ORIGINAL RESEARCH PAPER

Clinical Microbiology

MYCOLOGICAL PROFILES IN COVID-19 PATIENTS: A CROSS SECTIONAL STUDY FROM EASTERN MAHARASHTRA

KEY WORDS: COVID-19, Mucor, SARS-CoV2, Pandemic

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ABSTRACT

Introduction: 2019 novel coronavirus (2019-nCoV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China that rapidly spread to other parts of the world resulting in pandemic. Increase in fungal superinfections among COVID-19 patients created panic during pandemic times in India. Secondary fungal coinfections are important factors leading to morbidity and mortality in COVID infected patients. The aim of this study was to assess the mycological profile and distribution of fungal pathogens among COVID-19 patients admitted in Government medical college, Gondia, Maharashtra. **Material and methods:** All clinical specimens received in Microbiology department were studied for fungal growth. Macroscopic and microscopic characteristics were observed. **Results:** Total 97 Covid positive patients with suspicion of fungal infections were studied out of which 26 showed positive for fungal growth. 9 (34.61%) were females and 17 (65.39%) were males. Mucor and Aspergillus were predominantly isolated. **Conclusion:** Early Diagnosis plays a key role in managing SARS-CoV-2 associated fungal infections. Formulation of stringent infection control protocols, antimicrobial usage policy helps reducing infections.

INTRODUCTION:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously designated as novel coronavirus (2019-nCoV), single-stranded positive-sense RNA virus was the cause for COVID-19 pandemic[1]. SARS-CoV-2 infections are associated with secondary infections due to misuse of antibiotics in many health care centres, irrational antibiotic prescription in many countries, especially in low and middle-income countries, widespread antibiotic usage in hospital settings, veterinary settings and animal husbandry.COVID-19 symptoms include fever, chest pain, cough, muscle and joint pains, memory loss, depression and difficulty breathing[2] .SARS-CoV-2 can present as asymptomatic or mild form to life-threatening pneumonia. Fungal co-infections have been noted in COVID-19 infected patients with many predisposing factors such as co-morbidities including Diabetes Mellitus, COPD, Sinusitis, previous lung pathologies, immunosuppressive therapy, nosocomial infections,attenuation in the immune system due to SARS-CoV-2 infection.[3] Rhino-orbital mucormycosis cases have seen a rise in India among COVID-19 patients. It is a rare, fatal, invasive fungal infection that originates in the paranasal sinuses and may frequently extend into the orbits and cerebral parenchyma.[4] Clinical manifestations of fungal sinusitis may not provide clear differentiation from other infections pathology; clinical diagnosis needs to be strengthened by laboratory and imaging services for prompt diagnosis. Fungal infections can be diagnosed by staining techniques, culture, serological methods, and imaging modalities. Government medical college Gondia is serving as a dedicated COVID-19 hospital since COVID-19 pandemic.

The present study was conducted aiming to determine the distribution of fungal infections according to age and gender, their causative agents among COVID-19 infected patients.

MATERIALS AND METHODS

Present Cross sectional Prospective Hospital-based study was conducted in the Department of Microbiology, Government Medical College Gondia, Maharashtra on 97 Covid-19 Positive patients with suspected fungal infections.

Inclusion Criteria

All ages of both sexes. COVID-19 infected patients. Suspicious fungal etiology.

Exclusion Criteria

Patients without any history of SARS-CoV2. Patients who are on anti-fungal medications.

COVID-19 infection was detected using RT-PCR and Rapid antigen test of COVID-19. Various clinical specimens were received in microbiology department from respective hospital units in sterile normal saline leak-proof containers. All specimens were processed by 10% KOH mount and fungal culture immediately after receiving at the lab. Microscopy was done and observed for fungal hyphal filaments and other fungal elements. Culture samples were inoculated on two sets of Sabouraud dextrose agar (SDA) and incubated at 25°C and 37°C. Fungal cultures were followed till 28 days. Positive fungal growth was identified by macroscopic and microscopic characteristics. The data was collected into a spread excel sheet and analyzed.

All descriptive variables were presented as numbers or percentages.

RESULTS:

Ninety seven covid positive patients with suspected fungal infections were enrolled in this study. Cumulative incidence rate were calculated for study period of May and April months of 2021. Number of new cases during study period was 26; the cumulative incidence was 0.005. 26 (25.7%) patients were diagnosed with fungal infections out of 97 suspected covid patients with fungal infection. Out of 26 covid patients, 9 (34.61%) were females and 17 (65.39%) were male. The youngest age with fungal infection was 32 years and the eldest 71 years. Age and Sex distribution among Covid fungal infection patients showed in Table 1.

Table 1: Age And Gender Distribution of Covid-19 Fungal Infection Patients

AGE (YEARS)	MALES	FEMALES	TOTAL (%)
Upto 30 yrs	-	-	-
31 – 40 yrs	2 (13.52%)	1 (11.11%)	3 (11.53%)
41 – 50 yrs	7 (41.17%)	2 (22.22%)	9 (34.61%)
51 – 60 yrs	5 (29.41%)	4 (44.44%)	9 (34.61%)
61 – 70 yrs	3 (17.64%)	2 (22.22 %)	5 (19.23%)
Total (%)	17 (65.39 %)	9 (34.61%)	26 (100%)

Table 2: Distribution of Fungal Pathogens In Covid-19 Patients

CLINICAL SPECIMENS	No. of Patients	Percentage (%)
Tissue bits from Nasal cavities and Sinuses	70	72.1%
Sputum	8	8.2%
Endo-Tracheal secretions	13	13.4%
Nasal swabs	6	6.1%
Total	97	100%

As described above in Table.2., tissue bits from nasal cavities and sinuses were the most common specimen received from suspected patients followed by Endo-Tracheal secretions, sputum and nasal swabs.

Table 3: Statistical Association of Koh Mount And Fungal Culture

DIAGNOSTIC METHOD		CULTURE		Total
		Positive	Negative	
KOH MOUNT	Positive	21	4	25
	Negative	5	67	72
Total	26	71	97	

Culture and KOH positivity was observed in 25 patients and culture-positive, and KOH negative were in 6 patients. On statistical association between KOH and culture assessment, the p-value was found to be <0.00001 and considered significant

Mucor was the most common fungal pathogen isolated from COVID-19 patients in the present study, followed by Aspergillus species including Aspergilus niger, Aspergillus fumigatus, Candida albicans, Non-albicans candida and Rhizopus as detailed in Figure:1.

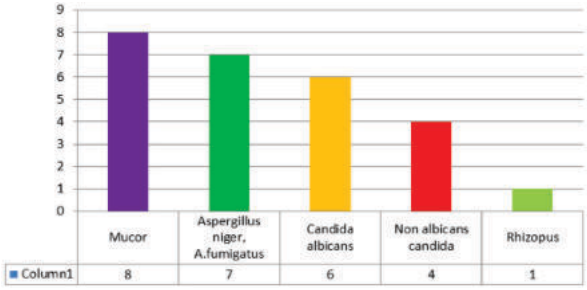

Figure 1: Distribution of Fungal Pahtogens In Covid-19 Patients

DISCUSSION:

Co-infections and superinfections in COVID -19 patients can be either bacterial, viral or fungal pathogens or a combined pathogenic manifestation. Other than SARS-CoV-2, various pathogens can infect a patient following an initial COVID-19 disease[5].While there were rampant SARS-CoV-2 cases in India, mucormycosis emerged as a significant public health problem. The dual infection causes exaggerated human body causes, including dysregulation of the immune system, ciliary dysfunction, cytokine storm, thrombo-inflammation, microvascular coagulation, and eventual immune exhaustion. The two most important clinical presentations of mucormycosis were rhino-orbital mucormycosis and pulmonary mucormycosis. The usual time of presentation of mucormycosis is the third week of onset of COVID- 19 symptoms.[6] Rhino-orbital mucormycosis involves the paranasal sinuses or orbits and may extend into the cerebral parenchyma.[7,8]. Mucormycosis is an aggressive, highly lethal, angioinvasive, opportunistic fungus that invades rhinal, occipital, and cerebral areas. This pathogen can invade craniofacial compartments such as paranasal sinuses, pharynx, orbit, and intracranial cavity via spore spread.[9] Delta and Delta Plus variants of COVID-19 virus were involved in majority of COVID-19 cases during first and second waves of Pandemic.These waves affected adult population in majority.This could be the reason for nil samples from paediatric age group in the present study.

The incidence of fungal infection in SARS CoV2 patients has been studied in other countries where Zhang Y et al reported 14.80% incidence[10]Yin CH et al. found the incidence of fungal infection to be 21.9-33% in severely ill patients[11]. Li CS et al. found fungal infection as major cause for increased mortality rate in COVID patients[12] In the present study, out of 97 post covid fungal infection patients, 9 (34.61%) were females and 17 (65.39%) were males. Chakrabarti A et al. studied Mucormycosis in India and documented that India has the second-largest diabetic population (65.1 million) worldwide[13]. Deepak Garg et al. reported COVID-19 associated mucormycosis caused by Mucorales after 10-14 days of hospitalization.[14] A retrospective study conducted on COVID- 19 critically ill pneumonia patients in Wuhan, China, found that 3 out of 52 (5.8%) patients had fungal infections, including Aspergillus flavus, Aspergillus fumigatus, and Candida albicans.[15] A study from Germany on COVID-19-associated invasive pulmonary aspergillosis found that 5 out of 19 (26.3%) had fungal culture positive.[16] Most severe and critical COVID-19 patients had poor diabetic control and corticosteroid usage as risk factors. Rapid progression of angioinvasive fungus to multiple organs, late manifestations, and shortage of anti-fungal drugs during the COVID-19 pandemic has been a burden in treating the patients. These global pandemic times need current and effective anti-fungal drugs and other management approaches for the needy population.[17]

CONCLUSION:

Mucor, Rhizopus and Aspergillus species including A. niger and A. fumigatus were predominantly isolated in COVID-19 associated fungal infections. Males were most affected with majority in age group of 41-60 years. Most common samples processed were nasal tissue bits. Early diagnosis and treatment helps to manage SARS-CoV-2 associated fungal infections. Rhino-orbital mucormycosis is a rapidly progressive condition, so empirical management with a KOH stain report highly benefits patients before confirmatory culture report. The formulation of stringent infection control protocols, antimicrobial usage policy, and updated COVID-19 management guidelines by government authorities helps reduce infections.

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Conflicts of interest: Nil

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