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SPECTRUM OF OPPORTUNISTIC FUNGAL INFECTIONS IN COVID-19 AFFECTED PATIENTS IN A TERTIARY CARE HOSPITAL

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ABSTRACT **Background and aim:** Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread around the world. COVID-19 patients particularly those with Diabetes, Critically ill and Immunocompromised patients have higher probability of suffering from opportunistic fungal infections. Treatment of COVID-19 with immunosuppressive agents (e.g. corticosteroids and cytokine blockers) remains a complicated challenge, especially among patients with severe disease which may increase the risk of invasive fungal infections. We studied the association of risk factors in COVID-19 affected patients leading to opportunistic fungal infections. **Materials and Methods:** A cross-sectional observational study was conducted on a total number of 500 clinically suspected cases of fungal infection attending ENT outpatient department during May 2021 to August 2021. Samples were processed and identified by direct microscopy and culture. **Results:** A total number of 500 clinically suspected cases were screened for microscopy and culture. Of which 324 cases (64.8%) identified with fungal growth. Among these 324 cases, 51.5% were Aspergillosis, 31.4% were Mucormycosis, 12.9% were Candidiasis, 2.4% were mixed infection with Mucormycosis and Aspergillosis and 1.5% were identified with other opportunistic fungi. Out of 324 cases, known diabetics were 50% and non-diabetic with increase blood glucose levels were 22.8% and on steroid therapy were 66.3%. Most of the cases were presented in the recovery phase of COVID-19 (74%) than during the active phase (26%) of the infection. **Conclusion:** COVID-19 is associated with secondary fungal infections due to immune dysregulation and the wide spread use of immunomodulatory drugs along with certain risk factors like diabetes mellitus and immunocompromised conditions. Judicious use of steroids and stringent glycemic control can prevent the severity of the disease. Early recognition of this life threatening infection is the key to allow for optimal treatment and improved outcomes.

KEYWORDS : Opportunistic fungal infections, COVID-19, Diabetes, Corticosteriods.

INTRODUCTION

The Opportunistic Mycoses are those fungal infections which are found among patients with underlying predisposing conditions like Malignancy, Leukemia, Diabetes Mellitus, Immunosuppressive therapy, Solid organ transplantation, Hematopoietic stem cell transplantation, AIDS/HIV, Trauma and Malnutrition etc. Such clinical entities may essentially hasten the disease processes produced by fungi of lesser pathogenic potential in the host.

These Opportunistic fungi are found to occur in any geographical area and their clinical manifestations are variable depending on the underlying predisposition and immune status of the patient. The spectrum of diseases caused by these fungi ranges from superficial mycoses to deep mycoses, like Candidiasis, Cryptococcosis, Pneumocystosis, Microsporidiosis, Talaromycosis, Aspergillosis, Mucormycosis, Entomophthoramycosis etc, and these diseases are responsible for high morbidity and mortality among the hospitalized patients.

The Coronavirus disease 2019 (COVID-19) is caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has diverse Sequelae in all ages. However older individuals (> 60 years old), males, and patients who have comorbidities or co-infections, such as pulmonary diseases, chronic kidney disease, diabetes, hypertension, and cardiovascular diseases, have higher risk of severe infection. Close contact and respiratory droplets are the main route of COVID-19 transmission through breathing, sneezing, coughing, and even normal speech.

Pulmonary system is the most commonly affected organ, and therefore, the main clinical manifestations of COVID-19 are respiratory signs, including cough, dyspnea, sore throat, and fever. In the severe COVID-19 state, development of pneumonia with acute respiratory distress syndrome (ARDS), hypoxic respiratory failure, and/or death have been occurred. The disease is characterized by a variety of symptoms, of which breathing difficulties are typical of severe cases. Such severe cases require urgent intervention in hospitals, including oxygenation and mechanical ventilation, and such patients are prone to complications, including secondary infections with opportunistic fungal pathogens. On the other hand, extra-pulmonary organ involvement like cardiac, neurologic, endocrine, gastrointestinal, hepatic, renal, ocular, and dermatologic associated with loss of smell and taste has significant health threat.

Patients with COVID-19, are at increased risk of secondary fungal infections due to complex interplay of multiple risk factors, including pre-existing diseases [Diabetes Mellitus], use of Immunosuppressive drugs, Hospital acquired infections and systemic immune alterations by COVID-19 itself. Besides this, the widespread use of Broad-spectrum antibiotics, Corticosteroids, or Monoclonal antibodies (that inhibits binding of IL-6 to the membrane and soluble receptors) was significantly linked with the risk of developing secondary fungal infections[5] in the management of COVID-19 or exacerbation of pre-existing fungal diseases[8].

The main Opportunistic fungal infections that were reported among severe COVID-19 patients or those recovering from the disease are Aspergillosis, Mucormycosis, Invasive Candidiasis and penicilliosis. Of all these, Mucormycosis is most severe fungal co-infection associated with COVID-19 with high morbidity and mortality rates, followed by aspergillosis and candidiasis. India has reported a recent surge in these fungal co-infections during this COVID-19 pandemic.

MATERIALS AND METHODS

A cross-sectional observational study was conducted on a total number of 500 clinically suspected cases of fungal infection, attending ENT outpatient department during May 2021 to August 2021. Samples were taken either by tissue biopsy, scrapings from nasal and oral mucosa, deep nasal swab, Broncho-alveolar lavage fluid (BAL), CSF, swab from Paranasal sinuses and sputum. Informed consent was obtained from patients and the study was approved by the Institutional Ethics Committee.

The specimens were processed for fungal elements by direct microscopic examination with 10% potassium hydroxide (KOH) and culture. Direct microscopy is followed by inoculation of specimen on two SDA slopes (Sabouraud's dextrose agar) with antibiotics and without cycloheximide and incubated at 25°C and 37°C respectively and growth was seen within 2-4 days. Lactophenol cotton blue (LCB) mount was done from the culture growth for observing the microscopic morphology of the fungal growth. Slide culture was done on cornmeal agar to know the undisturbed morphology of fungal isolate.

The diagnosis of Mucormycosis was made by the finding of characteristic broad, aseptate or sparsely septate, ribbon like hyphae with wide-angle or right angle branching at irregular intervals with KOH and on culture floccose, dense, fibrous or cotton candy growth filling the entire tube and the diagnosis of Aspergillosis with 10% KOH shows narrow hyaline septate hyphae with dichomatous acute angle branching. Culture grew easily and relatively quickly. On culture Aspergillus flavus shows velvety, yellow to green or brown colour colonies, Aspergillus fumigatus shows velvety or powdery at first, turning to smoky-green colonies, Aspergillus niger shows woolly at first white to yellow, then turning dark brown to black colonies, Aspergillus terreus shows usually velvety cinnamon brown colonies. Candida species on KOH shows budding yeast cells with pseudohyphae, and on culture the colonies appear as cream colored, smooth and pasty appearance.

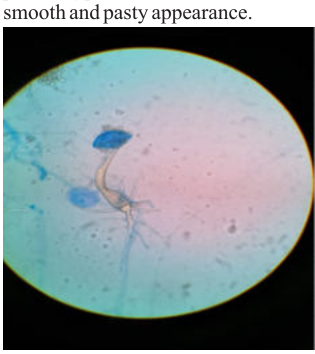


Fig.1. KOH mount showing broad, non-septate hyphae with wide angle branching.
Fig.2. SDA Slope Showing Fluffy Dense Cottony Growth

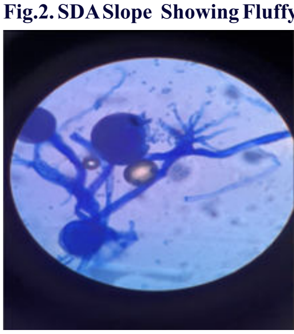
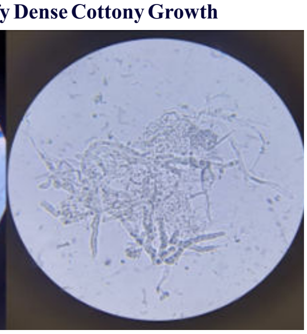

Fig.3. LCB mount of Rhizopus spp
Fig.4. KOH mount of aspergillus spp showing septate hyphae with acute angle branching

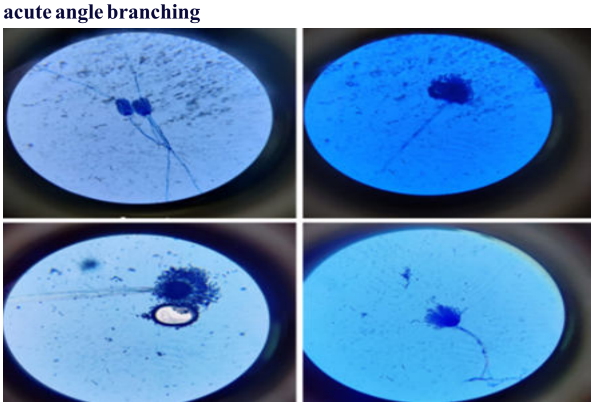

Fig.5. LCB mount of A. fumigatus, A. flavus, A. niger, A. terreus

RESULTS:

A total number of 500 clinically suspected cases, attending ENT outpatient department were screened for fungal infection by microscopy and culture.

Table 1: Distribution of Fungal positivity

Total samples tested	Fungal culture positivity	No fungus grown
500	324	176

Table 1 shows, out of the 500 samples tested, 324 cases were found to be positive for fungal growth on culture and no growth was observed in 176 cases.

Table 2: Distribution of Fungal Isolates

Culture positivites	Members of mucorales	Aspergillus species	Candida species	Mucorales & Aspergillus species	others
324	102	167	42	8	5

The distribution of fungal species is shown in table 3, Out of 324 culture isolates, 102 cases were found to be positive for Mucorales, 167 were found to be positive for Aspergillus species, 42 cases were found to be positive for candida species, 8 cases were found to be positive for both Mucorales and Aspergillus species, 5 cases were positive for other opportunistic fungi like Penicillium, Curvularia, and Syncephalastrum.

Table 3: Species wise distribution of Fungal Isolates (n = 324)

Isolate	No. of positives	Percentage
Mucorales (102)		
Rhizopus species	82	25.3%
Mucor species	17	5.24%
Rhizomucor species	3	0.92%
Aspergillus species (167)		
Aspergillus fumigatus	73	22.5%
Aspergillus flavus	52	16%
Aspergillus niger	40	12.3%
Aspergillus terreus	2	0.6%
Aspergillus species + Mucorales	8	2.46%
Candida species	42	12.9%
Curvularia	2	0.6%
Penicillium	2	0.6%
Syncephalastrum	1	0.3%

Table 3 shows, Out of 102 Mucorales, predominant isolate was found to be Rhizopus spp (25.3%), followed by mucor spp (5.24%), and around (0.92%) were Rhizomucor spp. Out of 167 Aspergillus species, predominant species was found to be A. fumigatus (22.5%), followed by A. flavus (16%), A. niger (12.3%) and A. terreus (0.6%). Around 2.46% of cases have mixed infection with both mucorales and aspergillus species. Candida species were found around (12.9%) and around 1.5% isolates were found positive for other opportunistic fungi.

Table 4: Association of opportunistic fungi with blood glucose levels (n=324)

Diabetic status	Total	No. of cases
Known diabetic	228	162 (50%)
Non diabetic	272	
With increased blood glucose levels	126	74 (22.8%)
With normal blood glucose levels	146	88 (27.2%)

Table 4 shows, Among 324 cases, known diabetics were 162(50%) and non-diabetics with increased blood glucose levels under treatment of COVID-19 were 74(22.8%) and non-diabetic with normal blood glucose levels 88(27.2%). Majority of cases were found to be associated with increased blood glucose levels.

Table 5: Association of opportunistic fungi with steroid therapy (n=215)

No. of cases on steroid therapy	known diabetics on steroid therapy	Non-diabetic on steroid therapy and developed drug induced hyperglycaemia	Non-diabetics on steroid therapy and with normal blood glucose levels
215	100(46%)	47(22%)	68 (32%)

Table 5 shows, among 350 cases, 215 cases were on steroid therapy during COVID-19 treatment. Out of these 100 cases (46%) were found to be known diabetics and 47 cases (22%) were non-diabetics but developed high blood glucose levels during the treatment. Remaining 68 cases (32%) were non-diabetics with normal blood glucose levels even with the steroid therapy.

Table 6: Association of opportunistic fungi with oxygen therapy

Total positives	No. of cases on oxygen	No. of cases not on oxygen
324	192(59.2%)	132(40.7%)

Table 6 shows, among 324 cases, 192 cases were found to be on oxygen therapy during COVID-19 treatment and 132 were found to be without oxygen therapy.

Table 7: Association of opportunistic fungi with active COVID and post COVID period (n=324)

Stage of infection	No. of positives	Percentage
Active COVID (120)	84	26%
Post COVID (380)	240	74%

Table 7 shows, among 324 cases, 84 cases were in active phase of covid-19 infection and 240 cases, were found to be in the recovery phase of covid-19. Majority of the cases were presented in the recovery phase of the covid-19 than during active phase.

Table 8: Site Of Distribution Of Disease

Diagnosis	No. of patients
Rhino-cerebral mucormycosis	8
Rhino-orbital mucormycosis	56
Rhino-orbital-cerebral mucormycosis	6
Pulmonary mucormycosis	11
Pulmonary mucormycosis with Pulmonary aspergillosis	3
Pulmonary aspergillosis	18
Rhinosinusitis	92

The clinical presentation included Rhino-orbital mucormycosis (n=56) cases, Rhino-cerebral mucormycosis (n=8) cases, Rhino-orbital-cerebral mucormycosis (n=6) cases, Pulmonary mucormycosis (n=11) cases, (n=3) patients were diagnosed with both pulmonary mucormycosis and pulmonary aspergillosis, pulmonary aspergillosis (n=18) patients and (n= 92) patients were diagnosed with fungal Rhinosinusitis.

DISCUSSION

Severe cases of coronavirus disease 2019 (COVID-19) who were managed in intensive care units are more prone to complications, including secondary infections with opportunistic fungal pathogens. The combined risk factors of diabetes mellitus, COVID-19, and recent corticosteroid treatment contributed to airway epithelial damage and immune dysfunction which are known complications of COVID-19 may provide an opportunity for fungal pathogens.

Most of the current treatment options for managing patients with severe COVID-19 infection are Immunomodulators (such as dexamethasone, methylprednisolone, prednisone, hydrocortisone, tocilizumab, azithromycin etc). The anti-inflammatory properties of these immunomodulators are important to counteract the heightened and unregulated release of pro-inflammatory cytokines also known as CYTOKINE STORM in the lungs during SARS-CoV-2 infection. Hyperinflammation and cytokine storm syndrome (CSS) are among the major causes of acute respiratory distress syndrome (ARDS), multiorgan failure, and death due to SARS-CoV-2 infection. Thus, immunosuppressants such as corticosteroids constitute the most common treatment options for managing hyperinflammation and CSS in COVID-19 patients which hampers both innate and adaptive immune responses through sophisticated qualitative and quantitative mechanisms of immune dysregulation, thereby increasing patient's susceptibility to invasive fungal infections [5].

In the present study, most of the patients who were identified with mucormycosis, aspergillosis, candidiasis and other fungal co-infections, were found to have elevated blood glucose levels. Hyperglycaemia is found to be an important risk factor for developing opportunistic fungal infections, in several recent studies both in known diabetics and non-diabetics who developed hyperglycaemia secondarily to disease or treatment. Opportunistic fungal infections were noted in hyperglycaemic patients in known diabetics is 68.8% and in non-diabetics is 70.1% in a study by Khichar et al. [10] similar findings were noted, by Umang Arora et al. [7] (55.7% and 35.5% respectively) and Ritesh Gupta et al. [9] (71.3% and 13.9% respectively). In the present study elevated blood glucose levels in known diabetics was 50% and in non-diabetics 22.8% ($p<0.05$) which shows that, the patients with elevated blood glucose levels were at significant risk of developing invasive fungal infections. Persistent hyperglycaemia in diabetes is thought to be responsible for impaired chemotaxis and phagocytosis of neutrophils and degranulation of NK cells [8]. It promotes surface glucose-regulated protein (GRP78) expression on the endothelium which is essential for invasion of fungal pathogens. Besides this, acidosis in diabetic ketoacidosis impairs the binding of iron to transferrin, increasing free iron that promotes fungal multiplication [9]. Along with this, physiological stress and viral mediated islet cell damage during COVID-19 infection also plays role in elevated blood glucose levels.

Corticosteriods have been used extensively and inadvertently for the treatment of COVID-19 infection to reduce the severity and mortality rate of the infection, but prolonged corticosteroid intake is a risk factor for the invasion of fungal pathogens. In the present study, the usage of systemic corticosteroids was found to be 66.35% several other studies also reported the similar findings. A study by Khichar et al. [10] 70.1% of corticosteroid usage was found in patients with opportunistic mycosis. Similar findings were noted in P. Lewis White et al. [4] (57.1%), in Umang Arora et al. [7] (65.8%) respectively. Corticosteroids contribute to elevated blood glucose levels by enhancing hepatic lipolysis, proteolysis and glycogenolysis as well as by insulin resistance. In diabetic patients, both hyperglycaemia and insulin deficiency decrease the release of protective cytokines, which further reduces the macrophage activity and allow the pathogens to invade the immune system. In the present study, steroids can led to elevation of blood glucose levels in known diabetics 46% and in non-diabetics 22% ($p<0.05$) was associated with significant risk of opportunistic fungal infections.

Severe COVID-19 patients with viral pneumonia and respiratory rate of more than 30 breaths/min and oxygen saturation<90% requires oxygen supplementation, but inappropriate Oxygen supplementation to mild and moderate cases is irrational. Queries has also risen that the use of supplemental oxygen allows the growth of fungus in the nasal mucosa; however no studies are available to support this idea [11]. In the present study, significant risk of opportunistic fungal infection was found among those patients who received any form of oxygen supplementation in hospital settings (59.2%) compared to cases not on oxygen supplementation (40.7%). Furthermore prolonged use of ventilators and humidifiers, unhygienic use of masks, poorly managed diabetes and the prolonged usage of high dosage steroids in treating COVID-19 are considered as causative factors for fungal colonization.

CONCLUSION:

The COVID-19 patients with underlying predisposing conditions like Diabetes mellitus, Immunosuppressive therapy, History of chronic respiratory disease, prolonged hospital stays, ICU admissions on Mechanical ventilation have an increased risk of COVID-19 associated opportunistic fungal infections. Among all the opportunistic fungal infections, Mucormycosis being the most dangerous infection leading to high morbidity and mortality within a short span of time followed by Aspergillosis, but can be readily treated if diagnosed early. Strategies for early screening, detection and differential diagnosis of opportunistic infections are essential and challenging issue in COVID-19 Patients. Early detection can helps in early treatment and consequently leads to better outcome of COVID-19 patients.

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