

Original Research Article

A Microbiological Study on Abrupt Rise of Mucormycosis Cases in the Backdrop of Covid-19 in a Tertiary Care Hospital in West Bengal

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Abstract

Background: There is increasing evidence of mucormycosis in COVID-19 patients during or post treatment caused by different microorganisms and for their culture, specimens from certain sites are collected. The present study was conducted to assess different genera causative of mucormycosis, to describe the suitability of specimens for their culture and to determine the relationship between mucormycosis and COVID-19. **Methods:** The descriptive cross sectional study was conducted among all the 50 samples which were received at Microbiology laboratory, Bankura Sammilani Medical College and Hospital during May to September 2021 inoculating them in SDA and SDCA media within a class II bio safety cabinet and after incubating for at least 7 days at room temperature, processing for 40% KOH mount and LCB staining were done. Slides were observed under light microscope. Principles of descriptive statistics were used. Chi square test was done where applicable. **Results:** 44% of samples showed no growth while 30% and 26% of them exhibited the growth of *Mucor* and *Rhizopus* respectively. Majority of the mucorales were seen growing on the samples collected from nasal septum followed by middle meatus and maxillary sinus. Significant association was noted between the occurrences of mucormycosis and infection with COVID19 ($p < 0.001$). **Conclusions:** *Mucor* and *Rhizopus* are the main causative genera of mucormycosis and the suitable specimens for culture of them are tissue from nasal septum, middle meatus, maxillary sinus etc. Mucormycosis is significantly associated with COVID positivity.

Keywords: Mucormycosis, COVID19, *Mucor*, *Rhizopus*, Nasal septum

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Introduction

Mucormycosis is a devastating and angio-invasive infection with high mortality rates. In Corona virus disease 2019 (COVID19) pandemic situation, the incidence of mucormycosis has increased rapidly. Post SARS COV-2 invasion, Immune-suppression, hyperglycaemia and elevated serum ferritin level along with steroidal treatment creates a storm for opportunistic infection like Mucormycosis. There is increasing evidence of Mucormycosis co-infection in COVID-19 patients during or post treatment. It is caused by the fungi of the Mucorales order of the class of Zygomycetes[1]. It is an opportunistic infection; mainly affecting the patients of Diabetes Mellitus (DM), patients with malignancy, chronic renal failure, acquired immunodeficiency syndrome, neutropenia, patient receiving steroids and having history of organ transplant[2]. The incidence of mucormycosis is gradually increasing because of increasing incidence

of DM[3].

Human mucormycosis is caused by pathogenic species like *Rhizopus*, *Mucor*, *Rhizomucor*, *Lichtheimia*, and *Cunninghamella*[4]. *Rhizopus* oryzae is the most common type and responsible for around 60% of human mucormycosis cases and almost 90% cases of rhino cerebral mucormycosis is caused by this fungus[5]. Mode of transmission is Inhalation of fungal spores followed by ingestion of spores, direct inoculation of spores into injured skin (burns), trauma with contaminated soil and intravenous transmission (IV drug users)[6]. For the laboratory diagnosis of mucormycosis, fungal culture is preferable. Specimen like tissues from nasal septum and para nasal sinuses are ideal. Samples are inoculated on Sabouraud dextrose agar (SDA) and Sabouraud dextrose chloramphenicol agar (SDCA) media and incubated at 25 to 30 degree C and then fungal colonies are processed by potassium hydroxide mount. Microscopic findings are short and broad aseptate or pauci-septate obtuse angle branching ribbon like hyphae with sporangiospores[7]. Lacto phenol cotton blue mount is also useful for detection of hyphae[8]. Other methods for detection are imaging study (MRI or CT scan), PCR, Histopathology. The first line antifungal agent is liposomal amphotericin B along with surgical debridement[9]. For step-down therapy, Posaconazole and Isavuconazole are used as parenteral and oral formulation for patients

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who have responded to lipid formulation of amphotericin B [10,11,12]. There were a few many studies across India describing different types of causative microorganisms and determining the association of COVID 19 with mucormycosis though these kinds of researches are still lacking in this part of the country. Bankura Sammilani Medical College was designated as one of the nodal centres for the treatment of patients infected with Mucormycosis in West Bengal after the sudden surge of few cases during and after the second wave of COVID-19. With this background of literature, the present study was planned to assess different types of genera causing mucormycosis and to describe the suitability of different types of specimens for the culture of the causative organism along with the determination of relationship between the disease and infection with COVID 19. So, the **objectives** of the present study were as follows:

1. To assess the proportion of different genera of Mucorales as causative agents
2. To describe the suitability of different specimens for culture of mucorales
3. To determine the relationship between mucormycosis and infection with COVID 19.

Materials and methods

Study type and design

It was a Descriptive Cross-Sectional study.

Study settings

This was an Institutional study conducted in the Microbiology laboratory of Bankura Sammilani Medical College and Hospital.

Study period

The study was conducted from the month of May to September 2021.

Study population

All the samples from the patients with suspicion of mucormycosis which were received at Microbiology laboratory, BSMCH during the study period were considered as study subjects.

Inclusion criteria

Samples from the patients with suspicion of mucormycosis.

Exclusion criteria

Samples (Tissue) which are inadequate in amount.

Sample size and sampling technique

All the 50 samples which were received during the study period were included in the study using complete enumeration method.

Study tools and techniques

Study tools

1. Specimen (Tissues) from different sites of involvement
2. Bio-safety cabinet II
3. Sterile scalpel
4. Spirit lamp
5. Teasing loop

6. Straight wire loop
7. Sabouraud dextrose agar (SDA)
8. Sabouraud dextrose chloramphenicol agar (SDCA)
9. 40% Potassium Hydroxide (KOH) solution
10. Lactophenol cotton blue stain
11. Glass slides
12. Cover slips

Study techniques

All the tissue samples will be cut into smaller pieces by the help of sterile scalpel blade and inoculated in the Sabouraud dextrose agar (SDA) & Sabouraud dextrose chloramphenicol agar (SDCA) media with in a class 2 Bio-safety cabinet. Then the inoculated media will be incubated at room temperature for at least 7 days. If the cotton woolly growth appears, it will be processed for 40% KOH mount. Lactophenol cotton blue stain will also be done. Then the slides will be observed under light microscope to identify short, broad aseptate hyphae with wide angle along with sporangiospores. Presence or absence of rhizoid will also be noted.

Study variables

Independent variables

- a. Socio-demographic variables: Age, gender, religion of the patients
- b. Proportion of different type of samples collected
- c. Proportion of samples collected from COVID positive patients

Dependent variables

- a. Proportion of samples positive for Mucormycosis.
- b. Proportion of the samples having no Growth.
- c. Proportion of different genera found to be causative

Ethical clearance

The ethical clearance was obtained from Institutional Ethics Committee of Bankura Sammilani Medical College.

Data collection

The socio-demographic data along with site of collection site of specimen and COVID positivity status were collected during the arrival of sample at laboratory. Growth of the microorganisms if any, were noted after KOH mount and LCB staining.

Data analysis

Data were entered into Microsoft excel sheet, checked twice to detect any erroneous entry and were organised later on. Principles of descriptive statistics were used to analyse the data. Chi square test was done to determine association as and when applicable.

Results

Mean age of the patients from whom samples were collected was 51.5 \pm 13.6 years. Majority of the patients (48%) affected with mucormycosis were belonging to the age group of 50-70 years. 62% of the patients were male while 86% of them belonged to Hinduism. (Table 1)

Table 1: Socio-demographic distribution of study subjects: (n=50)

Variables	Number (%)
Age	
<50	21 (42)
50-70	24 (48)
>70	5 (10)
Sex	
Male	31 (62)
Female	19 (38)
Religion	
Hinduism	43 (86)
Islam	7 (14)
Total	50 (100)

44% of samples showed no growth of any mucorales while among the rest 30% exhibited the growth of Mucor and 26% showed the growth of Rhizopus (Figure 1 & Table 2).

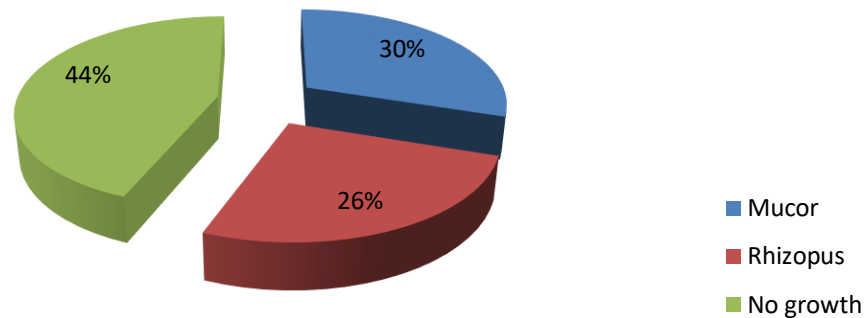


Fig.1: Proportion of different genera as causative of mucormycosis

Table 2: Distribution of different genera of Mucorales as causative agents :(n=50)

Genera of Mucorales	Number (%)
Mucor	15 (30)
Rhizopus	13 (26)
No growth	22 (44)
Total	50 (100)

Among the 28 samples showing growth of mucorales, 15 (53.6%) showed short and broad aseptate obtuse angled hyphae with sporangiospore devoid of rhizoid suggesting that of Mucor and rest 13 (46.4%) revealed similar kind of hyphae with sporangiospore and nodal rhizoid suggesting that of Rhizopus.

Table 3: Distribution of different genera of Mucorales according to site of specimen collection and COVID positivity :(n=50)

Variables	Different genera of mucorales		
	Mucor (15)	Rhizopus (13)	No growth (22)
Site of specimen collection			
Nasal septum	8	5	6
Middle meatus	3	5	6
Sphenoid sinus	0	0	3
Lip	0	0	1
Maxillary sinus	2	1	4
Ethmoid sinus	1	0	1
Hard palate	1	0	0
Orbital area	0	2	1
$\chi^2 = 14.212$, df = 14, p = 0.434			
COVID positivity			
Positive	12	7	1
Negative	3	6	21
$\chi^2 = 22.561$, df = 2, p = < 0.001			

Majority of the growth of mucorales were exhibited among the samples collected from the Nasal septum followed by middle meatus and maxillary sinus. (Table 3) Thus in the present study Nasal septum was the most suitable site for specimen collection followed by middle meatus and maxillary sinus.

Table 3 also revealed that occurrence of mucormycosis was significantly associated with COVID positivity of the patient (p = <0.001).

Discussion

Mucormycosis which is caused by Mucorales are angio-invasive in nature. During the second wave of COVID-19, this mucormycosis was increased abruptly. In post COVID phase, cytokines storm is

developed which can lead to immune-suppression. Use of systemic steroids and uncontrolled diabetes are also aggravating the opportunistic fungal infection like mucormycosis. Rhino- orbital mucormycosis is the commonest form.

Some studies were conducted from where we can get information about the predominant genera of Mucorales were involved, relation with COVID-19, involved sites of mucormycosis etc.

In the present study male predilection of mucormycosis was established as 62% of the patients were male. **Valliappan Muthu et.al**[13] showed in "Epidemiology and patho-physiology of COVID-19 associated Mucormycosis: India versus the rest of the world" that, in India, male was mostly affected (80.3%). Similar finding was found in a study done by Singh AK et.al[14] showing Mucormycosis

was predominant in male (78.9%). Similarly, a study from MAMC, New Delhi, India showed that male and female ratio was 3:1[15].

Valliappan Muthu et.al[13] also showed 28.8% COVID-19 positivity, to some extent resembling to the finding of the present study revealing 40% COVID-19 positivity. Similar finding was found in a study done by Singh AK et.al[14] (showed in "Mucormycosis in COVID-19: A Systematic review of cases reported worldwide and in India") showing 59.4% of mucormycosis were COVID positive.

In the present study 56% cases (28 out of 50) of mucormycosis exhibited the growth of mucorales among which *Mucor* was the predominant (15 out of 28). This finding contradicts to that of **Valliappan Muthu et.al** where total number of cases were 233 and organisms were identified from 41 cases (only 17.6%) and also in contrary to our study they found the predominant organisms were *Rhizopus* (20 in numbers)[13]. Another study from MAMC, New Delhi, India showed that out of 60 cases 19 cases (31.7%) showed non-septate or ribbon like wide angled branching hyphae on direct KOH mount[15].

In the present study 45 out of 50 i.e. 90% samples were from nose and para nasal sinuses. Also it was found that 25 out of 28 cases which exhibited the growth of mucorales were having the samples taken from nose and para nasal sinuses. This finding is in resemblance with that of Singh AK et.al[14] which showed that, in case of mucormycosis, 88.9% of samples were found from nose and para nasal sinuses.

Conclusions

The present study concluded that *Mucor* and *Rhizopus* were the causative mucorales behind this suddenly emerging burden of mucormycosis during the time period after the pandemic of COVID 19. For the laboratory based diagnosis of aetiopathogenic microorganism, tissue from nasal septum, middle meatus, maxillary sinus etc. were the suitable sites for specimen collection. Our study also infers a significant relationship between occurrences of mucormycosis and the infection with COVID 19. Further researches will be helpful to establish other associated variables of mucormycosis and its causative organism.

Declarations

Funding

None

Conflicts of interest

None

Ethical approval

Ethical clearance was sought from Institutional Ethics Committee of Bankura Sammilani Medical College, Bankura.

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