



COVID Induced Functional Exhaustion and Persistently Reduced Lymphocytes as Vital Contributing Factors for Post-COVID Rhino-orbital and Cerebral Mucormycosis in Patients with Diabetes: Report from the Indian Sub-continent

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Abstract

The current pandemic of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a global, unanticipated public health crisis. Another emerging challenge is the prevalence of “black fungus”, or mucormycosis, among patients who recovered from COVID-19 infection. A retrospective study was conducted on 12 patients in a post-COVID recovery phase who developed mucormycosis. The study parameters evaluated lymphocyte count, CD4+ T cell status, and associated systemic co-morbidities for the patient, namely diabetes. The interventions during the treatment for COVID were also recorded to include administration of oxygen, ventilator assistance (invasive and non-invasive)/oxygen support, and steroid use. The possible relationship between low lymphocyte and CD4+ counts with diabetes and fungal growth was evaluated. It was observed that the majority of the patients who had a positive history for diabetes with low lymphocyte and CD4+ counts were more susceptible to opportunistic fungal infections. Most of the patients, but not all, had a history of receiving oxygen or assisted ventilation, as well as steroids, during the treatment for COVID infection. These interventions may be considered as accessory contributing factors for fungal infection. Post-exposure to SARS-CoV-2, therapies should be targeted at prevention of functional exhaustion of lymphocytes and maintaining optimal lymphocyte and subset counts in susceptible hosts for the prevention of opportunistic fungal infections. The relationship between functional exhaustion of the lymphocyte, diabetes, and COVID mandates further research.

Keywords COVID · SARS-CoV-2 · Fungal · Infection · Mucormycosis · Coronavirus · Lymphocytes · CD4 · T cells

Introduction

The ongoing pandemic, caused by the novel strain of coronavirus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), is posing a public health and economic

challenge globally [1]. A secondary condition arising in India is a large number of post-COVID fungal infections. Fungal spores are present in the environment and humans are often exposed to them. Immune-competent hosts safeguard from these fungal infections through natural defence

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mechanisms. Reports of rhino-orbital cerebral mucormycosis (Fig. 1) are common in immune-compromised hosts in sparse numbers. Even with appropriate treatment, this disease has high morbidity and mortality if not detected early [2].

Mucormycosis represents acute or sub-acute, rapidly progressive infections caused by angio-invasive fungi in the order of Mucorales [3]. A rapid surge in cases of the otherwise rare “black fungus” was observed in India in patients recovered from COVID-19 after the second wave of the viral infection. The diagnosis of mucormycosis may be confirmed microscopically using KOH mounts to demonstrate broad aseptate or sparsely septate ribbon-like hyphae with right angled branching in a tissue specimen or aseptically aspirated material (Fig. 2). Alternatively, routine stains, such as hematoxylin and eosin (H&E) stains, may be used to visualize mucor hyphae (Fig. 3). The medical community is considering the increase in fungal infections to be multifactorial, with theorized contributory factors of use of industrial oxygen, compromised oxygen transportation during an emergency, use of steroids to manage the COVID induced cytokine storm, nosocomial fungal exposure due to prolonged hospital stay, or contaminated oxygen humidifiers, tubing and masks. The co-morbidities of a patient also contribute to complications of COVID-19 disease including post-COVID fungal infections.

One aspect that is neglected as a contributing factor of fungal infections is the lymphocyte and lymphocyte subset, including CD4+ and CD8+ T cells, statuses of the patient during the active phase of the SARS-CoV-2 and the post-COVID recovery. Depressed CD4+ T cell subsets of lymphocytes is a known cause for fungal infections in patients with human immunodeficiency virus (HIV) [4]. It is well documented that absolute numbers of lymphocytes, CD4+ T cells, and CD8+ T cells are decreased in almost all COVID-19 patients, with significantly lower numbers in patients with severe cases [5].

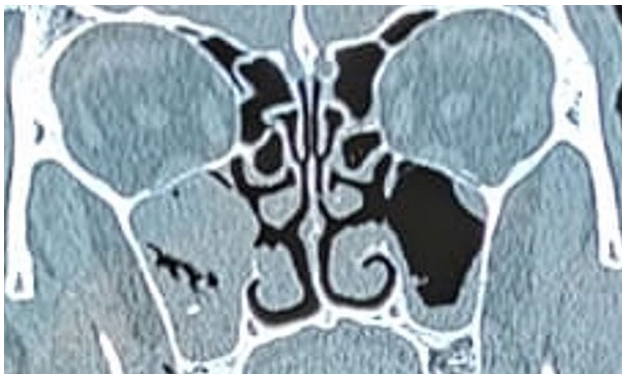


Fig. 1 Computed tomographic (CT) coronal section demonstrating classic mucor growth in the right maxillary sinus



Fig. 2 KOH mount demonstrating mucormycosis with broad aseptate or sparsely septate, ribbon-like hyphae with right-angled branching

From the literature thus far, it may be postulated that reduction and functional exhaustion of lymphocytes (including CD4+ T and other subsets) is the vital contributing factor for the surge in cases of rhino-orbital and cerebral mucormycosis post-SARS-CoV-2 infection.

Materials and Methods

A retrospective data compilation was done for patients diagnosed with ‘mucormycosis’ of the maxillofacial region post-recovery from COVID infection. Data was collected on a random selection basis after stratification by disease

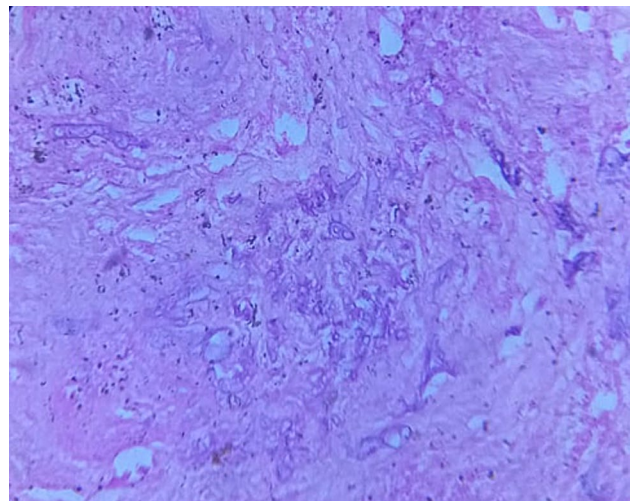


Fig. 3 Hematoxylin and eosin (H&E) stained specimen, demonstrating mucor hyphae

severity from the cases reported to the senior consultant and one of the authors of the present paper (DB) for opinion and consultation. All of the patients included for this study had a history of COVID infection that was confirmed using RT-PCR test results. The variables for which data was collected included age and sex of the patient, diabetic status, and treatment for COVID infection including oxygen administration, assisted ventilation, steroid administration, lymphocyte count, and CD4+ T cell count. All data collection was compiled by a single qualified research assistant.

Results

The observations gathered from the current study indicate that reduced lymphocyte and subset counts qualify as vital contributing factors for development of post-COVID rhino-orbital and cerebral mucormycosis. Twelve study samples were included (N = 12). Among the 12 patients, 8 (66.6%) were males and 4 (33.3%) were females. All the patients had a history of COVID infection preceding mucor infection, confirmed by RT-PCR. Eleven patients (91.6%) had a history of diabetes. Mucormycosis was diagnosed in the study population after COVID recovery and discharge from the hospital. Four (33.3%) patients developed mucormycosis less than one week after recovery from COVID, two (16%) patients in the first week, four (33.3%) in the second week, and two (16%) patients were three weeks post-COVID recovery (Table 1).

Only one (8%) patient did not receive any kind of supportive intervention such as oxygen supplementation or assisted

ventilation. The rest of patients, n = 11 (91%), required oxygen or assisted ventilation. The same eleven patients were also treated with steroids. Eleven patients (91%) had reduced lymphocyte count for weeks after COVID recovery (Table 1). Eleven patients (91%) also had reduced lymphocyte count below the normal cut-off value, with only one patient having a lymphocyte count in the normal reference range (at the lower limit side) at the 3rd week post recovery (Fig. 2).

Data was available for two (16%) patients regarding the CD4+ cell count and it was observed that it was significantly reduced. This suggested a possible correlation between reduced lymphocyte and CD4+ counts, along with co-morbidities, as contributing factors for mucormycosis. The use of medical oxygen and steroids in an immune-compromised host may also pave the way for fungal infections (Table 1; Fig. 4). Out of the 12 study patients, five (41.6%) fungal infections proved fatal within 2–3 weeks due to cerebral involvement.

Discussion

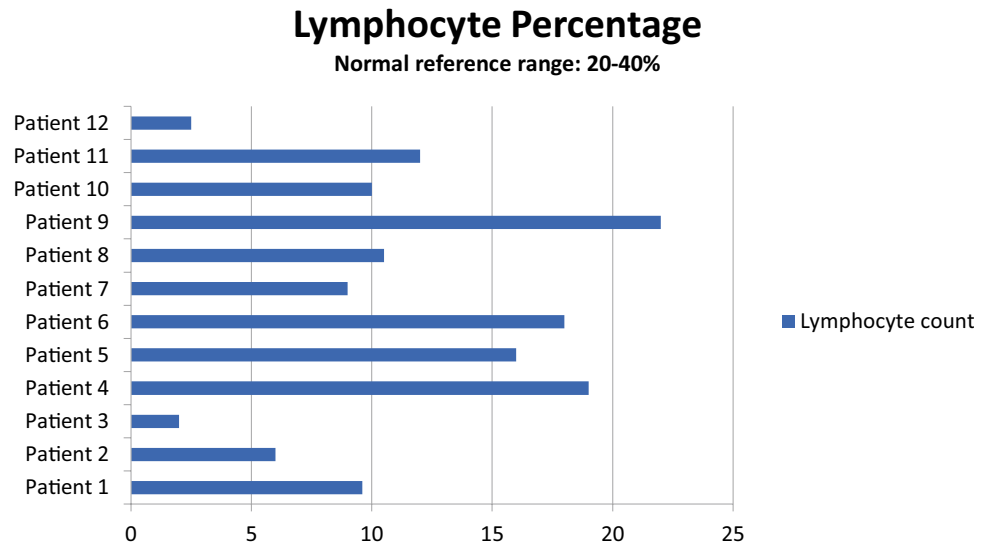
The possibility of opportunistic fungal infections in patients recovering from the COVID infection is now documented in the literature. Associated fungal infections are not just restricted to mucormycosis, but include a wide variety of organisms such as invasive aspergillosis, candidiasis, and cryptococcosis. It is essential to assess the risk factors, types of invasive mycosis, appropriate diagnostic methods, and the

Table 1 Summary of post-COVID patients, diagnosed with mucormycotic infection of the maxillofacial region

S. no.	Age	Sex	Diabetic history	Assisted ventilation/ supplemental oxygen support	Steroid use	Establish- ment of mucor infection(duration- post recovery from COVID) (in week post recovery)	Lymphocyte count(at the time of mucor diagnosis)(% using flowcytometry); nor- mal reference range 20–40%	CD4+ T cell status(at the time of mucor diagnosis)
1	60	M	Yes	Yes	Yes	Less than 1 week	9.6	NA
2	43	M	Yes	Yes	Yes	Less than 1 week	06	264
3	68	F	Yes	No	No	2nd week	02	NA
4	46	M	Yes	Yes	Yes	3rd week	19	NA
5	44	M	Yes	Yes	Yes	2nd week	16	NA
6	27	M	No	Yes	Yes	2nd week	18	NA
7	69	F	Yes	Yes	Yes	1st week	09	NA
8	63	M	Yes	Yes	Yes	2nd week	10.5	NA
9	55	M	Yes	Yes	Yes	3rd week	22	NA
10	55	F	Yes	Yes	Yes	Less than 1 week	10	NA
11	45	F	No	Yes	Yes	1st week	12	NA
12	57	M	Yes	Yes	Yes	Less than 1 week	08	412

NA data not available

Fig. 4 The lymphocyte percentage in the patients at the time of diagnosis of mucormycosis infection



requirement for individual treatment protocols in COVID-19 patients [2].

Diao et al. observed that the number of total T cells, including CD4+ and CD8+ counts, were intensely reduced in COVID-19 patients, especially in patients admitted to the Intensive Care Unit (ICU). T cells from COVID-19 patients had significantly higher levels of the exhausted marker PD-1, demonstrating functional exhaustion [6]. The absolute number of T lymphocytes, CD4+, and CD8+ T cells were markedly lower in severe COVID-19 cases than moderate cases, and were associated with markedly higher levels of IL-2R, IL-6, IL-10, TNF-alpha, and other inflammatory markers [7].

Liu et al. analysed 95 patients confirmed for COVID with and without diabetes mellitus (DM) and found that lymphocytes and subsets of lymphocytes, especially B and T cells, were reduced in COVID-19 patients, especially in those with DM. Patients with severe COVID-19 and DM had the lowest lymphocyte counts compared to patients with severe COVID-19 without DM and those with non-severe COVID-19 with or without DM. Observations indicate that immune system dysfunction or dysregulated host immune response is commonly noted in individuals affected with COVID-19. The authors concluded that the immune-modulatory treatment should be considered to reduce disease progression and prognosis of COVID-19 patients, especially in patients with systemic co-morbidities [8].

Evaluation of dynamic changes in peripheral blood lymphocyte subsets in adult patients with COVID-19 demonstrated that the various lymphocyte subsets (CD3+, CD4+, CD8+, CD19+, and CD16/56+) were below normal ranges at one week after the onset of illness. The lowest point was reached during the second week. The subsets increased gradually during the third week and returned to normal levels in the fifth week, but remained lower than those of the healthy

controls. The CD3+, CD4+, and CD8+ counts were lower in patients with severe disease compared to those with non-severe disease, and in patients who died compared to those who recovered [9].

Liu D et al. demonstrated that critically ill patients with COVID-19 and DM had lymphocyte and subset counts that were significantly reduced at one week, rapidly elevated to peak at 2 weeks after onset, and then gradually declined during recovery. The COVID-19 critically ill patients with DM had the lowest decline at one week and a slow and lowest rise at two weeks after onset, while COVID-19 non-critically ill patients with DM had the highest rise at two weeks after onset. Both these groups had similar lymphocyte and subset counts at five weeks after onset, which were lower than patients without DM [10]. In addition to the decrease in T cell count, there was a disruption of the T cell differentiation balance in COVID-19 patients, which affects T cell function [11].

In a multicenter, retrospective study conducted by Wen et al., the absolute numbers of T-lymphocytes, CD4+ T cells, and CD8+ T cells were decreased in almost all COVID-19 patients, with significantly lower numbers in patients with severe COVID-19 (294.0 , 177.5 , and $89.0 \times 10^6/L$) than in patients with moderate COVID-19 (640.5 , 381.5 , and $254.0 \times 10^6/L$). This finding suggested that SARS-CoV-2 infection majorly affects T-lymphocytes, especially CD4+ T cells and CD8+ T cells [12].

The significant reduction of the absolute number of lymphocytes, specifically T cells, described in the most of the severe COVID-19 cases, may be associated with the worse outcome and a higher risk of developing opportunistic infections. Mucormycosis is a fungal infection caused by a group of opportunistic molds, i.e., mucormycetes [13]. The role of T-cells in invasive mucor as part of the adaptive immune system is highlighted by Potenza L et al. in a brief report on

a group of haematologically ill patients who suffered from mucormycosis. Their researchers described Mucorales-specific T-cells (CD4+ and CD8+) that produced cytokines, such as IL-4, IL-10, IL-17 and IFN- γ , that directly damaged Mucorales hyphae [14]. It is essential to monitor the moderate to severely ill COVID patients with fungal pathogen surveillance through clinical and histopathologic examination, as well as PCR based assays.

Through the observation of this study and previously reported data by various authors, the reduction and the functional exhaustion of lymphocytes (including CD4+ T and other subsets) should be considered as the vital contributing factor for the surge in cases of rhino-orbital and cerebral mucormycosis post SARS-CoV-2 infections, apart from the established predisposing factors that include: ketoacidosis, high blood sugar, iron metabolism related aberrancy, role of ferritin, high serum iron, free radical-induced endothelitis, hepcidin activation, and upregulation of glucose receptor protein (GRP78) [15].

Conclusions

With the limitation of our small sample size, the relation between functional exhaustion with persistently reduced lymphocytes and subset counts in SARS-CoV-2 in diabetics and vulnerability to post COVID rhino-orbital and cerebral mucormycosis should be considered as a vital parameter for further research. In patients post- SARS-CoV-2 who experienced moderate to severe symptoms, future research, interventions, and therapies should be targeted at prevention of functional exhaustion of the lymphocytes and maintaining the optimal lymphocyte and subset counts with intended effort to prevent opportunistic fungal infections in susceptible hosts. Furthermore, aseptic hygiene protocols are required for the care of such patients, as endorsed through observations of this study and the previously reported data.

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Code Availability NA.

Declarations

Conflict of interest None.

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Consent for Publication NA.

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