these cases were reported from India[1,6,23–31] (Table 4). Mucormycosis was seen mainly in males (78.9%). The most common risk factor was DM, seen in 80% of cases. Corticosteroid therapy was used in 76.3% of cases. Nose and sinuses (88.9%) were the most common site followed by rhino-orbital (56.7%).[21]

Mucormycosis was first described by Fürbinger in a patient who died of cancer and in whom the right lung showed a haemorrhagic infarct with fungal hyphae and a few sporangia. In 1885, Arnold Paltauf published the first case of disseminated mucormycosis, which he named "Mycosis mucorina."[32] The gold standard for mucormycosis diagnosis is histopathology followed by culturing, both of which are time-consuming, and culture has a high false negativity rate, and thus these methods are not suitable for rapid diagnosis of mucormycosis. Histological examination of biopsied tissue is the preferred diagnostic method but is variably invasive. Patients with mucormycosis require early and accurate diagnosis to receive timely and optimal antifungal treatment.[33] If treatment is not initiated promptly, Mucorales species may cause acute and highly invasive disease in predisposed patients and prove to be fatal.[34] Cytology plays an important role, including conventional and LBC preparation, but detecting Mucorales in conventional cytopathology smears is challenging due to overpowering background features.[35]

In the present study, conventional cytology, KOH preparation, LBC, culture, and biopsy of 34 patients admitted to our hospital for COVID-19 or COVI- related disease were analysed. Patients had

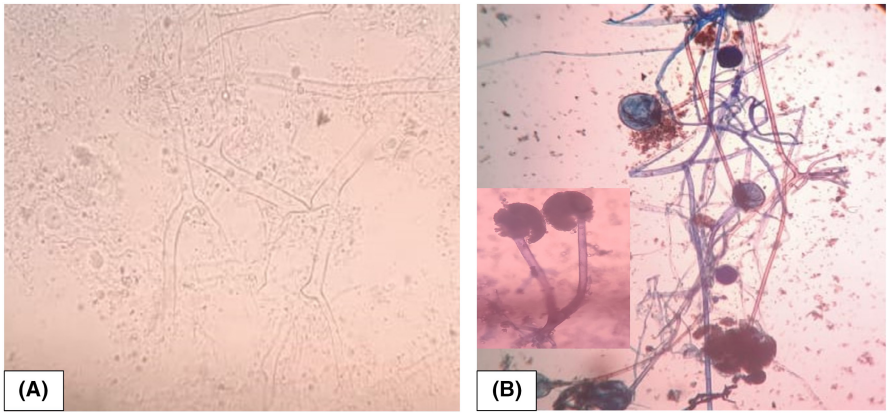

FIGURE 3 (A) *Mucor* hyphae on KOH preparation (400×). (B) Hyphae of *Rhizopus* species (lactophenol cotton blue [LPCB], 400×) with inset showing sporangium (LPCB, 400×)

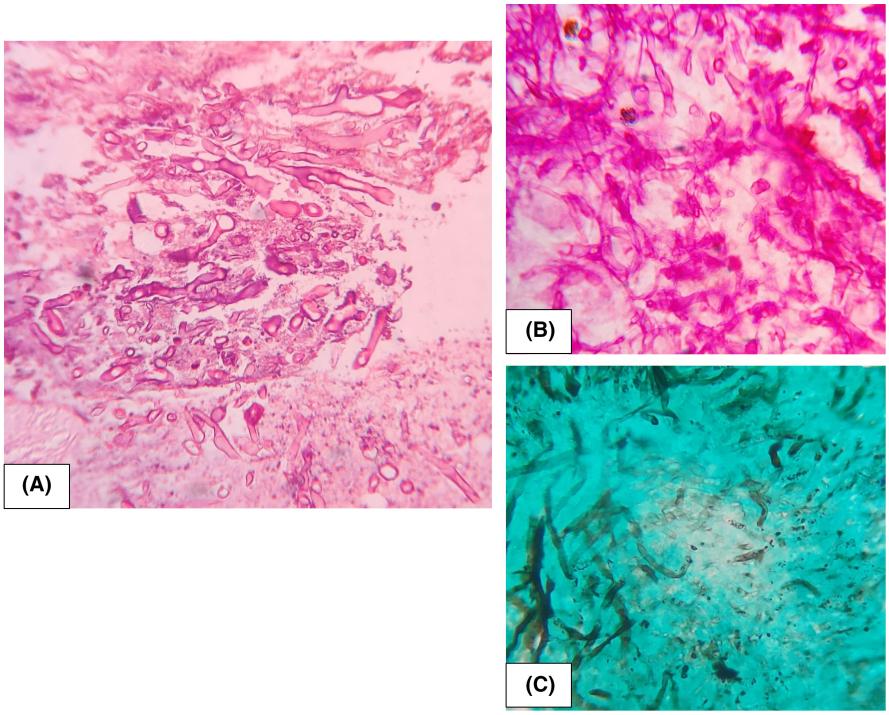

FIGURE 4 (A) *Mucor* hyphae on histopathology slide (HE 400×). (B,C) *Mucor* hyphae showing periodic anti-Schiff (PAS) and silver methenamine positivity on histopathology slides (B: PAS, 400×; C: silver methenamine, 400×)

TABLE 3 Comparison of positive predictive value, negative predictive value, sensitivity, and specificity of various modalities for *Mucor* detection

	Histopathology	LBC	Culture	Conventional cytology	KOH
Sensitivity	75.9%	72.4%	58.6%	44.8%	31%
Specificity	100%	100%	100%	100%	100%
Positive predictive value	100%	100%	100%	100%	100%
Negative predictive value	41.7%	38.4%	29.4%	23.8%	20%

Abbreviations: KOH, potassium hydroxide preparation; LBC, liquid-based cytology.

TABLE 4 Reports of mucormycosis in COVID-19 patients in India

Author	Number of cases	Age, sex	Risk factors	Site
Mehta et al[1]	1	60, M	Diabetes, Steroid	Nasal/Sinus, Orbit
Garg et al[6]	1	55, M	Diabetes, Steroid	Lung
Maini et al[23]	1	38, M	Steroid	Nasal/Sinus, Orbit
Saldanha et al[24]	1	32, M	Diabetes	Nasal/Sinus, Orbit
Revannavar et al[25]	1	F	Diabetes	Nasal/Sinus, Orbit, brain
Sen et al[26]	6	46.2–73.9, M	Diabetes, Steroid (5)	Nasal/Sinus, Orbit, brain
Sarkar et al[27]	10	27–67, M/F = 8:2	Diabetes, Steroid	Nasal/Sinus, Orbit, brain
Mishra et al[28]	10	37–78, M/F = 9:1	Diabetes (8), Steroid (6)	Nasal/Sinus, Orbit, bone
Satish et al[29]	11	30–74, M/F = NR	Diabetes, Leukaemia (1)	Nasal/Sinus, Orbit
Moorthy et al[30]	17	39–73, M/F = 15:2	Diabetes (15), Steroid (15)	Nasal/Sinus, Orbit, brain, bone
Sharma et al[31]	23	Age NR, M/F = 21:2	Diabetes (21), Steroid	Nasal/Sinus, Orbit, brain

a variety of comorbidities, namely DM, solid tumour, leukaemia, and tuberculosis. Histopathology showed a sensitivity of 75.9%, while LBC had sensitivity of 72.4%, and they were statistically significant with *P*-values of 0.003 and 0.005, respectively (with confidence interval of 95%). The sensitivity of culture, conventional cytopathology, and KOH preparation were very low compared to histopathology and LBC. Thus, this study showed that LBC can accurately and promptly diagnose mucormycosis.

LBC for fungal detection has not been extensively investigated, and few studies were conducted in the pre-COVID-19 era.[20,36] Shen

et al studied respiratory samples of 171 patients (117 sputum and 54 bronchial brushing samples) using conventional cytopathology and LBC for aspergillus.[20]

Jiang X et al used LBC for *Mucor* detection in biopsy-confirmed *Mucor* cases.[36]

They studied 27 patients diagnosed as mucormycosis on histopathology, with a female:male ratio of 4:23 and median age of 55.1 years. Comorbidities noted were diabetes in 18 (66.67%) cases, solid tumour in 6 (22.22%), haematological malignancy in 2 (7.41%), renal transplant in 1 (3.70%), while 8 (29.63%) cases had other comorbidities. A total of 33 pairs of bronchial brushing samples of conventional cytopathology and LBC from 27 patients were included in their study. The LBC platform detected Mucorales in 28 of 33 samples, corresponding to a sensitivity of 84.85%, while conventional cytopathology had a sensitivity of 45.5%. These findings were comparable to the present study.

Philip et al conducted a study in a tertiary health care hospital.[37] Thirty-two nasal swabs/scrapings/biopsy samples from patients suspected to have mucormycosis were subjected to KOH testing. Sixteen of the cases were positive while 16 were negative on KOH preparation. May-Grünwald-Giemsa (MGG) and PAP staining was done on both KOH positive and negative samples. Six of the KOH positive samples were positive for *Mucor* on MGG and PAP staining while mixed infection was seen in two cases, having both *Mucor* and *Aspergillus*. Four out of these six cases were positive on culture.

Four of the KOH negative samples were positive for *Mucor* on MGG and PAP staining while mixed infection was seen in one case, having both *Mucor* and *Aspergillus*. Two out of these six cases were positive on culture. Repeat sample from 16 KOH negative cases were stained by MGG and/or PAP stain. Three of the 16 samples were positive for *Mucor*. Two out of these three cases were positive on culture. These findings were similar to the present study.

The limitations of this study were the relatively small sample size, and the reliability of operators who were overworked during the pandemic conditions, and not all sites were amenable to sampling for LBC.

5 | CONCLUSION

The present study shows that LBC with its sensitivity of 72.4% can be a good alternative to histopathology, which had a sensitivity of 75.9%, for diagnosis of *Mucor* infection, with the added advantage of a shorter turnaround time and being less invasive.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Cytopathologists (conventional cytology, LBC): Rabish Kumar, Meeta Singh. Histopathologist (biopsy): Nita Khurana. Microbiologists (KOH, culture): Tanu Sagar, Sonal Saxena. Sample processing: M. Bharanidharan. Sample collection: Vikas Kumar, Vikas Malhotra. Clinical details: Vikas Malhotra, Ravi Meher, Ruchi Goel. Radiological details: Ritu Arora, Jyoti Kumar.

PATIENT CONSENT STATEMENT

Proper informed consent was taken.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not needed.

CLINICAL TRIAL REGISTRATION

Not needed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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