



A RETROSPECTIVE STUDY OF INVASIVE MUCORMYCOSIS IN COVID-19 PATIENTS FROM A TERTIARY CARE HOSPITAL, UT OF J&K, INDIA.

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ABSTRACT Mucormycosis is an angioinvasive disease caused by saprophytic fungi of the order Mucorales. There are growing reports of rhino-orbital mucormycosis in people with SARS COV-2, especially those from India. Diabetes mellitus (DM) is a very dangerous factor in COVID-19 and mucormycosis. We aim to conduct a retrospective study to determine patient characteristics of mucormycosis and COVID-19 from December 2020 to December 2021 at our tertiary care hospital, North India. During our study time, we have come across 7 such patients. Diabetes mellitus was a major risk factor for all patients with rhino-orbital cerebral mucormycosis. There was a high prevalence among males and the average age of presentation was 51 years. All patients received the same treatment with steroids and amphotericin B as well as local anesthetics. Deaths were 42.8%. Our study concludes that patients infected with COVID 19 are easily infected with mucormycetes due to immunosuppression, dysfunction of phagocytes and lymphocytes, and the use of antibodies and steroids.

KEYWORDS : Mucormycosis, diabetes, Rhizopus, Apophysomyces, Covid-19, fungal infections

1. INTRODUCTION

Coronavirus 2019 (COVID-19) caused by severe acute respiratory syndrome (SARS-CoV-2) has been linked to a wide range of opportunistic infections [1]. Mucormycosis is an angioinvasive disease that is characterized by vessel thrombosis and tissue necrosis. A recent increase in cases of mucormycosis in people with COVID-19 has been reported worldwide in particular from India. [2] . Mucormycetes are characterized by the presence of a wide range, aseptate hyphae (coenocytic mycelia) and the formation of zygospores. Among the mucoraceae species, Rhizopus are the most distinctly found followed by the genus Mucor. A very small percentage of Cunninghamella, Apophysomyces, (Lichtheimia), Saksenaia, Rhizomucor, and Cokeromyces and Syncephalastrum are also isolated from clinical settings [3,4,5]. Rhino-orbital-cerebral (ROCM) type is the most common type of disease in India, followed by lung and skin types [6,7]. As COVID-19 leads to levels of respiratory disorders, endothelial barrier disorders, alveolar-capillary dysfunction, oxygen transfer helps the Mucorales seeds to germinate [8]. Diabetes mellitus is the most dangerous complication associated with mucormycosis in India, unlike hematological-malignancy (leukemia, lymphoma, and multiple myeloma) aplastic anemia, myelodysplastic syndromes, and recipients of solid organ transplants in developed countries [9]. The current study is designed to understand clinical behavior, genetic history, epidemiology, clinical course, and the outcome of this disease. Here we presented 7 case series of patients with COVID-19 infection, who had rhino-orbital mucormycosis.

MATERIALS AND METHODS:

Study Design:

This is a retrospective study conducted in the postgraduate Department of Microbiology and the department of Pathology of Government Medical College in Srinagar, Kashmir, North India from December 2020 to December 2021. In the present study, enrolled patients were Covid -19 positive and comprised both immunocompromised and immunocompetent individuals.

Sample processing:

Visual biopsy data from patients with SARS COV-2 with suspected clinical mucormycosis were collected in two empty containers. One contains common salt and the other containing 10% formalin and was processed by the Department of Microbiology and Pathology, respectively. Experimental sampling in the Department of Microbiology includes direct KOH testing (10-20% potassium hydroxide) and mold culture. Fungal elements from the clinical specimen were directly identified and a diagnosis of mucormycosis was obtained. A broad, aseptate, ribbon-like hyphae with wide-angle or right-angle branching at irregular intervals was found under microscopic examination of KOH (10–20%) wet mount (Fig. 1). For fungal culture, the tissue sample was cut into small pieces and injected

without crushing into two tubes containing Sabouraud Dextrose Agar (SDA) with antimicrobials (containing chloramphenicol and gentamicin, without cycloheximide) and two tubes free of antibiotics, and one tube per set was incubated at 37° C and at 22°C respectively. The sample was also inoculated in the brain heart infusion broth (BHIB) and blood agar medium and was incubated at 37°C. In the first week, the growth culture was tested daily followed by twice a week at a later time. Finally, fungal culture was identified by common techniques using lactophenol cotton blue (LCB) mounting, and size, morphology, and the number of any fungal features were subsequently noted [10]. For histopathological examination tissue sections were stained with Hematoxylin and Eosin (H&E) stain, Periodic acid Schiff stain (PAS), and Grocott methenamine silver stain (GMS) (Fig. 2). These were evaluated and interpreted for the presence or absence of specific fungi [11,12,13].

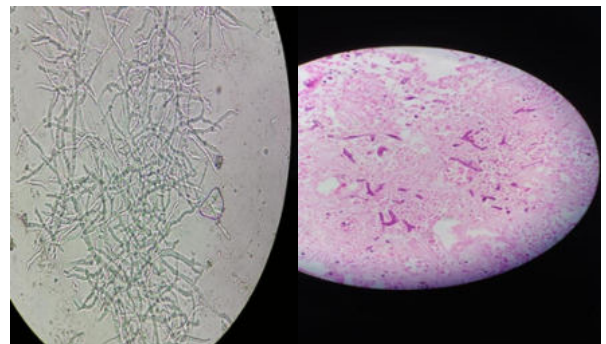


Figure:1 KOH MOUNT

Figure:2 H&E STAINING

SERIES OF CASES:

Patients with rhino-orbital-cerebral mucormycosis (ROCM) and COVID-19 infection admitted and managed at our tertiary care center from December 2020 to December 2021 were retrospectively reviewed. From the retrieved medical records, we had reviewed the necessary demographic findings along with the clinical, histopathological, and radiological data. All 7 patients were diagnosed and managed for COVID-19 at our hospital. Mucormycosis was diagnosed and treated for all the patients in our hospital. The mean age of our patients was 51 years (range 33 to 65 years), Diabetes Mellitus was reported among 5 out of 7 patients. Eye pain, facial pain, and nasal block were the presenting complaints. The radiological evidence from all the 7 patients had in the form of CT PNS and MRI brain, showed mucosal thickening of sinuses and adjacent bony erosions. Microbiological identification of the hyphae based on diameter, absence of septa, branching angle (right angle branching), and pigmentation, differentiates it from other fungal infections and

histopathological examination interpreted the presence of specific fungi in the specimen. Loss of vision was reported in 3 out of 7 patients. All the patients received steroids and one patient received tocilizumab for the management of COVID-19. All the patients except one had underlying comorbidities like diabetes mellitus, hypertension, and chronic kidney disease. Four patients at presentation had severe

COVID-19 disease, rest of the patients had the mild and moderate disease. All the patients were treated for the infected and necrotic tissue along with IV amphotericin B in addition to the treatment of COVID-19 disease. The overall mortality was 42.8% (3 of the 7). Details of these patients are described in Table 1.

Table 1: Baseline Characteristics of Covid-19 Patients With Mucormycosis

S.NO	1	2	3	4	5	6	7
Age	65	60	33	48	39	64	52
Gender	Female	Male	Female	Female	Male	Male	Male
Comorbidity	DM	DM	None	DM	DM	CKD	DM
Presentation	Facial Pain And Nasal Block	Holo- Cranial Head Ache	Stroke, Loss Of Eye Sight	Nasal Injury	Pain And Loss Of Eye Sight	Eye Swelling And Loss Of Vision	Epistaxis And Chemosis
Covid-19	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Radiological evidence	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Severity	Moderate	Severe	Moderate	Severe	Severe	Severe	Moderate
O2 requirement	No	Yes	No	Yes	Yes	Yes	No
Hpe and fungal smear(koh): broad aseptate hyphae	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Steroids	Yes	Yes	Yes	Yes	Yes	Yes	Yes

DISCUSSION:

The current study focuses mainly on the possible link between COVID-19 and Mucormycosis infection. Out of a total of 7 confirmed cases of mucormycosis in people with COVID-19, DM was reported in 93% of cases, while 88% received corticosteroids [14]. Mucormycosis was first reported as the cause of human disease in 1885 [15]. As a result of the worldwide increase in the number of dangerous agents associated with mucormycosis worldwide, there has been a dramatic increase in the number of invasive fungal infections. Among the various risk factors associated with the development of mucormycosis, uncontrolled diabetes is considered to be an important factor in promoting angioinvasive mucormycetes infection. Since macrophages and neutrophils are the first line of defense against mucormycosis, diabetic ketoacidosis leads to low serum pH and reduces the phagocytic effect of macrophages and chemotactic and oxidative outbreaks of neutrophils. Excessive hyperglycemia leads to transferrin glycosylation which reduces the ability to bind iron and increases free iron to circulate in the blood. In addition in patients with Covid-19, there is an increase in cytokines especially interleukin-6, leading to an increase in free iron by increasing ferritin levels due to increased concentration and decreased iron transport. Concomitant acidosis, therefore, reduces the ability of transferrin to chelate iron and increases free iron, thereby increasing the growth of mucormycetes.

As SARS COV-2 causes endothelial damage, endothelialitis, thrombosis, lymphopenia, and decreased CD4 β and CD8 β T-cell levels that further predispose to secondary or opportunistic infections [16,17]. Covid 19 is a pro-coagulable condition and provides a favorable site of mucor angioinvasion due to vessel thrombosis thus leading to widespread infections [18]. Since the hallmark recovery trail for COVID-19 patients emphasized the use of steroids in reducing the need for invasive ventilation in hypoxic patients and the hospital stay and ultimately decreasing the mortality due to the disease. The administration of steroids causes neutrophilic leukocytosis leading to many opportunistic infections. According to a hypothesis from a case report published by Mehta et al, patients with Covid-19 received steroids according to the protocols after which he developed mucormycosis it may be due to the alterations in the immunity especially T cells and innate immunity resulting in invasive fungal infection[19]. That is similar to our series of case studies in which most patients with angioinvasive mucormycosis had diabetes and received steroids for the management of Covid-19. Hence unholy intersection of the trinity of diabetes and rampant use of corticosteroids should be controlled in COVID-19 patients in order to prevent the burden of mucormycosis.

CONCLUSION:

The present study, therefore, emphasizes that patients with COVID-19 infection with comorbidities may have aggressive mucormycosis. There is a need to raise awareness about the disease and to take precautionary measures in order to achieve early diagnosis and management that can significantly reduce morbidity and mortality.

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Conflict of interest:

Nil

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