

# Crush cytology smears – A novel approach to rapid diagnosis of rhino-orbital mucormycosis in post-COVID-19 patients: A single-center study



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## ABSTRACT

**Background:** With recent surge in SARS-COVID-19, cases of rhinocerebral mucormycosis are on rise. Rapid diagnosis of mucormycosis is essential as delay in the treatment is associated with dreadful outcome. Many studies have found that crush cytology smears can rapidly diagnose mucormycosis as compared to histopathology, thereby reducing the time for arriving at final diagnosis, here, we present a novel approach to the rapid diagnosis of mucormycosis by crush cytology and analyze whether it has a higher sensitivity in diagnosing cases of mucormycosis. **Aims and Objectives:** This study aims to analyze the efficacy of crush cytology smear in rapid diagnosis of mucormycosis. **Materials and Methods:** Biopsies received from all the clinically suspected cases of mucormycosis with a history or associated present COVID-19 infection. The biopsy was processed for KOH preparation, crush cytology smear and confirmation were done by histopathological examination. Special stains were done wherever necessary. **Results:** Out of 50 cases of clinically suspected mucormycosis, 43 confirmed on histopathology. Out of 43 confirmed cases of mucormycosis, 33 (76.74 %) were male and 10 (23.25%) were female with male: female ratio of 3.3:1. The mean age of the studied cases was found to be  $52.32 \pm 12.59$  years. The most common presenting complaint was fever (79.07%), followed by headache/retro-orbital pain (62.79%), rhinorrhea (58.14%), and facial swelling (53.49%). Out of 43 patients, 41 recovered from COVID-19 disease with mean duration of 12 days. Hospital stays of patients with for COVID-19 varied from 7 days to maximum of 35 days. Out of 43 cases of mucormycosis, 24 cases were diagnosed with KOH mount with sensitivity of 55.81% and 39 cases with crush cytology smears with sensitivity of 90.69%. **Conclusion:** The diagnosis of mucormycosis is done with the gold standard methods such as histopathological examination and culture studies but the present study emphasizes on use of crush cytological smears prepared from sinonasal biopsies in suspected cases of mucormycosis in rapid and accurate diagnosis of mucormycosis.

**Key words:** Mucormycosis; COVID-19; Cytology; Early diagnosis

## INTRODUCTION

Mucormycosis is a mycotic disease caused due to fungal mold of order Mucorales, class Zygomycetes, and genus *Mucor*, *Rhizopus*, *Rhizomucor*, and *Absidia*. Rhino-orbito-cerebral mucormycosis is the most common form of mucormycosis seen commonly in diabetics.<sup>1</sup> These Mucorales are largely ubiquitous and commonly found in soil and infects individuals mainly by inhalational route. In

immunocompromised patients, these Mucorales develop hyphal forms and eventually spore formation starts within the tissues. These spores subsequently grow to form hyphae and start invading blood vessels causing serious consequences such as tissue necrosis, thrombosis, and secondary infections.<sup>2</sup>

In pre-COVID-19 era, the common predisposing factors for mucormycosis included patients on long-term immunosuppression, in cases of acquired immunodeficiency

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syndrome, patients having malignancies and individuals with conditions causing iron overload such as transfusion-dependent thalassemia patients.<sup>3</sup> The common sites affected by mucormycosis include nasal sinuses, lungs, soft tissues, and gastrointestinal system. The most dreadful involvement include central nervous system which is a serious life-threatening situation.<sup>4</sup> With recent surge in COVID-19, cases of rhino-orbital mucormycosis are on rise. Important factors contributing to increased risk of mucormycosis in patients of COVID-19 include dysregulation of immune system as well as use of immunomodulatory drugs and steroids in these cases. Associated comorbidities such as diabetes or chronic obstructive pulmonary disorders and use of corticosteroids during the treatment of COVID-19 are linked to increased morbidity due to mucormycosis. A high index of suspicion is needed for early diagnosis, particularly in patients of COVID-19 who have a history of receiving immunomodulatory drugs, steroids, or has been on ventilator.<sup>5</sup>

Rhinocerebral involvement is the most common form of presentation in cases of mucormycosis though other anatomical locations such as lungs (pulmonary), gastrointestinal tract (gastrointestinal), and skin (cutaneous) may also be affected.<sup>6</sup> In severe and untreated cases, disseminated form of mucormycosis can also be seen. Rhinocerebral mucormycosis usually presents with signs and symptoms such as headache, facial pain, and constitutional symptoms such as fever and malaise.<sup>7</sup> Untreated cases may progress to serious complications such as invasion of orbital structures, sinuses, and intracranial extension. Mucormycosis can spread rapidly and an early diagnosis and aggressive treatment is cornerstone of therapy. Any delay may have catastrophic consequences. In many cases, destructive surgeries are undertaken to save the life of patients.<sup>8</sup> Combined systemic antifungal treatment as well as surgical management and debridement forms the cornerstone of therapy. In addition to systemic antifungal treatment and surgical debridement, a strict glycemic control and restricted use of corticosteroid in COVID patients is also necessary in prevention of mucormycosis in patients recovering from COVID-19. Despite all the efforts, prognosis is poor in cases of rhinocerebral mucormycosis and mortality up to 45% of cases has been reported.<sup>9</sup>

The diagnosis of mucormycosis is usually done by histopathological examination and imaging studies such as computerized tomography as well as magnetic resonance imaging can be useful in knowing the extent of involvement, particularly in knowing intracranial extension in cases of rhinocerebral mucormycosis. Culture and polymerase chain reaction (PCR) are gold standard in diagnosis, but they are

time consuming and expensive.<sup>10</sup> We studied efficacy of crush cytology smears prepared from biopsy specimens taken from suspected lesions of mucormycosis from sinonasal tract for rapid diagnosis of mucormycosis in post-COVID-19 patients with histopathological correlation.

### Aims and objectives

The aims of the study were as follows:

1. To analyze efficacy of crush cytology smear in rapid diagnosis of mucormycosis
2. To compare sensitivity of KOH mount versus crush cytology in studied specimens.

## MATERIALS AND METHODS

This was a prospective study conducted in the department of pathology in a tertiary care hospital. The Institutional Ethical Committee approved the study (BVDUMC&H/Sangli/IEC/272/17). The study period was 6 months extending from December 2020 to August 2021. Biopsy specimens from 43 patients of COVID-19 presenting with clinical suspicion of rhino-orbital mucormycosis were analyzed in this study. Sample size calculation was calculated on the basis of pilot studies done for diagnosis of mucormycosis by cytology smears. Keeping power (1-Beta error) at 80% and confidence interval (1-alpha error) at 95%, the minimum sample size required in each group was 30 patients; therefore, we included 43 (more than minimum required number of cases).<sup>10</sup> All the patients were tested positive for COVID-19 by RTPCR either during present illness or in the recent past. Biopsies from sinonasal lesion site were collected in normal saline with rapid transport of specimen to the laboratory, where crush smears from the biopsy samples were done as well as the KOH mount was prepared. Remaining specimen was immersed on 10% formalin and processed by routine tissue processing. Histopathological examination was done with H&E (hematoxylin and eosin) and special stains like periodic acid Schiff (PAS) and Gomori's methenamine silver (GMS) wherever necessary and results were recorded.

While preparing crush cytology smears, macroscopic examination of the submitted specimens from nasal cavities, maxillary, sphenoid, or ethmoid sinuses was done as proper sampling for crush smear preparations is a key in the good yield of fungal elements. Submitted tissue was examined thoroughly and purulent material, yellowish necrotic areas, blackish areas were selected. Tiny tissue bits of approximately 1–2 mm were taken from these areas and crushed in between two glass slides held perpendicular to each other. Gentle pressure was applied for specimens of purulent material. Little more pressure was applied to crush if the tissue appears firm. The smears were

fixed immediately with alcohol spray fixative and stained with rapid H&E. The sensitivity of crush cytology was compared to that of KOH mount. SSPS 21.0 software was used for statistical analysis.  $P < 0.05$  was taken as statistically significant.

## RESULTS

We studied 50 cases of sinonasal biopsies with clinical suspicion of mucormycosis during a period of 3 months out of which, 43 were diagnosed as mucormycosis on histopathology. All the 50 patients included in the study were tested positive with COVID-19 either during the disease course or in the recent past. Out of 43 confirmed cases of mucormycosis, 33 (76.74%) were males and 10 (23.25%) were female with male: female ratio of 3.3:1 (Figure 1).

The analysis of age distribution of the studied cases showed that the most common affected age group was between 51–60 (27.91%) years followed by 61–70 (20.93%) years and 41–50 (20.93%) years. Mucormycosis was uncommon in patients below 30 years of age (2.32%). The mean age of the studied cases was found to be  $52.32 \pm 12.59$  years (Table 1).

All the patients included in the study were infected with COVID-19. Out of 43 patients, 41 recovered from COVID-19 disease with mean duration of 12 days. Hospital stays of patients with for COVID-19 varied from 7 days to maximum of 35 days. The analysis of the cases based on the presence of predisposing factors showed that all the patients included in the study were having high blood glucose levels during their hospital stay or during the period of recovery from COVID-19. The mean blood glucose level was found to be 248 mg/dl. Out of 43 patients of mucormycosis, 36 were known diabetic while seven patients exhibited high blood glucose during the disease course. Thirty-three patients were having high serum ferritin levels with average of 384 mg/dl, with 10 patients having normal serum ferritin (Figure 2). All the patients were given corticosteroids during their COVID-19 disease as per treatment protocols given by ICMR.

The analysis of clinical presentation of the studied cases showed that the most common presenting complaint was fever (79.07%), followed by headache/retro-orbital pain (62.79%), rhinorrhea (58.14%), facial swelling (53.49%), peri-orbital swelling (48.84%), loss or diminution of vision (20.93%), and proptosis (16.28%) (Figure 3).

Out of 43 cases of mucormycosis, 24 cases were diagnosed with KOH mount with sensitivity of 55.81% and 39 cases

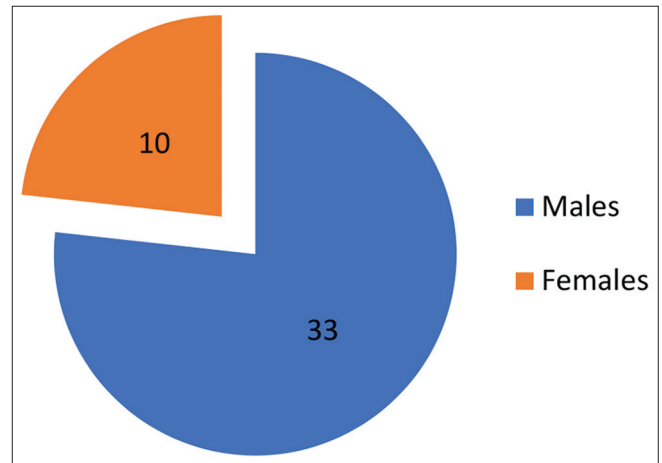


Figure 1: Gender distribution of studied cases

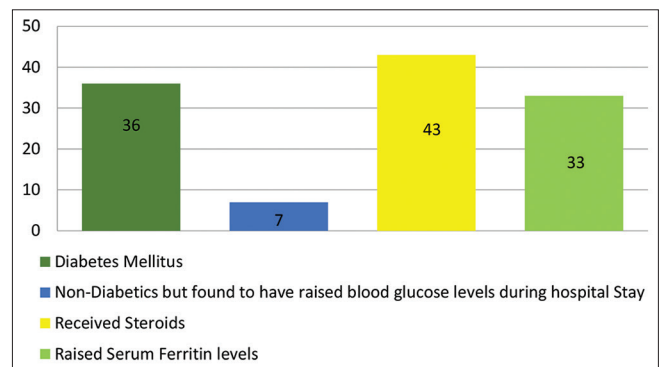


Figure 2: Predisposing factors for mucormycosis in studied cases

Table 1: Age group and gender-wise distribution of patients with mucormycosis

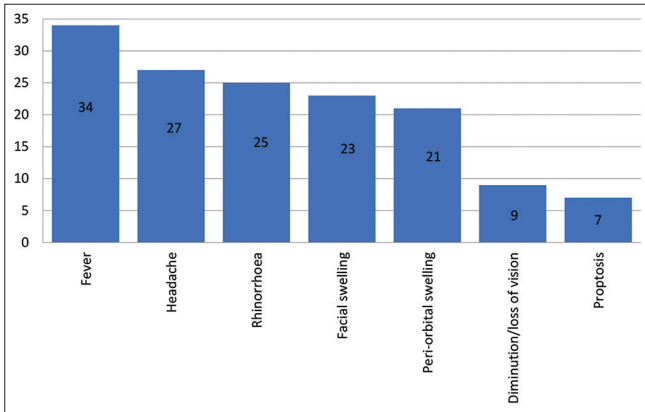
Age group (years)	Number of cases	Percentage
<20	0	0.00
21–30	1	2.33
31–40	7	16.28
41–50	9	20.93
51–60	12	27.91
61–70	9	20.93
71 onwards	5	11.63
Total	43	100.00

Mean age =  $52.32 \pm 12.59$  years

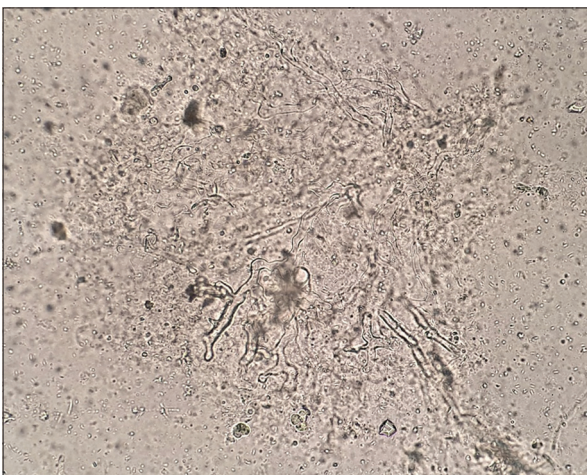
with crush cytology smears with sensitivity of 90.69%. All 43 cases were confirmed with histopathological examination and special stains. In five cases, there was associated aspergillosis coinfection as well. Crush cytology was found to be more sensitive for the diagnosis of mucormycosis as compared to KOH mount and the difference was found to be statistically significant ( $P = 0.0001$ ) (Table 2).

For rapid diagnosis of mucormycosis, KOH mount (Figure 4) as well as crush cytology smears (Figure 5) were prepared from biopsy specimen, and rest of the

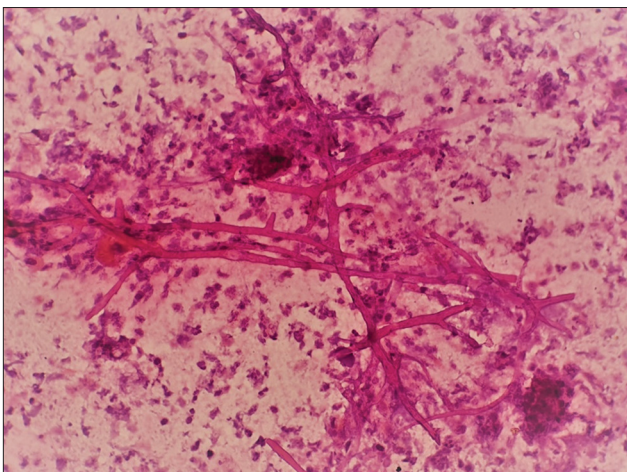




**Figure 3:** Clinical presentation in the studied cases

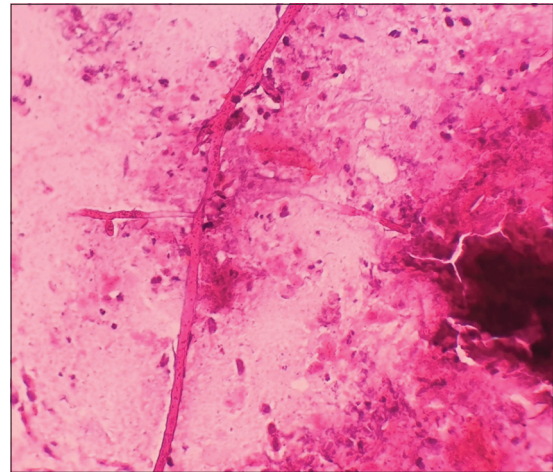


**Figure 4:** KOH wet mount preparation showing direct evidence of presence broad aseptate fungal hyphae suggestive of mucormycosis (KOH, x40)

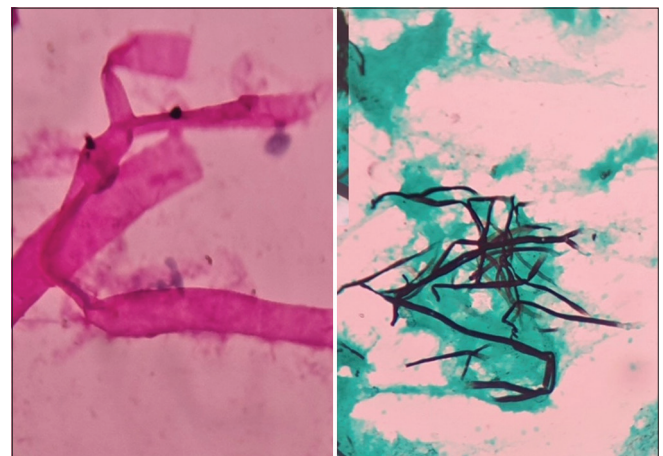


**Figure 5:** Crush cytology smear showing broad, ribbon-like aseptate fungal hyphae with the right-angled irregular branching (H&E stain 40)

tissue was processed by routine tissue processing with HE (Figure 6). Special stains as GMS and PAS were used wherever necessary to enhance the visibility of fungal hyphae (Figure 7).



**Figure 6:** Histology section showing broad, aseptate hyphae with the right angled, irregular branching (H&E stain x40)



**Figure 7:** Crush cytology smears stained with special stains as periodic acid-Schiff (PAS) and Gomori's methenamine silver (GMS) highlighting broad ribbon-like aseptate hyphae with the right angled branching (PAS, stain x40); (GMS, stain x20)

**Table 2: Comparison of sensitivity of KOH mount versus crush cytology in studied specimens**

	True positive	False negative	Sensitivity (%)
KOH mount	24	19	55.81
Crush cytology	39	3	90.69

P=0.0001 (significant)

## DISCUSSION

Mucormycosis is caused commonly by fungi of the order Mucorales including *Rhizopus*, *Mucor*, *Rhizomucor*, *Cunninghamella*, and *Absidia*. Zygomycosis and Mucormycosis occur in soil forming airborne spores. These spores can contaminate food and laboratory specimens causing rhino-orbital mucormycosis. It is a fulminant disease with high rates of morbidity and mortality. In the background of

the COVID-19 pandemic, increasing number of cases of mucormycosis is getting reported.<sup>11</sup>

Mucormycosis was identified as a disease-causing agent in humans in 1885. Since then, the incidence of mucormycosis is on rise. In the past two decades, there is worldwide increase in the prevalence of invasive mucormycosis probably because of the increase incidence of uncontrolled diabetes. With the outbreak of COVID-19, the cases of mucormycosis are rapidly increasing. Concomitant diabetes, immunosuppressive drugs used in treating COVID-19 infection, and severity of COVID-19 patients with poor oral and nasal hygiene are the reason for upsurge in the cases of mucormycosis. In India, mucormycosis affects approximately 0.14 patients per 1000 population,<sup>12</sup> which is almost 80 times higher than the prevalence in developed countries.<sup>13</sup>

In the present study, there was male preponderance with male: female ratio of 3:1 including 33 (77%) males and 10 (33%) females. The patient age ranged from 22 to 78 years with larger incidence in the age group of 41–80 years. Goel et al., got similar findings with male preponderance (70%, n=23), with the male: female ratio being 2.3:1.<sup>14</sup> The patients age ranged from 15 to 75 years in their study with the larger incidence in the 41–60 years (48%) age group. Approximately 94% (n=31) were immunocompromised with diabetes mellitus. Similar male preponderance in cases of mucormycosis was also reported by the authors such as Sarvestani et al.,<sup>15</sup> and Prakash H et al.<sup>16</sup>

In our study, the most common presenting complaints were fever (78.79%) followed by headache/retro-orbital pain (63.64%), rhinorrhea (57.58%), facial swelling (54.55%), periorbital swelling (48.48%), loss or diminution of vision (21.21%), and proptosis (15.15%). In a similar study, Camara-Lemarroy et al., analyzed data of 14 consecutive patients diagnosed to be having mucormycosis. The authors found that the most common presenting complaints in studied cases was fever (71.4%) followed by rhinorrhea (57.1%). The authors also reported that other common complaints in patients were headache, ocular pain, facial edema, and visual abnormalities.<sup>17</sup> Similar clinical features were also reported by the authors such as Petrikos et al.,<sup>18</sup> and Lin et al.<sup>19</sup>

For diagnosis of mucormycosis, various methods used are KOH preparation, histopathological examination, and culture studies. As rhino-orbital mucormycosis is extremely dangerous rapidly spreading disease, it is very important to start the treatment as early as possible. A lot of importance is given to the early timely diagnosis of mucormycosis which plays an important role in outcome of the disease.

Along with KOH preparation and histopathological examination, we performed crush cytology smears of biopsy samples received and found crush cytology smears to be more sensitive for the diagnosis of mucormycosis as compared to KOH mount and the difference was found to be statistically significant. Philip et al., conducted a similar study to investigate the role of stained cytology smears in the rapid diagnosis of mucormycosis. In this study, the authors performed May Grunwald Giemsa (MGG) and Papanicolaou stains on the remnant samples of nasal swabs as well as scrapings and biopsies after KOH test and fungal culture. The authors found that six out of 16 KOH-positive samples were positive on stained cytology smears and two were found to have mixed infections wherein *Aspergillus* were seen in addition to mucormycosis. The 4 KOH-negative samples were positive for mucormycosis with one sample having both mucormycosis and *Aspergillus*. Three out of 16 repeat samples which were earlier negative on KOH test were positive for *Mucor*. The findings of this study were similar to our study.<sup>20</sup>

#### Limitations of the study

A relatively small number of cases were studied in this case. A study comprising larger number of patients would further substantiate the results of this study.

## CONCLUSION

Although for diagnosis of mucormycosis, histopathological examination and culture studies are gold standards, the present study emphasizes on use of crush cytological smears prepared from sinonasal biopsies in suspected cases of mucormycosis in rapid diagnosis of mucormycosis. We conclude that crush cytology smears when used in conjunction with KOH test can result in increased sensitivity of detection of mucormycosis.

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**Authors Contribution:**

**AP** – Concept and design of the study, interpreted the results, prepared first draft of manuscript, and critical revision of the manuscript; **SM** – Statistically analyzed and interpreted, reviewed the literature, and manuscript preparation; **AP** – Design of the study, statistically analyzed and interpreted, preparation of manuscript, and revision of the manuscript; and **AS** – Concept and coordination of the overall study.

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