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Original Research Article

A Clinicomycological Study of Suspected Mucormycosis in Post COVID Patients Attending a Tertiary Care Hospital

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Abstract:

Background: Most of the studies done on this emerging disease in India, as well as investigations throughout the world, are retrospective. Due to increase in number of cases within a short duration, diverse risk factors and inclusion of immunocompetent and immunocompromised patients, there is need of prospective study so that suspected cases can be diagnosed in a timely manner, various risk factors can be analyzed and accordingly patients appropriately treated, which should results in the increase of patient survival.

Methods: A prospective observational study was undertaken over a period of ten months from March to December 2021. A total of 175 biopsy samples were collected from 150 suspected cases of post covid mucormycosis patients in sterile normal saline at the ENT department and received in the department of microbiology. MRI of brain, orbital and paranasal sinus was done in all patients.

Results: The prevalence of mucormycosis laboratory confirmed was found to be 42.66% . Out of 150 Patients, 47 were KOH positive while 33 culture positive. The most common isolate was *Rhizopus arrhizus* (84.85%) followed by *R.microsporus var. rhizopodiformis* (9.09%) , *Rhizomucor pusillus* (3.03%) and *Saksenaea vasiformis* (3.03%). Rhino-orbital-cerebral mycormycosis was the most common clinical form (33 cases). Diabetes mellitus was the most common predisposing factor n=112, (74.66%) . Many patients had multiple symptoms like periorbital swelling (52%) , headache (44.7%) , fever (41.3%) , loss of vision (16%) , facial swelling (27.3%) and nasal pain (8.7%). Invasive fungal sinusitis was seen in MRI in 116 (77.33%) cases. Tissue debridement was done in all positive patients along with treatment with liposomal amphotericin B (1.5- 5.0 mg/kg/d) and then switched over to oral posaconazole 300 mg BD on first day then 300 mg OD for several weeks. Inspite of rigorous treatment in 4.6% cases the prognosis was not good resulting in mortality. Also in 40 % of the patients who recovered, long-term morbidity related to vision was observed.

Conclusion: Present study determined the fungal etiology of post covid mucormycosis with *Rhizopus arrhizus* being the most common cause in our region comparable to other studies from India. The identification of risk factors, clinical features and laboratory findings helped in early diagnosis, leading to prompt treatment and favourable outcome. With diabetes mellitus being the most common predisposing factor seen, it is suggested that holistic approach to prevent, revert and treat this condition need to be strengthened.

Key words: Mucormycosis, COVID, Lab finding

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome. Coronavirus 2 (SARS-CoV-2) has been associated with a wide range of opportunistic bacterial and fungal infections. Both Aspergillus and Candida have been reported as the main fungal pathogens for co-infection in people with COVID-19.[1]

Recently, several cases of mucormycosis in people with COVID-19 have been increasingly reported world-wide, in particular from India. The primary reason that appears to be facilitating Mucorales spores to germinate in people with COVID-19 is an ideal environment of low oxygen (hypoxia), high glucose (diabetes, new-onset hyperglycemia, steroid-induced hyperglycemia), acidic medium (metabolic acidosis, diabetic ketoacidosis [DKA]), high iron levels (increased ferritins) and decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated or background comorbidities) coupled with several other shared risk factors including prolonged hospitalization with or without mechanical ventilators.[2]

It is mainly diagnosed by laboratory analysis of the biopsy isolated from the site of infection. In addition, other imaging tests like CT are also beneficial for diagnosis. This condition can be classified into six forms namely rhinoorbital cerebral mucormycosis (ROCM), pulmonary, cutaneous, gastrointestinal, disseminated and uncommon sites based on the location of their occurrence. Among them, ROCM is the most commonly occurring one. Among the species that cause mucormycosis, the Rhizopus species was linked with ROCM. At the same time *Cunninghamella* was found in the pulmonary or disseminated form, while *Apophysomyces* and *Saksenaea* were seen in the cutaneous type. The most common sites of infection are sinuses (39%), lungs (24%), disseminated (23%); and skin and soft tissue infection (19%).[3]

Importantly, DM has been the most common risk factor linked with mucormycosis in India, although hematological malignancies and organ transplant takes the lead in Europe and the USA.4,5 India is already having second largest population with diabetes mellitus (DM) and was the diabetes capital of the world, until recently.[4]

While long term use of corticosteroids have often been associated with several opportunistic fungal infection including aspergillosis and mucormycosis, even a short course of corticosteroids has recently been reported to link with mucormycosis especially in people with DM.[4]

Moreover, rapidity of dissemination of mucormycosis is an extraordinary phenomenon and even a delay of 12 h in the diagnosis could be fatal, the reason 50% of cases of mucormycosis have been historically diagnosed only in the post-mortem autopsy series.[4]

Signs and symptoms of rhino-orbito-cerebral mucomycosis (ROCM) are diplopia, proptosis, ptosis, ophthalmoplegia and neurological involvement. Although not very common, but the invaded necrosed tissue may appear as ―black eschar, due to which this condition has been commonly referred to as ―Black Fungus.[6] Mucormycosis is an angioinvasive fungus that is usually present in the environment and grows on wet surfaces and dead and decaying vegetable matter. The term ―black fungus being used for mucormycosis is incorrect as the term ―black fungus is used for dematiaceous

fungi, which are an entirely different group.[7]

High degree of clinical suspicion and endoscopic findings supported by microbiological staining and culture reports confirm the diagnosis. CT scans help surgical planning by delineating the extent of the disease. For assessing intracranial and orbital involvement, MRI proved superior to CT, besides having role in follow-up. Prompt diagnosis followed by immediate and aggressive management is key to prevent morbidity and mortality.[8]

Most of the studies done on this emerging disease in India, as well as investigations throughout the world, are retrospective. Due to increase in number of cases within a short duration, diverse risk factors and inclusion of immunocompetent and immunocompromised patients, there is need of prospective study so that suspected cases can be diagnosed in a timely manner, various risk factors can be analyzed and accordingly patients appropriately treated, which should results in the increase of patient survival.

Material and Method

A prospective observational study was undertaken over a period of ten months from March to December 2021. A total of 175 biopsy samples were collected from 150 suspected cases of post covid mucormycosis patients in sterile normal saline at the ENT department and received in the department of microbiology. MRI of brain, orbital and paranasal sinus was done in all patients.

Collection and transportations of sample:

Biopsy material from clinically suspected individuals of mucormycosis was transported in sterile, leak-proof container containing 2-3 ml normal saline and processed in mycology laboratory.

Processing of sample:

1. Direct microscopic examination by KOH MOUNT:

The first portion of specimen was first teased (don't grind) with the help of teasing niddle then prepare direct KOH mount. Few drops of 20-40% potassium hydroxide (KOH) was added to the material and kept for 10-30 min to dissolve the tissue upon the thinkness of scale & gently heating the slide to clear the tissue. The slide was examined under microscope using LP (10x) and HP (40x) for the presence of fungal elements. The fungal hyphae are identified as broad, non-septate, ribbon-like hyphae with wide-angle or right-angle branching at irregular intervals.

2. Culture and Incubation:

Second portion of specimen was cut into small pieces and inoculated into pair of tubes of the following media: Sabouraud's Dextrose Agar with 0.05% chloramphenicol, one tube from each set incubated at 37°c and at 22°c for a minimum 5-7 days. The sample was also be inoculated in brain heart infusion broth (BHI) and incubated at 37°c and was observed periodically for growth.

Fungal isolate was identified based on colony morphology, growth rate, pigmentation, microscopy (LPCB), Growth temperature test. Sporulation may be stimulated by the use of nutrient-deficient media, such as yeast extract agar.

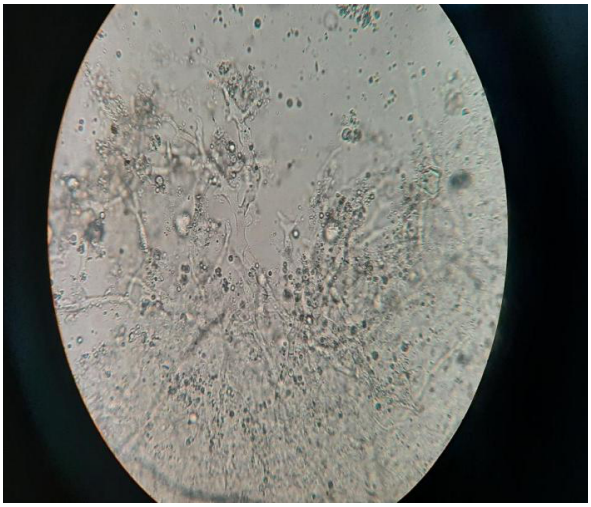

Figure 1: (a) KOH wet mount preparation of tissue sample showing broad sparsely septate fungal hyphae

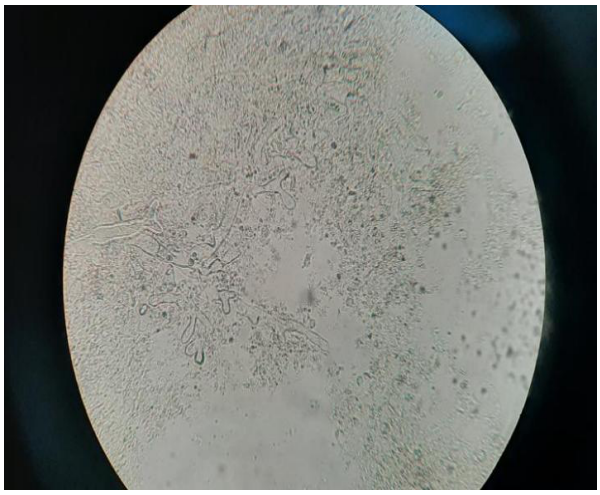

Figure 1: (b)KOH wet mount preparation of tissue sample showing broad,ribbon-like , non septate hyphe wide angle branching (400x magnification)

Results & Observation

The prevalence of mucormycosis laboratory confirmed was found to be 42.66%. Out of 150 Patients, 47 were KOH positive while 33 culture positive. The most common isolate was *Rhizopus arrhizus* (84.85%) followed by *Rhizopus microsporus var. rhizopodiformis* (9.09%) , *Rhizomucor pusillus* (3.03%) and *Saksenaea vasiformis* (3.03%).

Rhino-orbital-cerebral mycormycosis was the most common clinical form (33 cases). Diabetes mellitus was the most common predisposing factor n=112, (74.66%). Many patients had multiple symptoms like periorbital swelling (52%), headache (44.7%) , fever (41.3%) , loss of vision (16%) , facial swelling (27.3%) and nasal pain (8.7%). Invasive fungal sinusitis was seen in MRI in 116 (77.33%) cases.

Tissue debridement was done in all positive patients along with treatment with liposomal amphotericin B (1.5- 5.0 mg/kg/d) and then switched over to oral posaconazole 300 mg BD on first day then 300 mg OD for several weeks. Inspite of rigorous treatment in 4.6% cases the prognosis was not good resulting in mortality. Also in 40 % of the patients who recovered, long-term morbidity related to vision was observed.

Table 1

Complaints	Total (N=150)
Fever	62 (41.3%)
Headache	67 (44.7%)
Eye Edema	78 (52%)
Loss Of Vision	24 (16%)
Nasal Pain	13 (8.7%)
Face Swelling	41 (27.3%)

Table 2: Fungal Etiology of Culture Positive Mucormycosis

Isolates	Number Of Isolates	Percent
Rhizopus arrhizus	28	84.85%
Rhizopus microsporus var. rhizopodiformis	3	9.09%
Rhizomucor pusillus	1	3.03%
Saksenaea vasiformis	1	3.03%
Total	33	100.0

Rhizopus arrhizus is most commonly isolated from rhino-orbitocerebral mucormycosis.

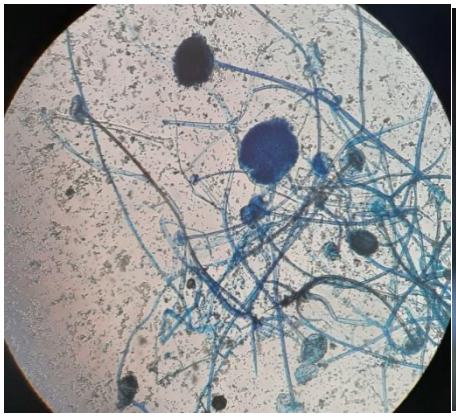

Figure 2: (a)

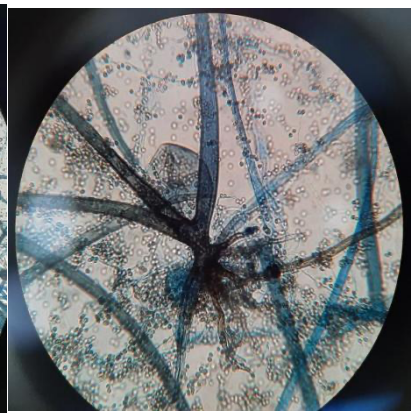

Figure 2: (b)

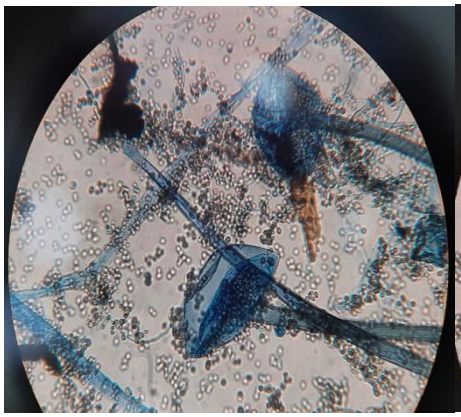

Figure 2: (c)

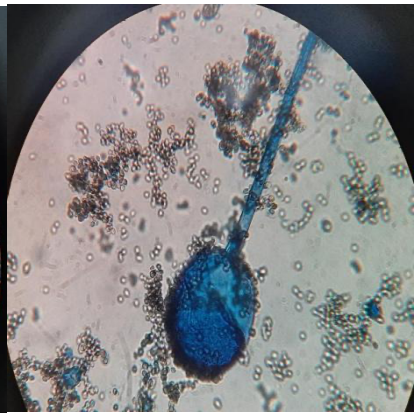

Figure 2: (d)

LPCB Mount of *Rhizopus arrhizus* showing
a) Unbranched sporangiophore, produced in groups (10x)
b) Nodal rhizoid seen (40x)
c) Umbrella shape of collapsed columella (40x)
d) Globose shape sporangium seen (40x)

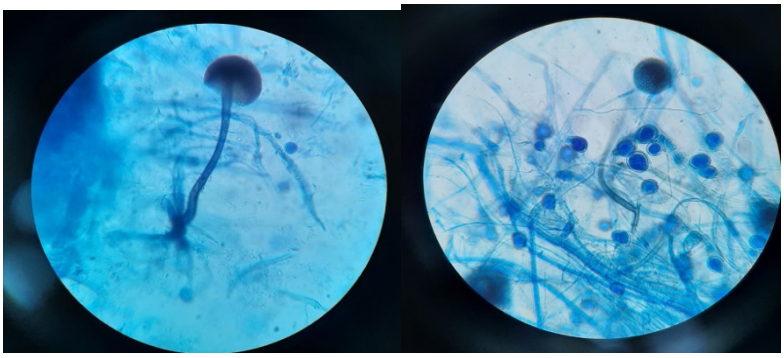

Figure 3(a) **Figure 3(d)**

LPCB Mount of *Rhizopus microsporus var. rhizopodiformis* showing

a). Short, brown sporangiophores with rhizoids (40x)

b). Thick –walled chlamydospores seen, (40x)

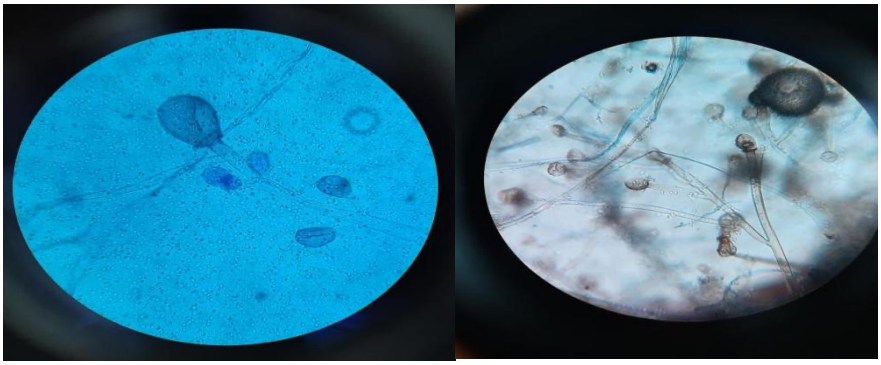

Figure 4: (a) **Figure 4: (b)**

(a)& (b) LPCB of *Rhizomucor pusillus* showing branched sporangiphores (40x)

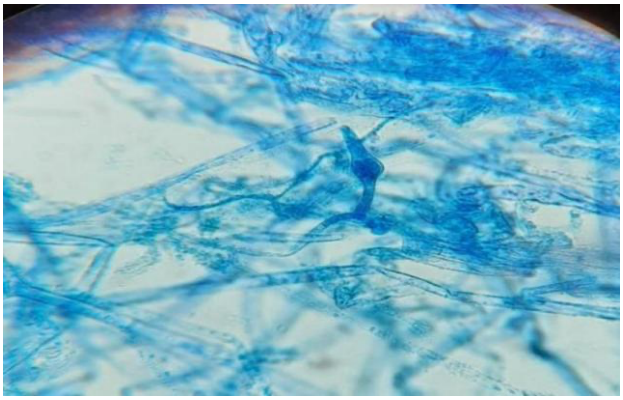

Figure 5.

LPCB of *Sakenaea vasiformis* showing flask-shape sporangium after 3 week of incubation (40x)

Discussion

In this study total 150 patients included on the bases of RT-PCR it's divided into two group 108 patients were positive and rest 42 patients were negative (suspected) for covid-19.

In our study the mean age was 45.6 years. Most of the patients (71.3%) were males. In the study by Noha Ahmed et al. (2021)[9], the mean age was 52.92 ± 11.30 years. More males were included. In the study of Dr Raghavi et al. (2019)[10] the mean age was 50.28 years. 58% patients were males.

Most of the patients (72%) were positive and 60% were not vaccinated against COVID-19 in the current study. Similarly 59.4% patients had active COVID-19 in the study of Awadesh kumar et al[11] while 101 patients out of 105 patients included were positive for COVID-19 in the study of Hardeva et al.(2021)[12]

In the current study, many patients had Diabetes N=112 (74.66%), hypertension N=14(9.33%), steroid users N=35 (23.33%) and immunocompromised N=16 (10.66%). Most of the patients had eye edema (52%), headache (44.7%) and fever (41.3%). In the study of Hardeva et al.12 diabetes was seen in 78.10% cases. 66.30% patients were steroid users. 80% patients had diabetes, 3% had malignancy and 1% were post-transplant patients out of 101 patients included in the study of Awadesh Kumar et al[11]. Diabetes mellitus was the most common co-morbidity (36%) of which 48% had diabetic ketoacidosis. 17% had malignancy and 7% underwent organ transplantation (7%), and no underlying condition was seen in 19% in the study of Maureen et al. (2005)[13]. In the study of Raghaviet al[10]. most common symptoms were nasal obstruction (88%) (Mostly unilateral), and headache (82%) (Especially in the frontal region).

In our study 31 patients (20.66%) were KOH positive but culture negative. 19 patients (12.66%) were positive for both KOH and culture. In the study of Kiran Bala et al.[14], the mucormycosis cases were positive by KOH (84%), and culture (61%). Histopathological and microbiological (KOH & Culture) investigations were positive in 34.2% cases, and those processed by only KOH and culture tests were positive in 23.7% cases. In our study, histopathology was not done. MRI, D - Dimer, CRP and IL6 were evaluated. Invasive fungal sinusitis was seen in MRI in 116 patients out of 150 patients included in the current study.

In our study, among 33 culture positive isotates were *Rhizopus arrhizus* (84.85%) ,*Rhizopus microspores var.rhizopodiformis* (9.09%), *Rhizomucor pusillus (*3.03%) and *Saksenaea vasiformis* (3.03%). In the study of KiranBala et al[14], among 23 culture isolates, *Rhizopus arrhizus* (37.5%) was the most commonly isolated fungus, followed by *Apophysomyces variabilis* (29.2%), *Lichtheimia* (16%), *Rhizopus microsporus* (4.2%) etc. No patient had *Apophysomyces* or *Lichtheimia* in the current study.

In current study majority of patients survived (95.31%) while 4.6% patients succumbed inspite of Amp + Posa. However majority of patients who survived complained of severe morbidity especially loss of vision, ptosis, loss the smell and facial palsy. In the study of Yogendra Mishra et al[15]., endoscopic debridement was done in 93.8% patients. Over all 12.5% patients did not survive, 5 (15.6%) were discharged and the rest are in hospital on parenteral antifungals. In the study of Noha Ahmed et al.[9], endoscopic debridement was done in 66% cases. 77.8% received Amphotericin B, 27.8% received Voriconazole and 8.3% received Posaconazole. 63.89% survived and 36% died. In the study of KiranBalaet al.[14], liposomal amphotericin B was given in dose of 5 mg/kg/day to 74% patients. Conventional amphotericin B was given in 11% patients. Out of 38 patients, 61% patients survived. 24% patients died due to mucormycosis.

Conclusion:

Present study determined the fungal etiology of post covid mucormycosis with *Rhizopus arrhizus* being the most common cause in our region comparable to other studies from India. The identification of risk factors, clinical features and laboratory findings helped in early diagnosis, leading to prompt treatment and favourable outcome. With diabetes mellitus being the most common predisposing factor seen, it is suggested that holistic approach to prevent,

revert and treat this condition need to be strengthened.

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