

Retrospective Study

Clinicopathological Features of Fungal Infections in COVID-19 Patients: A Retrospective Study in Yazd, Iran

Running Title: Fungal Infections in Patients with COVID-19

Maryam Vajihinejad¹, Faezesadat Heidari², Zahra Eslami³, Ali Sartipzadeh^{4*}

- ¹ Department of Pathology, Shahid Sadoughi Hospital, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
- ² Department of Infectious Disease, School of Medicine, Infectious Disease Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
- ³ General pharmacist, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
- ⁴ Medical Doctor, Shahid Sadoughi University of Medical Sciences, Yazd,Iran.

ARTICLEINFO Received: 02/08/2025 Accepted: 08/17/2025 **Corresponding author** Medical Doctor, Shahid Sadoughi University of Medical Sciences, Shohadaye gomnam Blvd, Yazd, Iran. Tel: +98-9134570722 **Femal** alisartipzadeh5924@gmail.com

Abstract

Patients with COVID-19 due to unspecified medical treatment, hospitalization in the intensive care unit, need for invasive or non-invasive ventilation, prescription of broad-spectrum antibiotics, use of corticosteroids, and suffering from other underlying diseases, especially diabetes, are opportunistic to fungal infection. This study investigated the clinicopathology of fungal infections in patients with COVID-19 in samples sent to the pathology department of Shahid Sadoughi Hospital in Yazd, Iran, from 2020 to 2022.

Methods: This is an analytical cross-sectional study. The information related to patients with COVID-19 and fungal infections who were referred to the pathology department of Shahid Sadoughi Hospital in Yazd, Iran, from March 2020 to March 2022 was analyzed. Age, gender, clinical manifestations, type of fungal infection, site of involvement, mortality, duration of hospitalization and hospitalization department, need for mechanical ventilation, and clinical symptoms were extracted from patients' files and recorded in a researcher-made questionnaire. The data were statistically analyzed using SPSS version 26.

Results: In this study, fungal infections were confirmed in 53 patients with COVID-19 based on pathological diagnosis. Among them, 30 (56.6%) were men and 23 (43.4%) were women. The average age of the patients was 54.26 ± 18.07 years. In the pathological examination of fungal samples, 50 (94.3%) cases of mucormycosis, 2 (3.8%) cases of Aspergillus, and 1 (1.9%) case of simultaneous mucormycosis and Aspergillus were reported. Fungal infections involved the nasal cavity in 31 (58.5%) cases and paranasal sinuses in 18 (34%) cases. After the treatment period, the patients were discharged, and 14 (26.4%) patients died.

Conclusion: We concluded that fungal infections following COVID-19 infection are associated with serious complications and a high mortality rate; therefore, it is recommended that patients who are liable to suffer from fungal infections avoid overtreatment and treat them more carefully for COVID-19 to prevent possible fungal infection.

Keywords: COVID-19, Fungal Infection, Mucormycosis, Epidemic

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Introduction

The emergence of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), precipitated an unprecedented global health crisis (1). Originating in Wuhan, China, in late 2019, the virus rapidly disseminated globally, impacting virtually every nation and challenging healthcare systems worldwide (2). COVID-19 is primarily characterized by respiratory manifestations, including fever, cough, shortness of breath. and fatigue, although gastrointestinal symptoms and other systemic complications have also been observed (3). The clinical presentation of SARS-CoV-2 infection often overlaps with other respiratory viral illnesses, underscoring the critical role of diagnostic testing, particularly molecular assays and imaging studies, for accurate diagnosis and effective management (4, 5). Beyond the immediate respiratory effects, severe COVID-19 can lead to a cascade of inflammatory responses, culminating in life-threatening conditions, such as acute respiratory distress syndrome (ARDS). ARDS, a severe form of lung injury, is a major contributor to morbidity and mortality in patients with COVID-19, characterized by widespread inflammation in the lungs, leading to fluid buildup and impaired gas exchange. The pathogenesis of ARDS in COVID-19 involves a complex interplay between immune dysregulation and a phenomenon known as cytokine storm. This uncontrolled release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN-y), contributes to the excessive inflammation and lung damage observed in severe cases (6).

The heightened inflammatory state and compromised immune defenses associated with severe COVID-19, coupled with interventions like mechanical ventilation and broad-spectrum antibiotic use, create a fertile ground for secondary infections. These secondary infections, often opportunistic, significantly complicate the clinical course of COVID-19, increasing the risk of adverse outcomes. Among secondary infections observed in patients with COVID-19, invasive fungal diseases have emerged as a particular concern. Although relatively rare in the general population, these infections pose a serious threat to individuals with weakened immune systems, such as those critically ill with COVID-19 (7, 8). Several factors contribute to the increased

susceptibility of COVID-19 patients to fungal infections. The use of corticosteroids, often employed to manage hyperinflammatory responses in severe COVID-19, can inadvertently suppress the immune system, increasing the risk of opportunistic infections. Similarly, prolonged hospitalization, particularly in intensive care units (ICUs), exposes patients to nosocomial pathogens, including fungi. Invasive medical procedures, such as mechanical ventilation and central venous catheterization, further increase the risk of infection (9-11).

Among the invasive fungal infections reported in patients with COVID-19, mucormycosis, caused by Mucorales fungi, has garnered significant attention. Often referred to as "black fungus," mucormycosis is a rare but aggressive infection that can manifest in various forms, including rhino-orbital-cerebral, pulmonary, cutaneous, and disseminated disease. The rhino-orbital-cerebral form, frequently observed in patients with

COVID-19, typically begins in the sinuses and can rapidly spread to the orbit and brain, leading to devastating consequences (12, 13). Symptoms may include facial pain, swelling, nasal congestion, vision changes, and neurological deficits (14).

Mucormycosis is associated with a high mortality rate, ranging from 40% to 80%, depending on the site of infection and the patient's underlying health status. Several factors, including uncontrolled diabetes mellitus, immunosuppression, iron overload, and trauma, predispose individuals to mucormycosis. The use of corticosteroids, a common treatment for severe COVID-19, has also been identified as a risk factor for mucormycosis. Early diagnosis and prompt antifungal therapy are crucial for improving outcomes in patients with mucormycosis.

Other fungal pathogens, such as Aspergillus and Candida species, have also been reported in patients with COVID-19. Invasive pulmonary aspergillosis caused by Aspergillus species can lead to severe pneumonia and respiratory failure. Candidemia, a bloodstream infection caused by Candida species, can also occur in critically ill patients with COVID-19, further complicating their clinical course. Managing these fungal infections often requires prolonged antifungal therapy and careful monitoring for adverse effects (15, 16).

This study investigated the clinicopathological features of fungal infections in COVID-19 patients referred to Shahid Sadoughi Hospital in Yazd, Iran, between March 2020 and March 2022. We aimed to characterize the spectrum of fungal pathogens encountered in this patient population, describe their clinical presentations, and assess the associated

outcomes. This information will contribute to our understanding of fungal coinfection's epidemiology and clinical significance in COVID-19, ultimately informing strategies for prevention, diagnosis, and treatment.

Materials and Methods: This study

This analytical cross-sectional study investigated fungal infections among patients with COVID-19 at Shahid Sadoughi Hospital in Yazd, Iran, between March 2020 and March 2022. Following approval from the Department of Pathology and the Ethics Committee of Shahid Sadoughi University of Medical Sciences, data were retrospectively collected from available patient files. All COVID-19 patients with confirmed fungal infections during the specified period were included in the study. We excluded cases with incomplete files or unclear diagnoses. Data were analyzed using the Statistical Package for the Social Sciences software version 26 (IBM SPSS Inc., Chicago, IL, USA). Descriptive statistics included means and standard deviations for continuous variables and frequencies and percentages for categorical variables. The chi-square test or Fisher exact test was used to compare categorical variables, whereas the t-test was used to compare continuous variables. A p-value less than 0.05 was considered statistically significant.

Results

Of the 13,824 confirmed COVID-19 cases, 53 patients (0.38%) were diagnosed with concurrent fungal infections based on pathological examination. The cohort consisted of 30 men (56.6%) and 23

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women (43.4%), with a mean age of 54.26 years. Mucormycosis, the most common fungal infection, occurred in 50 patients (94.3%). Aspergillus was found in 2 patients (3.8%), and a single case (1.9%) exhibited coinfection with both Mucormycosis and Aspergillus. The most common site of infection was the nasal cavity (58.5%), followed by the paranasal sinuses (34 %). The incidence of orbital involvement was lower (7.5%). The distributions of fungal types and anatomical locations are detailed in Table 1. The most common clinical presentations were decreased visual acuity (26.4%), nonspecific symptoms (26.4%), ptosis (24.5%), facial pain (17%), and cranial nerve palsy (18.9%). Other symptoms included headache (13.2%), otological symptoms (11.3%), fever (7.5%), nasal congestion (3.8%), and periorbital swelling (1.9%). Figure 1 presents a visual representation of the relative frequencies of these clinical symptoms. The average length of hospital stay was 24.67 days (SD ± 20.96). Most patients (66%) were treated in the general ward, whereas 34% required ICU admission. Mechanical ventilation was necessary for 30.2% of patients. Thirty-nine patients (73.6%) were discharged after recovery. Fourteen patients (26.4%) died. Figure 2 illustrates the distribution of patients among different hospital settings and the need for mechanical ventilation. Statistical analysis using the chi-square test revealed no significant association between gender or age and patient outcome (P > 0.05). While the frequency of decreased visual acuity, fever, ptosis, and nonspecific symptoms was higher in the deceased group, only cranial nerve palsy showed a statistically significant difference (P = 0.014). **Table 2** highlights the key differences in the prevalence of cranial nerve palsy between the groups. Other variables, such as decreased visual acuity, fever, and ptosis, showed higher frequencies in the deceased group but did not reach statistical significance.

Table 1. Type and Location of Fungal Infections

Category	Subcategory	Number of Patients	Percentage
Type of Fungus	Mucormycosis	50	94.3%
	Aspergillus	2	3.8%
	Mucormycosis + Aspergillus	1	1.9%
Anatomical Sites	Nasal Cavity	31	58.5%
	Paranasal sinus	18	34%
	Orbit	4	7.5%

Table 2. Key Differences Between Recovered and Deceased Patients

Variable	Recovered (n=39)	Deceased (n=14)	P- value
Cranial Nerve Palsy	4 (10.3%)	6 (42.9%)	0.014

Figure 1. Distribution of clinical status in patients with COVID-19 and fungal infections.

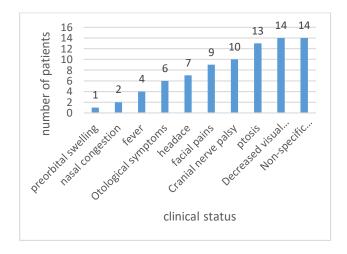
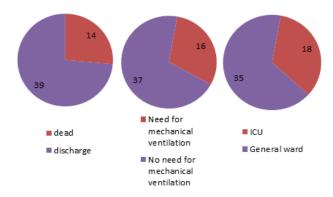


Figure 2. Distribution of current status, department, and need for mechanical ventilation among patients with COVID-19 and fungal infection.



Discussion

In this study, we examined patients with COVID-19 infection at Shahid Sadoughi Hospital, Yazd, Iran, between March 2020 and March 2022. The total number of patients with COVID-19 registered in the laboratory of Sadoughi Hospital was 13,824, with fungal infections confirmed in 53 patients based on pathological examination. Of these, 30 (56.6%) were men and 23 (43.4%) were women, indicating a near-equal distribution by gender.

The prevalence of fungal infections among patients with COVID-19, although not explicitly stated in our study, can be inferred from the ratio of fungal infection cases to the total number of COVID-19 cases, which was approximately 0.38% in our dataset. This rate aligns with emerging global trends in which fungal coinfections in patients with COVID-19 have been reported variably, with higher incidences noted particularly in regions with pre-existing high burdens of fungal diseases or where specific risk factors like diabetes and corticosteroid use are prevalent (17, 18). In a study conducted by Nejm et al. in 2021 at Zagaghi Hospital, Egypt, they showed that gender

does not have a significant difference in the rate of fungal infection(19). In addition, in a study conducted by Nazari et al. in 2021, they examined all Persian and English articles of Iranian patients and found no significant difference in men and women, which was similar to our study(20).

The average age of the patients was 54.26 ± 18.07 years. The highest rate of infection (37%) was observed in individuals aged 60–80 years and the highest mortality rate (50%) was observed in individuals aged 0–16 years. Simultaneous infection is more common in individuals aged >50 years than in young individuals.

According to a study in the United Kingdom on patients with COVID-19 and fungal infections, the age range of 55 to 81 years was associated with the highest percentage of co-infected patients(21).

In the pathological examination of fungal samples, 50 (94.3%) cases of mucormycosis, 2 (3.8%) cases of Aspergillus, and 1 (1.9%) case of simultaneous mucormycosis and Aspergillus were reported, indicating that the number of cases of mucormycosis was significantly (94.3%) higher.

In a study aimed at investigating the clinicopathology of sinonasal fungal infections in COVID-19 patients in India, Mundhe et al. observed fungal infection in 110 cases. 99 patients had mucormycosis infection, 8 patients had Aspergillus, and 3 cases were coinfections (22). Before the COVID-19 pandemic, India and Pakistan had the highest incidence of invasive mucormycosis, which occurs predominantly in men. Before the COVID-19 pandemic, India and Pakistan had the highest incidence of invasive mucormycosis, which occurs predominantly in men.

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The high incidence of mucormycosis is associated with environmental factors such as fungal spore exposure and chronic diseases such as uncontrolled diabetes mellitus. Other predisposing factors include the use of immunosuppressive drugs, such as corticosteroids and neutropenia. These known risk factors, along with changes in metabolism and immune system function caused by COVID-19 treatment, have led to increased cases of mucormycosis, particularly in low- and middleincome countries. While cases of mucormycosis occurred in patients with classic risk factors, such as underlying hematologic malignancies, in the first months of the epidemic, the number of reported cases of mucormycosis increased dramatically in the second wave of the epidemic, possibly due to overuse of systemic corticosteroids, increased exposure to Mucorales, and increased numbers of patients with uncontrolled or undiagnosed diabetes. Globally, the largest number of cases has been reported from India, with such a dramatic increase compared to pre-COVID-19 rates that the central government of India declared a mucormycosis epidemic on May 10, 2021(23).

In a review study conducted by Nazari et al. in 2022, in a review study that included 169 patients, the authors examined the relationship between fungal infection and COVID-19 in Iran. The reported CAFIs included candidiasis (85 cases), mucormycosis (35 cases), aspergillosis (29 cases), and other miscellaneous infections(20). In a study of papers on coronavirus and fungal coinfection in China during the coronavirus pandemic, Song et al. showed that the main fungal pathogens for fungal infections in severe

COVID-19 patients are Aspergillus and Candida. Other rare opportunistic fungi that cause lung infections should also be considered, such as Mucor and Cryptococcus(24). These two studies did not agree with our results, which could be due to the small sample size.

Mucormycosis fungal infection involved the nasal cavity in 31 (58.5%) cases, paranasal sinuses in 18 (34%), and orbital region in 4 (7.5%) cases. There was one case of Aspergillus involving the orbit and nose. The results of the studies showed facial pain in 9 (17%) patients, vision loss in 14 (26.4%) patients, ptosis in 13 (24.5%) patients, headache in 7 (13.2%) patients, and fever in 4 (7.5%) patients.

El-kholy et al. investigated 36 patients with an average age of 52.92 years who had a fungal infection in Covid. Mycological analysis showed infection with Mucor and Aspergillus species in 77.8% and 30.6% of the patients, respectively. Nasal sinus, orbital, cerebral, and palatal involvements were observed in 100%, 80.6%, 27.8%, and 33.3% of patients, respectively. The most commonly reported signs and symptoms were facial pain (75%), facial numbness (66.7%), ophthalmoplegia, and decreased vision (63.9%) (25). These data were consistent with the present study.

Inhalation of airborne fungal spores is the main route of infection, although infection can also occur through ingestion and skin contact. Rhino-orbito-cerebral mucormycosis (ROCM) is the most common form of mucormycosis, with very high mortality even with appropriate drug therapy(23). Therefore, in patients with COVID-19, symptoms such as facial pain, cheek swelling, orbital pain, and nasal discharge

should be evaluated with the possibility of fungal infection because if not diagnosed, it can be fatal, and if diagnosed on time and treated early, the invasion of infection (26).

In examining the clinical condition of the patients, the average length of hospitalization was 24.67 ± 20.96 days. 35 (66%) patients were in the inpatient ward, 18 (34%) were under observation in the ICU, and 16 (30.2%) were under mechanical ventilation. After the treatment period, 38 (72%) patients were discharged, and 14 (26.4%) patients died.

In a review by Ozbec et al., who studied mucormycosis in association with COVID-19, mucormycosis occurred on average 22 days after COVID-19 and 8 days after hospitalization. The diagnosis of mucormycosis in patients with coinfection of Aspergillus and pulmonary mucormycosis was accomplished at 15.4 days (range, 0-35 days) and 14.0 days (range, 0-53 days) after respectively. Cutaneous hospitalization, mucormycosis accounts for less than 1% of cases. The overall mortality rate was 38.9%, indicating that the mortality of mucormycosis was high, and most reports were from low- and middle-income countries(27).

Comparing the studied variables in patients with fungal infection after contracting COVID-19 between the two groups of dead and recovered patients using the Chi-square test showed that gender and age had no statistical relationship with the clinical condition of the patient (P-value <0.05). The frequency of decreased visual acuity, fever, ptosis, cranial nerve palsy, and nonspecific symptoms was higher in the group of dead patients than in the

recovered group, but only the frequency of cranial nerve palsy was statistically significant between the two groups (P-value<0.05).

Mucormycosis in patients with COVID-19 due to the high mortality rate, necrotizing nature of the disease, and need for invasive surgical debridement leads to deformities in patients (23).

I.Sree Lakshmi et al. confirmed that the histopathological features of mucormycosis, such as angioinvasion, perineurial invasion, heavy fungal burden, and extensive areas of necrosis, were directly proportional to the mortality rate. Therefore, histopathologists can help in prognostic assessment at the time of tissue diagnosis to optimize treatment (28).

Conclusion

The current study from Shahid Sadoughi Hospital, Yazd, Iran, highlighted the significant occurrence of fungal infections, predominantly mucormycosis, among patients with COVID-19 over a two-year period (March 2020 to March 2022). We observed a higher prevalence of fungal infections, particularly those involving the nasal cavity and paranasal sinuses, with notable clinical presentations, including facial pain, vision loss, and ptosis. The mortality rate associated with these infections was substantial, with 26.4% of patients dying despite treatment.

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Ethical considerations: The studies involving humans were approved by the Ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The studies were conducted in accordance with local legislation and institutional requirements. The participants provided written informed consent to participate in this study. A written informed consent was obtained from the individual(s) for the publication of any potentially identifiable data included in this article.

Authors' contribution: MV: Data curation, Investigation, Project administration, Writing – original draft, Writing – review & editing.AS: Data curation, Investigation, Writing – original draft.FH: Investigation, Writing – review & editing. ZE translating the manuscript

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