

MUCORMYCOSIS DURING THE SECOND WAVE OF COVID19 PANDEMIC IN SOUTH RAJASTHAN: AN ANALYTICAL CROSS SECTIONAL STUDY

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INTRODUCTION

Mucormycosis is an opportunistic infection caused by several fungi belonging to phylum Glomeromycota. They are found ubiquitously in the soil and environment.

They are classified under subphylum Mucoromycotina and Entomophthoromycotina. Mucormycosis caused by members belonging to subphylum Mucoromycotina, order Mucorales, is the most clinically important and includes the genera Lichtheimia, Mucor, Rhizomucor and Rhizopus species. It is a very rapidly progressive (because of its angioinvasive nature) thereby may prove fatal if timely diagnosis is not made entailing delay in institution of specific treatment. It presents as six clinical types Rhino-orbito-cerebral, Pulmonary, Cutaneous, Gastrointestinal, Isolated Renal and Disseminated Mucormycosis on the basis of anatomical site involved in a particular patient.^[3]

The advent of the second wave of Covid 19 in India has coincided with an unprecedented rise in the number of cases of mucormycosis in India. Almost all these cases are presenting as Rhino-orbito-cerebral mucormycosis. The Government of Rajasthan declare mucormycosis a notifiable disease on 19th May 2021.

Various causative factors have been proposed behind this geometric rise in the number of patients with invasive mucormycosis. These include, but are not limited to the often indiscriminate use of steroids for the treatment of Covid 19, uncontrolled diabetes both drug and disease induced, immunomodulatory therapy, prolonged oxygen therapy and possibly the virus induced changes themselves. However the exact contribution of these factors needs to be further explore in detail before conclusive association or causation can be determined.

Mucormycosis has been commonly caused by Rhizopus arrhizus. However, it remains to be established whether the causative agent causing the current cases are same or different from the above species.

The present study was therefore designed to find out KOH confirmed cases of invasive mucormycosis that presented at our institute and investigate the causative species causing the present surge in mucormycosis and the possible clinical and epidemiological factors contributing to the same.

MATERIAL AND METHODS:

The present study was an analytical cross sectional study in patients of mucormycosis presenting to RNT Medical College and associated group of hospitals, Udaipur, Rajasthan. It was commenced after obtaining clearance from institutional ethical committee.

CRITERIA FOR SAMPLE SELECTION:

Inclusion criteria - Amongst the samples received in the Microbiology laboratory from 20th May 2021 to 19th July 2021, samples with KOH confirmed mucormycosis were included in the study.

Exclusion criteria - Swab and Sputum samples were not included in our study. Patients with previously established fungal infections were excluded from the study.

KOH MOUNT AND CULTURE

The direct demonstration of fungal elements is essential in establishing diagnosis by detecting presence of causative agent in the clinical material. Slide and tube KOH mounts were prepared to establish the diagnosis. For tissue biopsy tube KOH method was used.^[5]

TUBE KOH TEST

1. Tissue material was placed in a test tube and 10%-20% KOH was added in a test tube. Preparation with KOH clear the tissue and cellular debris from all types of clinical specimens without damaging the fungal cells.
2. Clearing process required 20-30 minutes. Some samples were incubated overnight at 37°C.
3. Following incubation, a drop of suspension was placed on the clean slide and cover slip was placed over it.
4. The slide was examined under the microscope in 10X and 40X objectives.
5. In case of mucormycosis characteristic broad, non-septate, ribbon like hyphae with wide angle or right angle branching at irregular intervals were seen in KOH mount.

SLIDE KOH TEST

10-20% KOH was mixed with equal proportions with the specimen on a slide and a cover slip was placed over it. After that the slide was examined under the microscope in 10X and 40X objectives.

FUNGAL CULTURE

The hyphal elements of mucormycetes are prone to physical damage, so excessive grinding was avoided and specimens were directly inoculated onto the fungal culture media i.e. Sabouraud's dextrose agar with antibiotics and without cycloheximide and was incubated at both temperatures i.e. 25°C and 37°C. Cultures were examined for the expected growth, daily in the first week of incubation. All Mucorales grow rapidly within 3-5 days.

CLINICAL DATA COLLECTION

Data was collected from the patients through structured questionnaire by an investigator who did not take any further part in the study. This was done to prevent any bias.

Categorical data was expressed as absolute frequencies and percentage and compared using the Chi-square test/Fisher exact test as applicable. Relative risk and number needed to harm (NNH) was calculated for each potential risk factor for mucormycosis.

RESULT

The present study attempted to find a correlation between the occurrence of mucormycosis, its causative species and certain possible risk factors including prior COVID -19 infection and variables associated with COVID – 19 severity and treatment.

The microbiological findings of the 47 KOH positive cases of mucormycosis included in our study are detailed in Table – I. Of these 47 patients, 23 presented with mucormycosis within 2 weeks of RTPCR confirmed COVID – 19 infection while the remaining 24 presented after 2 weeks.

Table 1.

CULTURE POSITIVE	26
CULTURE NEGATIVE	21
TOTAL KOH POSITIVE SAMPLES	47

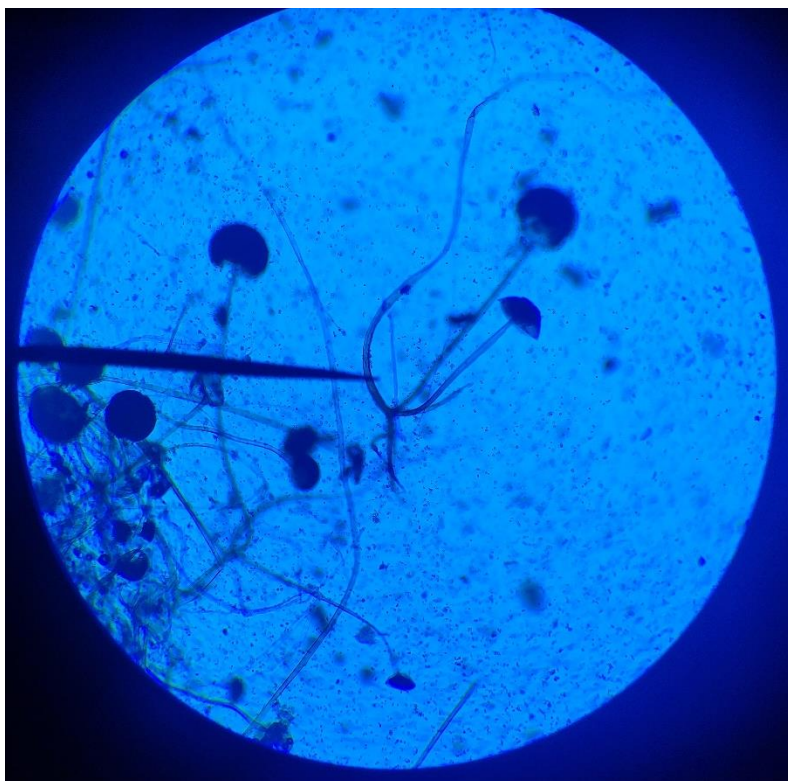
- In our study, Rhizopus species was found to be the causative agent of mucormycosis in all culture positive samples.

**Fig showing gross appearance
of mucor on SDA**



Fig showing broad aseptate hyphae in KOH

LPCB mount of *Rhizopus* species



We found that, of the total 47 patients included in the study, 40 had Diabetes mellitus (22 new onset, 18 known diabetic, Fig. 1). This was statistically significant ($P < 0.05$). Similarly, 45 patients had history of COVID – 19 infection (Fig. 2), a number that was highly significant ($P < 0.001$).

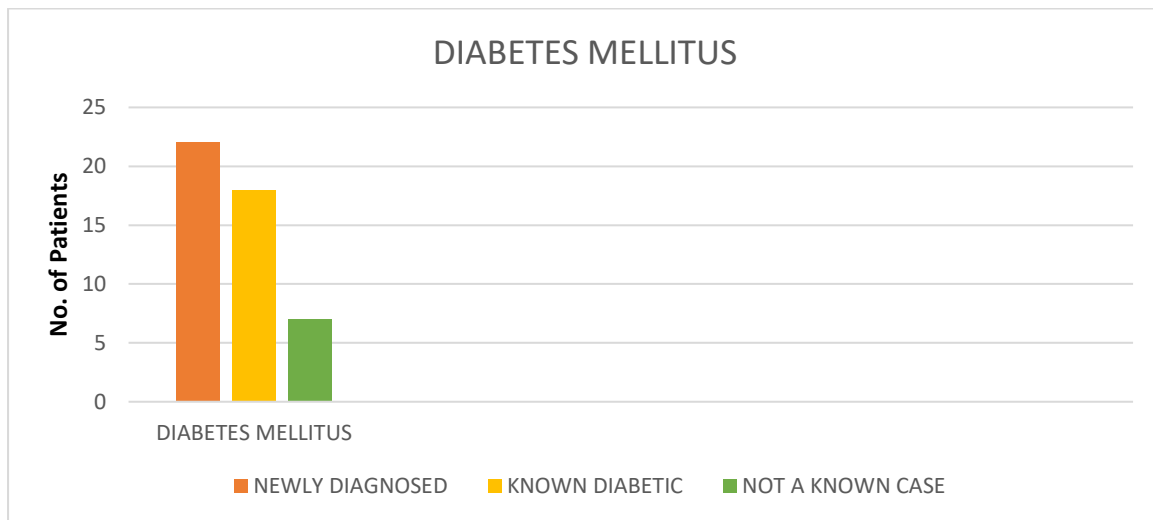


FIG: 1

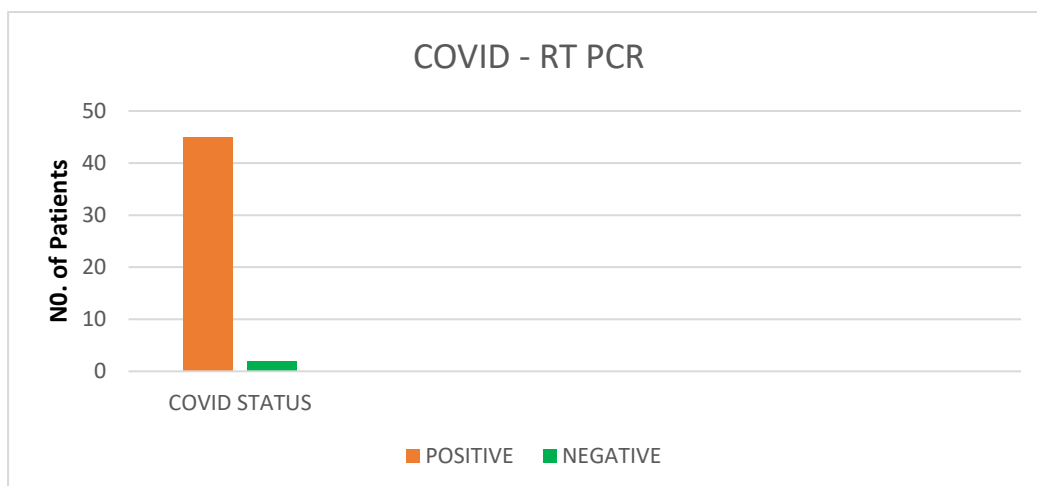


FIG:2

On the other hand among 24 patients out of 47 reported, there was no steroid (Fig. 3) use during COVID treatment and 31 patients didn't receive any oxygen therapy (Fig. 4) during COVID treatment. Both these values were statistically significant, with a P value of < 0.05 .

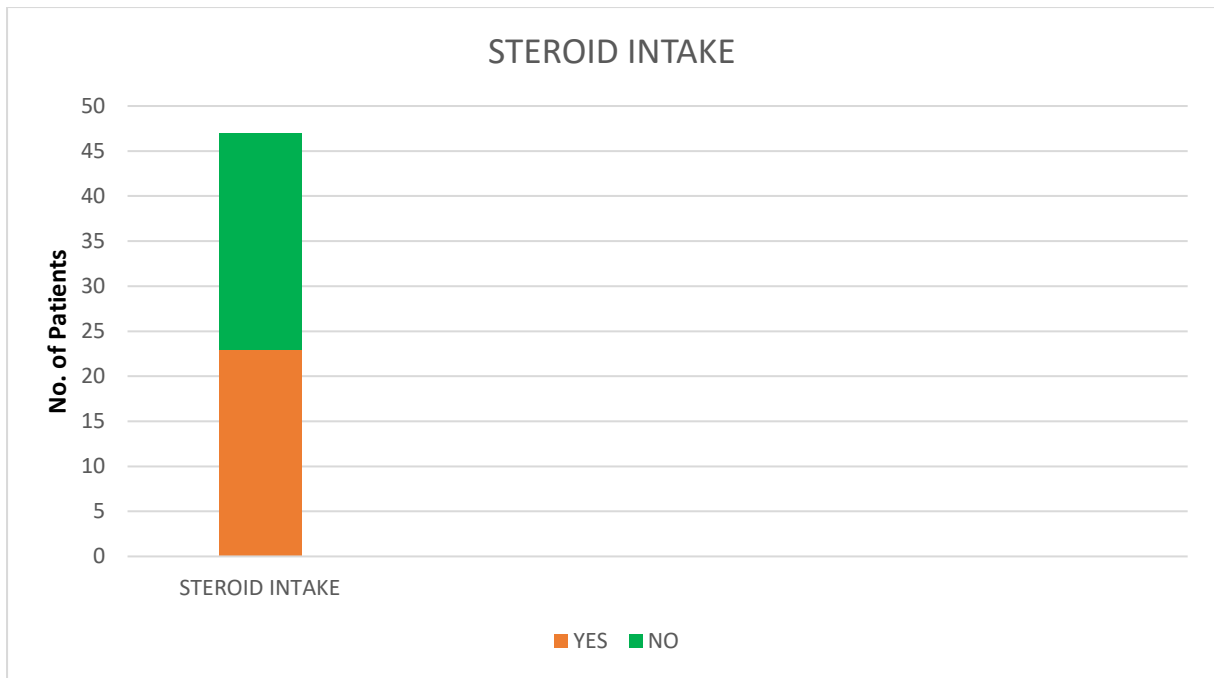


FIG:3

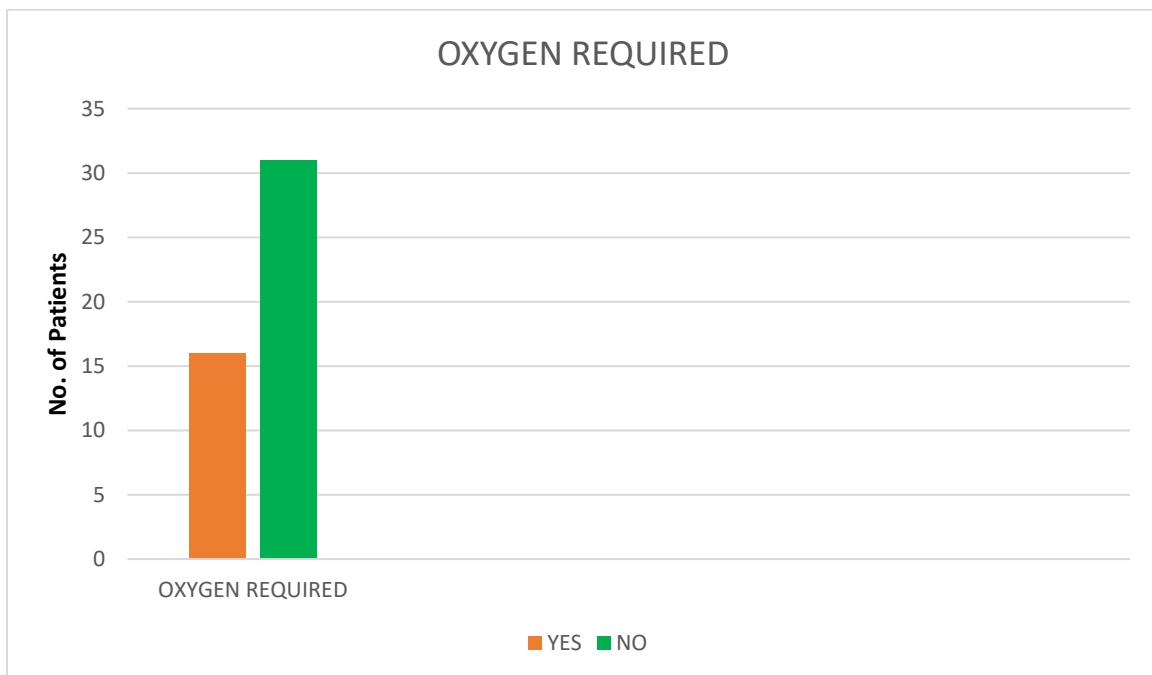
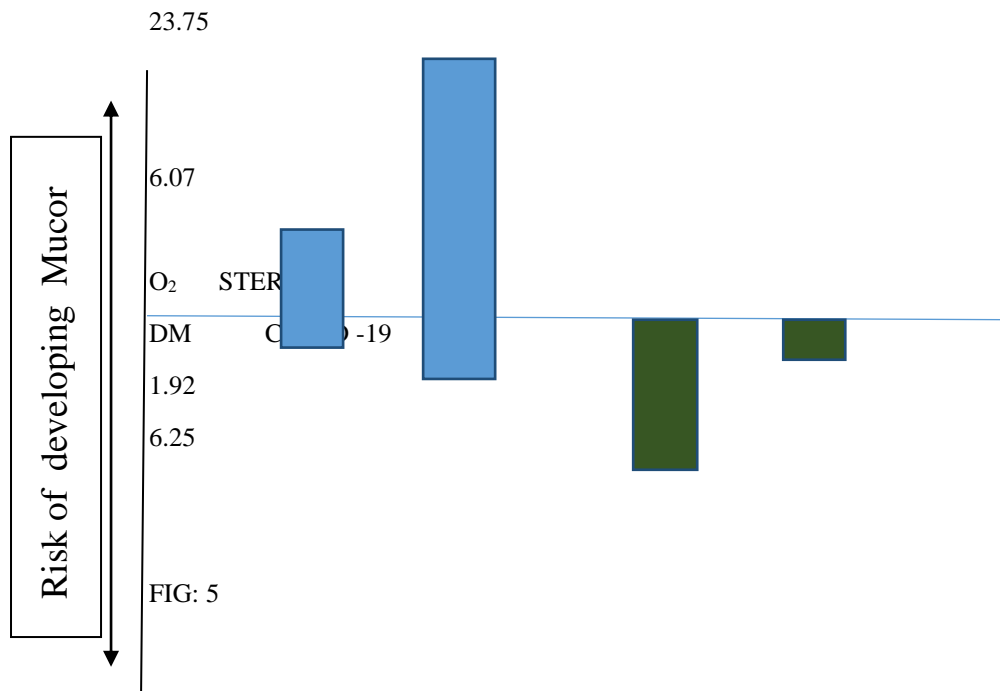


FIG:4

An estimation of the relative risk for each of these four factors in the development of mucormycosis showed that the presence of Diabetes mellitus and Covid positivity increased the chances of development of invasive mucormycosis by 6.07 and 23.75 times respectively.[Fig. 5]

Oxygen (6.25) and systemic corticosteroid therapy (1.92) instead had a negative correlation to the incidence of mucormycosis in our study.



The number needed to harm (NNH) for the development of mucormycosis was 1.40 and 1.09 for Diabetes mellitus and COVID – 19 infection respectively.

DISCUSSION

The unprecedented surge of invasive mucormycosis in India following the deadly second wave of COVID – 19 led to several attempts to identify the causative and contributing factors of this fungal disease and hence prevent its future recurrence. Our study is an attempt.

Rhizopus species was the causative agent in all culture positive cases in our study. Pal R et al^[8] also reported Rhizopus species as the predominant pathogen causing COVID associated Mucormycosis in their study.

The present study similar to studies conducted by Chauhan K^[4], Moorthy A^[6] and Nehra HR^[7] found that the two key factors that led to mucormycosis in patients that we studied were Diabetes mellitus (40/47 patients; 85.10 %; P < 0.05) and prior COVID – 19 infection (45/47; 95.74 %; P < 0.001). Both these factors conferred a 6.07 and 23.75 times increased risk of development of mucormycosis respectively.

However, contrary to other studies, like Sharma S et al^[9] and Al-Tawfiq et al^[1], we did not find an association between the administration of steroids and oxygen as part of covid treatment and the development of mucormycosis.

On the other hand, we found that steroid use and oxygen therapy reduced the incidence of mucormycosis by 1.92 and 6.25 times respectively.

Our results are in agreement with those of Arora et al^[2], who have also reported that 22 out of the 60 patients that they evaluated for mucormycosis did not receive any steroid and oxygen therapy in the preceding period.

One possible explanation of these findings maybe that the immunosuppressive effects of COVID – 19 infection per se and Diabetes mellitus may itself render the host susceptible to invasive mucormycosis. At the same time judicious use of oxygen and steroids may have contributed to a reduced impact of COVID – 19 in patients who

did not develop mucormycosis in our study. Further, factors such as recall bias and ignorance of steroid consumption by some of the patients may have contributed to these results.

A detailed study further evaluating the course, duration and dose of oxygen and steroid therapy in a longer cohort of COVID – 19 patients is warranted to further establish or refute the association with mucormycosis.

As all these predisposing factors were also present during first wave of Covid – 19, the occurrence of mucormycosis in second wave warrants further study on this new Delta variant of Corona virus.

CONCLUSION:

In our study, we found 26 culture positive cases out of 47 KOH positive cases and *Rhizopus* species was the causative agent in all culture positive cases.

Diabetes mellitus and previous COVID – 19 infection were associated with increased incidence of Mucormycosis.

The role of Oxygen and Steroid therapy need further evaluation as our study shows decreased incidence of mucormycosis in patients received oxygen and steroid therapy.

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