



# Mycological profile of fungi associated with rhino-orbital mycosis in post-COVID-19 patients

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## ABSTRACT

### Article info:

Received: 31 Jan 2023

Accepted: 06 Jun 2023

### Keywords:

Rhino-orbital mycosis

COVID-19

Rhizopus

Corticosteroids

Diabetes

Rhino-orbital mycosis is devastating fungal infection with high mortality and morbidity despite of recent advances in its diagnosis and treatment. It is caused by filamentous fungi of Mucorales order of the class of Zygomycetes. Rising number of cases presenting with fungal rhino-sinusitis with or without orbital involvement in patients recovered from coronavirus disease 2019 (COVID-19) infection was observed. Hence, present study was undertaken at a tertiary care hospital to know the mycological profile of fungi associated with these infections. Various clinical samples like deep nasal swabs, tissue from nasal cavity, nasal sinuses and orbital cavity were processed to isolate and identify fungi from suspected mucormycosis patients with standard mycological processes. Total 480 specimens from 226 patients suspected of mucormycosis were received in microbiology department of a tertiary care hospital, over 3 months period from April to June 2021. Rhino-orbital mycosis predominantly affected males and population over 50 years of age. Overall KOH positivity rate was 22.2% and culture positivity rate was 27.7% which was highest for tissue samples followed by deep nasal swabs. Most common isolate was Rhizopus spp. (51%) followed by Mucor (22%), Aspergillus (13%) and Rhizomucor (5%). Mixed infections with Mucor and Aspergillus were seen in 4% patients. Mucormycosis was observed in majority of post-COVID-19 patients and patients with high blood sugar. The majority of patients (64.1%) were suspected to have nasal involvement. Early diagnosis and prompt treatment play pivotal role in cases of mucormycosis. One should be vigilant to diagnose rhino-orbital mycosis as it is dreaded complication.

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## 1. Introduction

Mucormycosis is a devastating fungal infection with high mortality and morbidity despite of recent advances in its diagnosis and treatment. It is caused by the filamentous fungi of the Mucorales order of the class of Zygomycetes [1]. The order Mucorales includes the genera *Abisidia*, *Mucor*, *Rhizomucor* and *Rhizopus*. Although these organisms are ubiquitous in nature and are almost present everywhere, predominantly in soil, their propensity to cause infections in human beings is low and limited to immunocompromised hosts [2]. It is predominantly an opportunistic infection affecting immunocompromised hosts with diabetes mellitus (DM), neutropenia, malignancy, chronic renal failure, acquired immunodeficiency syndrome and those who have received organ or hematopoietic stem cell transplants. Few of the infections by Mucorales are seen in immunocompetent hosts as well (such as trauma patients) [1, 3].

During the second wave of the COVID-19 pandemic in India, there were reports about the rise of Rhino-orbital mucormycosis in COVID-19 patients [4]. Patients present with symptoms like headaches, ptosis, facial pain or weakness, blood-tinged nasal discharge, visual disturbances, and pain in the eye. We observed an increase in number of cases presenting with fungal rhinosinusitis with or without orbital involvement in patients recovered from COVID-19 infection. So the present study was undertaken at a tertiary care hospital to know the mycological profile of fungi associated with rhino-orbital mucormycosis.

## 2. Materials and Methods

This cross-sectional study was conducted at a tertiary care hospital (Post Graduate Institute, Yashwantrao Chavan Memorial Hospital, Pimpri, Pune), over 3 months period from April to June 2021. Various clinical samples from suspected cases of mucormycoses like endoscopic tissue from nasal cavity, nasal sinuses and orbital cavity, deep nasal swabs, nasal scrapes, were processed for microscopy and fungal culture. A detailed history was obtained from each patient.

A 10% KOH mount was prepared from direct samples and observed under microscope (10X, 40X). On KOH preparation, the samples which showed a characteristic broad, non-septate, ribbon like hyphae with wide angle branching at irregular intervals, were considered as KOH positive (Supplementary figures 1-4), and preliminary report was released [5]. The specimens were further cultured on Sabouraud's Dextrose agar (SDA) with chloramphenicol but without cycloheximide slants and kept for incubation at 30°C for initial three days and at room temperature (23-26°C) thereafter. Slide cultures were put on corn meal agar for detailed microscopic identification of etiological fungi. Fungal isolates were identified by using phenotypic characteristics such as growth rate, colony morphology, and reproductive structures [6]. Scotch tape method and lactophenol cotton blue stain were used for slide preparation from SDA agar and slide cultures from corn meal agar.

**Table 1:** KOH and culture positivity of different samples

Sample type	Total sample	KOH positive No. (%)	Culture positive No. (%)
Deep nasal swabs	329	57 (17.3)	88 (26.7)
Nasal tissue	117	42 (35.9)	36 (30.7)
Nasal scrape	15	2 (13.3)	4 (26.6)
Nasal crust	9	3 (33.3)	2 (22.2)
Sputum	6	0 (0)	0 (0)
Others	3	2 (66.6)	3 (100)
Orbital swab	1	1 (100)	0 (0)
Total	480	107 (22.2)	133 (27.7)

**Table 2:** Distribution of fungi causing mucormycosis

Isolates	Number	Percentage
<i>Rhizopus</i> spp.	49	51.0
<i>Mucor</i> spp.	22	22.9
<i>Aspergillus</i> spp.	13	13.5
<i>Rhizomucor</i>	5	5.2
Mixed <i>Aspergillus</i> and <i>Mucor</i>	4	4.2
<i>Syncephalastrum racemosum</i>	3	3.1

In the present study positive results were given when the given specimen was positive for fungal elements based on microscopy /KOH mount. It's very difficult for clinicians to wait for fungal culture results for treatment purpose as patient may land up in life threatening complications. In the present study fungal culture were done for all specimens as they help in identifying etiological fungus accurately. But many times especially in case of mucorales there is damage to fungal hyphae while handling the specimens resulting in positive microscopy and negative cultures. Vice versa negative microscopy with positive fungal cultures may be due to contamination of culture slants. In our study in such cases, histopathological diagnosis too was considered to rule out possibility of contamination.

Data was entered into Microsoft Excel® software and analyzed. Analyzed data were presented using simple descriptive statistics.

### 3. Results

A total of 480 specimens from 226 patients suspected of fungal rhinosinusitis/mucormycosis were received. The various sample included 329 (68.5%) nasal swabs, 117 (24.3%) endoscopic nasal tissue, 15 (3%) nasal scrape, 9 (1.8%) nasal crust and rest were other samples.

The analysis of demographic data revealed that mucormycosis predominantly affected males and population over 50 years of age. Amongst 226 patients 159 (70.3%) were male and 67 (29.6%) were female.

KOH and culture positivity was highest for nasal tissue samples followed by nasal swabs and nasal crusts (Table 1).

A total of 96 fungal isolates were recovered from 480 samples. The profile of fungi (Table 2) consisted predominantly of *Rhizopus* spp. followed by *Mucor* spp., *Aspergillus* spp., *Rhizomucor* spp. and *Syncephalastrum* spp. Mixed infections with *Mucor* and *Aspergillus* were seen in 4% patients.

Out of 226 patients, 219 patients had a history of COVID-19 illness in recent past while only 7 patients could not give history of COVID-19. Majority of the patients were treated by steroids and had raised blood sugar levels at diagnosis.

### 4. Discussion

Mucormycosis was first reported from humans as a pathogenic in 1885 [6, 7]. In present study, mucormycosis predominantly affected males. This finding is supported by previous studies conducted in India by Bala et al. (Chandigarh, 2010-2011) and also study by Kamath et al. (Jamshedpur, 2021) [7, 8]. In current study, majority of affected patients were over 50 years of age whereas Bala et al. proposed that median age of affected patients was 40 [7]. Kamath et al. also mentioned in their study that

86.7% patients were in 41 to 70 years age group [8].

In present study, it was observed that, patients suspected of mucormycosis had nasal involvement 64.1% followed by orbital involvement. This is correlating with study conducted by Bala et al. and with a case report of Zayet et al. (Tunisia, 2020) [7, 9]. Hoenigl et al. also mentioned similar findings in a review of cases (2019-2021 in various countries) [10, 11]. In current study, mucormycosis was observed in majority of post-COVID-19 patients and patients with high blood sugar. This is supported by study conducted by Monika et al., (Bangalore, 2021) and also by Bala et al., Singh et al. (Mumbai, New Delhi, 2019-2021) also found multifactorial association with development of mucormycosis in a systematic review carried out by them [4, 7, 12]. Overall KOH positivity rate was 22.2% and culture positivity rate was 27.7% in present study which is comparable with the study by Joshi et al. (Miraj, 2021); KOH and culture positivity rates being 68.7% and 25% respectively [13].

KOH mount microscopy is relatively inexpensive, simple, rapid method for presumptive diagnosis of devastating disease like mucormycosis. In the present study nasal tissue was a better sample than nasal swabs with better positivity. In current study, the most common isolate was *Rhizopus* spp. (51%) followed by *Mucor* (22%), *Aspergillus* (13%) and *Rhizomucor* (5%) [7]. The study conducted by Bala et al. proposed similar findings. Joshi et al. also identified *Rhizopus* spp. (66.6%) as the most common clinical isolate in their study [13].

Early diagnosis and prompt treatment play pivotal role in cases of mucormycosis. One should be vigilant to diagnose mucormycosis as it is a dreaded complication. KOH mount microscopy is relatively inexpensive, simple, rapid method for presumptive diagnosis. This test saves precious time while treating cases of mucormycosis.

### Supplementary files

Supplementary file 1.

### Authors' contributions

Conceptualization: SM. Data curation: SM, CJ, SD. Investigation: SM, CJ, SD. Methodology: SM, CJ, SD. Original draft preparation: SM, CJ, JP. Critical revision and editing: JP. All authors read and approved the final version of article.

### Conflict of interests

The authors have no conflict of interest to declare.

### Ethical declarations

This study was done as a routine diagnostic procedure and only used leftovers from clinical specimens, so ethics

approval was not taken. Institutional board of research studies approved the study design.

## Financial support

Self-funded.

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