

Mycological Profile of Acute Invasive Fungal Rhinosinusitis during COVID-19 Pandemic at a Tertiary Care Hospital

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Abstract

Introduction: During the coronavirus disease-19 (COVID-19) pandemic, a surge in acute invasive fungal rhinosinusitis (AIFRS) cases with high mortality was reported in India. **Objective:** The objective was to study the spectrum of fungus associated with AIFRS during the pandemic of COVID-19. **Materials and Methods:** A total of 51 patients who were clinically diagnosed as cases of acute invasive rhinosinusitis in the department of ear, nose and throat (ENT) were included in the study. The clinical data along with demographic details were noted, and fungal identification was done using a conventional method. **Results:** Out of 51 patients, 66.6% were males and 33.4% females. Predominantly affected age group was 41–50 years. Out of 51 patients, 92.15% (47/51) had suffered from COVID-19 and 7.8% (4/51) did not have COVID-19 infection previously. Thirty-seven patients out of 51 (72.54%) were diabetics. Out of 51 samples collected from patients, 94.11% (48/51) were fungal culture-positive and only 5.8% (3/51) were culture-negative. A total of 52 fungi were isolated from the 48 culture-positive samples. Mucormycetes were predominantly isolated from the samples followed by *Aspergillus* species and *Candida* species. Among mucormycetes, *Rhizopus* species was the predominantly isolated. **Conclusion:** Patients with COVID-19, especially those at high risk, need to undergo an ENT examination once they recover because an early identification of AIFRS and a strong clinical suspicion of the disease are crucial for a successful course of treatment and to improve patient prognosis.

Keywords: *Aspergillus*, *Candida*, COVID-19 infection, fungal rhinosinusitis, mucormycetes

INTRODUCTION

Rhinosinusitis is characterized as an inflammation of the nasal and paranasal sinus (PNS) mucosa, which is accompanied by changes in the mucosa ranging from inflammatory thickening to the production of noticeable nasal polyps.^[1,2] The etiology in the case of rhinosinusitis may be due to infectious agents (bacterial and fungal) and allergic immunological inflammation and sometimes due to nonallergic immunological inflammation.^[3] The term “fungal rhinosinusitis” (FRS) is used to characterize the condition when fungus is linked to the inflammation of nasal and PNS mucosa.

The classification of FRS is controversial, but according to the most widely accepted system, it can be divided into two types: noninvasive and invasive. Invasive form is categorized into three subgroups: chronic granulomatous invasive FRS, chronic invasive FRS, and acute invasive fungal rhinosinusitis (AIFRS),^[4,5] which has a significant

death rate and is described as having a temporal course of less than 4 weeks.^[6] Acute neutrophilic infiltrates with a necrotizing pathological reaction, hyphal invasion of blood vessels, vasculitis with thrombosis, hemorrhage, and tissue infarction are all symptoms of the infection, which is caused by the fungal species that quickly AIFRS spread from the PNS to the nearby orbits and central nervous system. Chronic forms of invasive FRS generally have indolent course with a slow destruction that may involve any PNS but most commonly affect the sphenoid and ethmoid sinuses.^[7,8]

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The invasive form of the disease is mostly seen in people with immunocompromised state or preexisting comorbidities especially diabetes mellitus. Once the fungus from the environment gets lodged in the sinuses of the individual, it releases several virulence factors that help hyphae to invade the blood vessels and local tissue. The extensive angioinvasion caused by the hyphae causes thrombosis, which ultimately causes the destruction of the tissue and necrosis. Because of angioinvasion, it disseminates to other nearby target organs very easily and can cause fatal complications.^[6]

The clinical picture and course of noninvasive FRS differ from those of invasive FRS. The symptoms of noninvasive FRS include recurrent nasal blockage, foul nasal discharge, postnasal discharge, and headache. The patients with invasive disease present with rapidly progressive swelling of the face with discoloration of the overlying skin, pain in the jaw, loosening of teeth proptosis, sudden visual loss, and ptosis.^[7,8]

Diagnosis of both noninvasive and invasive forms relies on high clinical suspicion, histopathology, microscopy, culture, and imaging technique. The imaging technique of choice for diagnosis is computed tomography scan of nose and PNS and magnetic resonance imaging of brain.^[8,9]

Although both medicinal and surgical treatments can be used to treat both noninvasive and invasive forms of the disease, the mortality rate for patients with invasive FRS is still significant (50%–80%), primarily because of invasion of the orbit and intracranial cavities. The prognosis is frequently determined by the prompt beginning of antifungal medication and/or surgical debridement; thus, an early and accurate diagnosis is actually crucial.^[10]

There is a wider range of fungi that is involved in rhinosinusitis. These can be hyaline or dematiaceous hyphomycetes (pigmented fungi causing pheohyphomycosis), dimorphic fungi, mucormycetes, as well as yeast-like fungi. However, the spectrum of fungi involved in noninvasive form differs from that of invasive form. *Aspergillus* species is considered as primary etiological agent of noninvasive allergic FRS, but nowadays, dematiaceous fungi such as *Bipolaris*, *Exserohilum*, *Curvularia*, and *Alternaria* species are increasingly being reported too. Mucormycetes are responsible for the more severe invasive infections followed by *Aspergillus* species.^[4]

Mucormycetes are the saprophytic fungi found in the soil and environment. In the past, these used to be considered as nonpathogenic to man and animals. They were once treated as fungal contaminants in the diagnostic microbiology laboratory, but in the past few decades, these mucormycetes have emerged as pathogenic organisms especially in patients suffering from comorbidities such as diabetes mellitus, leukemia, lymphoma, and other immunocompromised conditions.^[11]

The infection caused by mucormycetes is also known as mucormycosis. There are various clinical types of mucormycosis, which includes pulmonary mucormycosis, rhinocerebral mucormycosis, gastrointestinal mucormycosis, cutaneous mucormycosis, disseminated mucormycosis, and isolated renal mucormycosis. The most typical form of this illness progresses from the nasal mucosa to the turbinate bones, PNS, orbit, and palate before eventually spreading to the brain where a significant invasion of blood vessels occurs.^[11]

During the devastating coronavirus disease 2019 (COVID-19) in early 2021, there was a sudden surge in the cases of acute invasive fungal sinusitis in patients who had recently recovered from COVID-19. Mainly, the fungus of order mucorales (then labeled as “black fungus” by the media) was associated with a sudden rise of AIFRS disease although there were other fungi such as *Candida* species and *Aspergillus* species, which were also reported from the cases. The aim of the study was to study the spectrum of fungus associated with AIFRS during the COVID-19 pandemic.

MATERIALS AND METHODS

The study was planned from March 2021 to March 2022 as a prospective observational study in the Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, India. A total of 51 patients who were clinically diagnosed as cases of acute invasive rhinosinusitis in the Department of ENT were included in the study, and verbal consent was taken to participate in the study.

Ethical approval

Ethical approval was taken from the institutional ethical committee, vide letter no. MMIMSR/IEC/13XP dated 08/02/2021.

Inclusion criteria

Patients of both gender and all age group who were clinically diagnosed as cases of acute invasive rhinosinusitis after thorough clinical evaluation by an otorhinolaryngologist (or ENT surgeon) were included in this study.

Exclusion criteria

Patients who had already undergone surgical treatment for acute invasive rhinosinusitis prior to admission in the hospital were excluded from the study.

The demographic details and relevant clinical data were collected at the time of sample collection.

Methods

Samples collected included nasal swab and tissue specimens.

In the case of nasal swab, two swabs were collected. One nasal swab was used for microscopy for KOH (potassium hydroxide) mount, and the second swab was used for fungal culture following the standard protocol.^[12]

The tissue specimen was collected from the sinuses by endoscopic sinus surgery by ENT surgeon and was received in sterile container containing normal saline. The small portion of tissue was minced into small pieces (0.5–1 mm in diameter) using sterile scalpel. Then this minced tissue was prepared for microscopical examination by KOH wet mount. For fungal culture, the rest of the tissue was inoculated directly on Sabouraud Dextrose Agar (SDA) without mincing to increase the chances of isolation of fungi from the tissue as the hyphal elements of mucormycetes are prone to physical damage.

Potassium hydroxide wet mount

On KOH wet mount, mucormycetes had characteristic broad, aseptate or sparsely septate, ribbon-like hyphae with wide angle or right angle, branching at irregular intervals. *Aspergillus* species on the KOH mount was identified as narrow, septate hyphae with dichotomous branching. *Candida* species was identified by the presence of yeast cells with budding and pseudohyphae in microscopy. The presence of pseudohyphae denotes tissue invasion, and hence, their presence is considered significant [Figure 1].

Culture

The culture media used for fungal culture was SDA with antibiotic chloramphenicol to avoid bacterial contaminants. The other antibiotic cycloheximide

generally added to the SDA medium to avoid the growth of saprophytic fungi was not added as it inhibits mucormycetes and *Aspergillus*, which are saprophytic yet pathogenic fungi associated with the acute invasive fungal.

The inoculated tubes of SDA slants were incubated at 37°C and 22°C for 14 days and were checked on the third, seventh, and 14th day for any evidence of growth.

The growth of mucormycetes was observed within few days (1–5 days) and was typical white to grey cotton candy growth with mycelia elements expanding to cover entire fungal slant [Figure 2]. The reverse side of the slant was white to yellow for most of the species. The growth of *Aspergillus* species was observed within a week. The colonies of most *Aspergillus* species were velvety and varied in color from yellow to green to brown. The reverse of the growth also varied in color from yellow to red to brown depending on the species. On fungal culture, the colonies of *Candida* species appeared in 4–5 days as white-to cream-colored, smooth, and pasty.

The identification of molds in the fungal culture was done by colony characteristics, microscopically by lactophenol cotton blue (LPCB) mount, whereas the identification of *Candida* species was done by colony characteristics, microscopically by Gram staining, germ tube test, and using chromogenic media (CHROM) agar^[12] [Figures 3 and 4].

RESULTS

In this study, males were found to be affected more than females, i.e., out of the 51 patients studied, 66.6% (34/51) were males and 33.4% (17/51) were females.

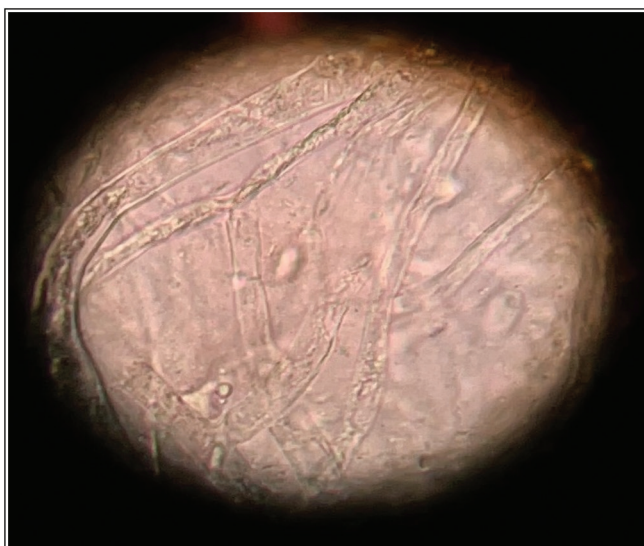


Figure 1: KOH wet mount from the tissue showing the characteristic broad, aseptate, ribbon-like, wide-angle branching hyphae suggestive of mucormycetes



Figure 2: Characteristic cotton candy growth of *Rhizopus* species on SDA slant

The age of patients ranged from 29 to 85 years. The mean age at the time of presentation was 51.47 years.

Out of 47 patients who gave a history of past COVID-19 episode, 85.1% (40/47) patients had received the steroid for the treatment. Only 8.5% (4/47) patients received

oxygen or mechanical ventilation during the treatment of COVID-19 infection [Table 1].

The most common associated disease was diabetes mellitus, 58.8% (30/51), followed by hypertension in 11.7% (6/51) cases and chronic kidney disease in 1.96% (1/51) patients.

A total of 52 fungal species were isolated from the 48 culture-positive samples. Mucormycetes were the predominant fungi isolated from the samples followed by *Aspergillus* species and *Candida* species. Among mucormycetes, *Rhizopus* species was the predominantly isolated [Tables 2 and 3].

Among *Candida* species, only *Candida albicans* was isolated from the samples. *Candida albicans* was isolated from samples of four patients, but there was coinfection with *Rhizopus* species in two patients and coinfection with *Aspergillus flavus* in other two samples.

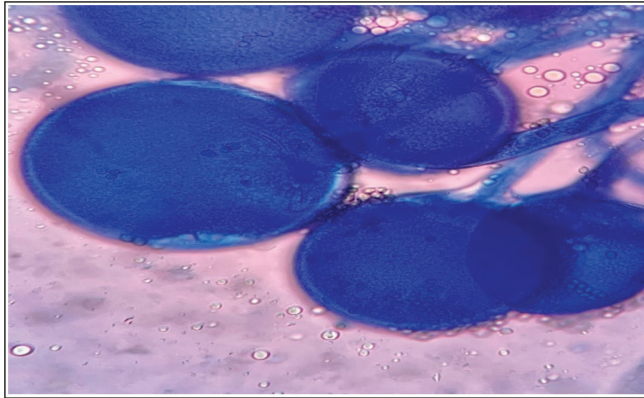


Figure 3: LPCB mount of *Rhizopus* species showing globose, smooth-walled sporangia

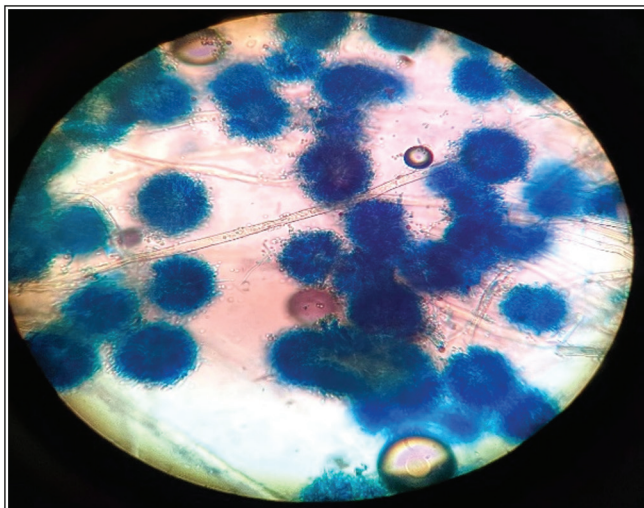


Figure 4: LPCB mount of *Aspergillus* species showing conidiophores, vesicles, and conidia

DISCUSSION

FRS is a mycotic infection of nasal and PNS. It exists clinically in two forms: noninvasive form, where the illness has benign course, and invasive form where there is progressive tissue damage and high mortality. A significant higher incidence is reported from countries where there is warm and dry climate such as Sudan, Saudi Arabia, and India.^[13,14]

The acute form of the invasive disease known as AIFRS caused by mucormycetes shows a sudden surge during COVID-19 pandemic in various states of India, which included Haryana, Rajasthan, Telangana, Odisha, Tamil Nadu, Gujarat, Uttarakhand, Bihar, Maharashtra, and Andhra Pradesh. As there is blackening or discoloration of the area infected by this fungus due to ischemic necrosis caused by the angioinvasion, the term “black fungus” was coined by media in India for this fungus. As a result of the sharp rise in cases, mucormycosis, known as “black fungus,” was classified as an epidemic and made reportable under the Epidemic Disease Act of 1897 in several Indian states.^[13,14]

Table 1: Distribution of patients pertaining to COVID-19 status along with preexisting diabetes or any other comorbidity

Total number of patients	COVID-19 status (RT-PCR) (n = 51)		COVID-19 history (A), preexisting diabetes, or any other comorbidity (B) (n = 51)		
	Positive	Negative	Positive (A and B)	Positive A but negative B	Negative (A and B)
51	47 (92.15%)	4 (7.8%)	37 (72.54%)	10 (19.6%)	4 (7.8%)

RT-PCR: real-time reverse transcription–polymerase chain reaction

Table 2: KOH wet mount and culture positivity

Total number of samples	KOH wet mount (n = 51)	Culture (n = 51)	
		No growth	Culture positive
51	51 (100%)	3 (5.88%)	48 (94.11%)

Table 3: Distribution of pure and mixed isolates

		Fungal isolate	Number of isolates
Number of culture-positive samples (<i>n</i> = 48)	Pure growth (<i>n</i> = 44)	<i>Rhizopus species</i>	32
		<i>Rhizomucor species</i>	4
		<i>Aspergillus flavus</i>	3
		<i>Aspergillus fumigatus</i>	3
		<i>Aspergillus niger</i>	1
		<i>Aspergillus versicolor</i>	1
	Mixed growth (<i>n</i> = 4)	<i>Candida albicans</i> with <i>Rhizopus</i> spp.	2
		<i>Candida albicans</i> with <i>Aspergillus flavus</i>	2

In the present study, males were more affected than females; this is in accordance with the study conducted by Sen *et al.* (2021) in India.^[15] However, in other study conducted by Michael *et al.* (2008) in India, females were more affected than males.^[16] In India, males being more active in fields and outdoor are more prone to the exposure of conidia and spores of the saprophytic fungus present in air than females.^[15]

In the current study, the age of patients ranged from 29 to 85 years with a mean age at the time of presentation of 51.47 years. A study conducted by Jadhav and Patwardhan (2021) in India reported 54.56 years as the mean age affected by AIFRS.^[17] Other multicentric study by Sen *et al.* (2021) in India reported 51.9 years as the mean age affected by rhino-orbital-cerebral mucormycosis following COVID-19 infection.^[15]

A majority of patients in the current study had a history of previous COVID-19 infection with preexisting comorbidity such as diabetes before the symptoms of acute fungal invasive sinusitis appeared. Although the exact mechanism involved is still unclear but the systemic use of steroids during COVID-19 treatment, preexisting diabetes and other comorbidities could be the possible mechanisms involved. In COVID-19 affected patients, there is an overexpression of inflammatory cytokines that circulate and there is an impairment of cell-mediated immunity as there is a decreased cluster of differentiation of four and eight positive T-helper (CD4+ T and CD8+ T) cell counts. This altered immune system could be the reason of increased susceptibility to fungal coinfection.^[18,19] Low oxygen (hypoxia), an acidic environment (metabolic acidosis, diabetic ketoacidosis, high iron levels [increased ferritin]), immunosuppression (severe acute respiratory syndrome coronavirus 2 mediated, steroid-mediated, immunomodulator-mediated, or background comorbidities), and decreased phagocytic activity of white blood cells are some additional risk factors.^[19]

However, in 7.8% of patients, there was no history of COVID-19 infection, diabetes, or any other comorbidity. Although the number of patients that suffered from AIFRS without a prior history of COVID-19 infection or the presence of any comorbidity was small but was

alarming as before this COVID-19 pandemic, it was considered to affect only individuals with preexisting comorbidities [Table 1].

In the present study, out of 51 samples collected from patients, 94.2% (48/51) were fungal culture-positive and only 5.8% (3/51) were culture-negative. There were three samples that were culture-negative despite the presence of fungal elements in KOH mounts indicative of mucormycetes. This could be attributed to the fact that during the processing of the tissue before inoculating the sample in the culture media, because of the absence of crosswalls, hyphae lose its fluid and collapse, thus losing the viability^[11] [Table 2].

A total of 52 fungi were isolated from the 48 culture-positive samples. Mixed fungal infection by two fungal species was seen in four patients. The presence of two fungi was seen in four samples [Table 3].

Rhizopus species of mucormycetes family was predominantly isolated from the samples followed by *Aspergillus* species and *Candida* species. A study conducted before the pandemic by Michael *et al.* (2008) in India reported *Rhizopus* species as the most common etiological agent in cases of acute fungal invasive rhinosinusitis followed by *Aspergillus* species and *Candida* species.^[16] A study conducted by Jadhav and Patwardhan (2021) in India during the second wave of COVID-19 pandemic also reported mucormycetes as the most common isolated fungi from patients of AIFRS^[17] [Table 3].

The most prevalent mucormycetes among *Rhizopus* species are *Rhizopus arrhizus*, formerly known as *oryzae*. *Rhizopus* species can infect people with preexisting diabetes mellitus because they have an active ketone reductase system and flourish in high glucose and acidic environments.^[11]

Aspergillus species a known saprophyte that generally colonizes the nasal cavity and PNS is mainly responsible for the noninvasive form of fungal rhinosinusitis, but in immunocompromised patients, it can behave aggressively like mucormycetes causing local destruction of tissue due to angioinvasion and thrombosis. In the current study, *Aspergillus flavus* was the predominant species isolated from the cases of AIFRS.

Candida species is not a common cause of AIFRS, but there have been various studies that have shown *Candida* as the etiological agent of AIFRS.^[17,20] In the current study, *Candida albicans* was isolated from samples of four patients, but there was coinfection with *Rhizopus* species in two patients and coinfection with *Aspergillus flavus* in other two samples [Table 3].

The limitation of this study was a limited number of patients and single tertiary care experience. Another limitation of the study is that samples were tested only to identify the fungal pathogen in AIFRS cases based on clinical discretion; thus, bacterial coinfection could not be ruled out.

It is advised to conduct more multicentric studies to update this experience with long-term follow-up and to target broader patient populations in comparison to determine the risk variables connected to both AIFRS and COVID-19 infection.

CONCLUSIONS

To conclude the overuse of corticosteroids, a history of diabetes mellitus and COVID-19 infection are the main risk factors of AIFRS. We suggest preventing the severe complications that involve the orbit and intracranial extension of the disease; it is necessary to increase the level of suspicion and awareness among the recovered COVID-19 patients.

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Conflicts of interest

There are no conflicts of interest.

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