Original Article

OPEN ACCESS

Study on Covid-19 Associated Mucormycosis in Patients Attending a Tertiary Care Hospital During Pandemic in India

Dr. S. Viji *[1], Dr. C. Nithya [2], Dr. K. Shanmugam [3], Dr. P. Suganthi [3]

[1]Assistant Professor, Government Thiruvannamalai Medical College, Thiruvannamalai, TN, India.
[2]Associate Professor, Govt Stanley Medical College, Chennai, TN, India.
[3]Assistant Professor, Government Krishnagiri Medical College, Krishnagiri, TN, India.

*Corresponding Author: Dr. S. Viji; *drvg2001@gmail.com*

Received: 02 January 2025; **Revised:** 03 February 2025; **Accepted:** 18 February 2025; **Published:** 25 February 2025

Abstract

Background: The Corona virus (Covid-19) infection is one of the cause for immunosuppressed state and increased risk for secondary infections. The Delta variant (B.1.617.2) was the most common variant circulating at that time. We have evaluated clinical features, predisposing factors, diagnosis and outcomes for mucormycosis among patients with Covid-19 infection. Mucormycosis is an opportunistic and angioinvasive fungal infection caused by the Mucorales family with Rhizopus and Mucor. It has the high morbidity and Mortality that requires prompt recognition and immediate intervention. The most important risk factors are uncontrolled blood sugar, the use of corticosteroids, and immune dysfunction due to Covid-19 infection. Poor glycaemic control is the single most cause for worse outcomes despite aggressive medical and surgical interventions. ***Aim & Objectives:*** To study the association between covid-19 and Mucormycosis in patients attending at Tertiary care centre during pandemic attack. ***Materials & Methods:*** This prospective observational study was conducted from May 2021 to October 2021.A total of 77 Covid-19positive patients with signs and symptoms suggestive of Mucormycosis were included in this study attending at Department of Microbiology in association with Department of Oto Rhino laryngology (ENT), Government Tiruvannamalai Medical College and Research Institute, Tiruvannamalai. As per the Standard aseptic precautions, Biopsy tissue samples were collected during FESS (Functional Endoscopic Sinus Surgery) from clinically and radiologically suspected cases of mucormycosis with Covid-19 infection after getting informed consent. All the samples were processed for Mycology culture isolation. The isolates examined under 10% KOH for detection of fungal elements and inoculated into Sabouraud's Dextrose Agar (SDA) and kept in Biological Oxygen Demand (BOD) for 2-4 weeks. The results were analysed in correlation with the clinical details and cultural growth identification. The cultural isolates were then subjected to LPCB (Lacto Phenol Cotton Blue) for Mucorales confirmation. ***Results:*** A total of 35 (45.4%) were positive for presence of fungal elements in either KOH mount or fungal growth on SDA. Gram positive budding yeast cells (ie) Candida species were most commonly isolated in culture, followed by Rhizopus spp, Aspergillus spp and Mucor spp. Mucormycosis was predominantly seen in males, 8 (10.38%), then in females 2 (2.6%). The commonest risk factors associated with mucormycosis were Covid-19 positivity and Diabetes mellitus (poor Glycaemic control). ***Conclusion:*** Mucormycosis can occur among Covid-19 patients, especially with poor glycaemic control, widespread and injudicious use of corticosteroids and broad-spectrum antibiotics, and invasive ventilation, owing to the high mortality. High index of suspicion is required to ensure timely diagnosis and appropriate treatment in high- risk populations to avoid mortality and reduce the complications of Mucormycosis.

Keywords: ***Mucorales, Covid-19 associated mucormycosis, angioinvasive fungal infections, poor glycemic control.***

Introduction

Corona virus disease (Covid-19), attributed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a global pandemic by the World Health Organisation (WHO) in March 2020. During the second wave of the COVID-19 pandemic, there was a sudden increase in the number of mucormycosis cases worldwide, presenting a new challenge to clinicians managing these patients (kumar *et al*, 2021; Mahalaxmi *et al*.2021). Covid-19 patients are more susceptible to develop secondary infections if they have decompensated pulmonary functions or require invasive mechanical ventilation. The median time for developing mucormycosis was two weeks after COVID-19 diagnosis.

Rhino-orbital- cerebral mucormycosis (44 - 49%) is the most commonly reported Clinical form followed by cutaneous (10%-19%), pulmonary (10%-11%), disseminated (6%-11%) and Gastrointestinal (2%-11%) [4]. Mortality rates remain high, exceeding 50% despite aggressive medical and surgical interventions (Kumar *et al*,2021; Sharma *et al*, Singh *et al*,2021).

Mucormycosis is known to affect Immunocompromised patients especially those with diabetes mellitus, prolonged corticosteroid use, solid organ transplant recipients, neutropenia and haematological malignancies.

Pathogenesis and associated risk factors

Mucorales are found in the environment, especially in decayed organic material such as bread, fruits, vegetables and soil (Kumar *et al*,2021) Mucorales can tolerate high temperatures, thus infections occur more in hot dry summer when the humidity is low, as it has been observed in India, a country with high prevalence (Kumar *et al*,2021).

Inhalation of fungal spores followed by repeated ingestion of contaminated products in immunocompromised patients, such as patients with diabetes.

The disease ranges from local sinusitis to a severe and aggressive form with dissemination and cerebral involvement. Fungal hyphae invade blood vessels, causing inflammation and necrosis of the vascular wall, which results in thrombosis, infarction or haemorrhage (Mahalaxmi *et al*.2021; Sen *et al*, 2021;).

Clinical features

Patients can present with facial pain or swelling, paresthesia, headache, or visual loss. On clinical examination, periorbital swelling, chemosis, proptosis, ophthalmoplegia and facial nerve palsy may found. The most common presentation reported by our Covid -19 patients had rhino-orbital mucormycosis (44 - 49%) by intracranial extension after 2-4 weeks of COVID Positive.

Mucormycosis mortality depends on the site of involvement. Mortality rates can be as high as 50-80%and associated with intracranial involvement, disseminated disease and presence of underlying comorbidities like uncontrolled diabetes.

The SARS-COv-2 Virus can itself infiltrate beta cells in the pancreas, which causes necroptosis cell death and thus metabolic dysfunction. This contributes to new-onset hyper-glycemia and poor control of Pre-existing Diabetes mellitus. (Gupta *et al*.,2021; Rao *et al*,2021) cytochrome storm can also lead to insulin resistance.

Diagnosis [11]

The diagnosis of COVID -19 was based on Real-Time Polymerase Chain Reaction (RT-PCR) test from nasopharyngeal or oropharyngeal swabs.

The diagnosis of Mucormycosis usually requires a combination of complete clinical assessment, Blood examination (fasting blood sugar level, HbA1c, C- Reactive protein level)), plain X-rays and further imaging (Eg. MRI scan, CT scan), bone, paranasal sinuses and/or soft tissue biopsies (FESS) and/or surgical sampling. Imaging is useful to assess the extent of infection and to rule out other potential causes of symptoms.

Tissue biopsy specimens received from Department of ENT for Fungal culture to the Microbiology Laboratory. The samples were subjected to various tests like 10% KOH examination for Fungal elements like broad non-septate, hyphae with right angle-branching and inoculated into two Sabouraud's dextrose agar (with and without antibiotics) tubes and one is incubated at room temperature (RT-37◦C) and another tube is incubated at Biological Oxygen Demand (BOD - 25◦C) for 2 weeks.

Treatment

The first step in the management of mucormycosis is to have a high index of clinical suspicion especially in those with COVID-19 who have diabetes mellitus, and who have received systemic corticosteroids.

Current management guidelines from India recommend intravenous methyl prednisolone (0.5-1mg /kg/day) or dexamethasone (0.1- 0.2 mg/kg/day) for 5-10 days for moderately severe cases of COVID-19 infection cases, especially for patients with escalating oxygen requirements or showing elevated biomarkers, intravenous methylprednisolone 1-2 mg/kg/day is recommended for severe cases. Accordingly, systemic corticosteroids were administered to all patients in our study irrespective of their COVID-19 disease severity.

However, the World Health Organization (WHO) recommends the administration of systemic corticosteroids for the management of patients with only severe COVID-19 pneumonia, to mitigate the effects of immune - related lung injury, especially for those requiring ventilatory support. High dose of steroids should be used cautiously in poorly controlled patients with DM, even if indicated in patients with severe COVID-19 [4].

AmBisome (the liposomal, less nephrotoxic form of Amphotericin B) at 5-10mg/kg/day is the antifungal therapy of choice. Posaconazole can be used in refractory cases.

Aim and Objectives

- To study the Fungal pathogens amongpatients with Covid -19 Positive confirmed by RT-PCR and isolate the pathogen from Biopsy material (FESS).
- To study about the associate risk factors of Mucormycosis among the study population.

Study design - Prospective -Cross sectional study

Place of study

Department of Microbiology in association with Department of Otorhinolaryngology, Government Thiruvannamalai Medical College and Research Institute, Thiruvannamalai.

Study Period

6 Months [May 2021 to October 2021]

Study population & sample Size

The total of 77 Covid-19 Positive Patients with clinical and radiological diagnosis of Mucormycosis.

Inclusion criteria

Covid -19 Positive patients with clinical and radiological signs of Mucormycosis

Exclusion criteria

Patients those who are not given consent to participate in the study

Ethical clearance

Institutional Ethics committee not applicable. Because, it's an fatal complication of Covid-19 during pandemic period, we have get consent only from patient ICMR Issued Covid-19 , RT-PCR request form. We have received the biopsy samples from ENT department during that pandemic period.

Materials & Methods

Specimen collection

Two swabs (throat & Nasal) were collected under aseptic precaution after obtaining informed consent for RT-PCR test (Real time-polymerase chain reaction) for Covid -19 diagnosis.

The biopsy tissue samples were collected during FESS (Functional Endoscopic Sinus Surgery) from clinical features suggestive of Mucormycosis and associated poor Glycaemic control.

Specimen processing and Identification of Fungal pathogens

The respiratory samples (Nasopharyngeal and Oropharyngeal swab) were subjected to two steps (ie) Viral RNA (ribonucleic acid) isolation by Extraction kit and for the detection of SARS-CoV-2 Virus by Real time- polymerase chain reaction (RT-PCR Kit)

system, Thermofisher Scientific, Waltham, MA was used in accordance with the manufacturer's instructions. The Assay targets three genomic regions of SARS-CoV-2 (S, N, ORF1ab genes) Amplification, RNA quantification detected.

Tissue biopsy specimens received from Department of ENT for Fungal culture to the Microbiology Laboratory. The samples were subjected to various tests like 10% KOH examination for Fungal elements like broad non-septate, hyphae with right angle-branching and inoculated into two Sabouraud's dextrose agar (with and without antibiotics) tubes and one is incubated at room temperature (RT-37◦C) and another tube is incubated at C (BOD - 25◦C) for 2 weeks.

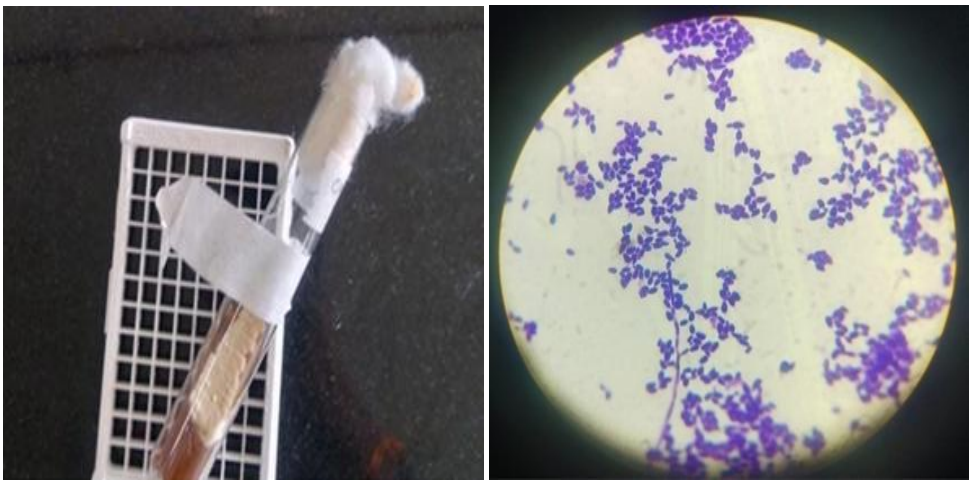
The culture on Sabouraud's dextrose agar (SDA) at 25◦C reveals characteristic white cottony woolly colonies with tube filling growth (hence called Lid lifters). In some species, eg. Rhizopus the colonies become brown black later, due to sporulation giving rise to Salt and pepper appearance.

The microscopic appearance of LPCB (Lactophenol Cotton Blue) mount colonies reveals broad Aseptate hyaline hyphae with right angle-branching. Some species bear a unique root like growth arising from hyphae called rhizoid which provides initial clue for identification of the fungus.

Most of the Sabouraud's dextrose agar (SDA) culture showed creamy white colonies, on Gram stain reveals Gram positive budding Yeast cells probably Candida Species isolated. It is also considered as most common fungal pathogen in Immunodeficient patients like Diabetes.

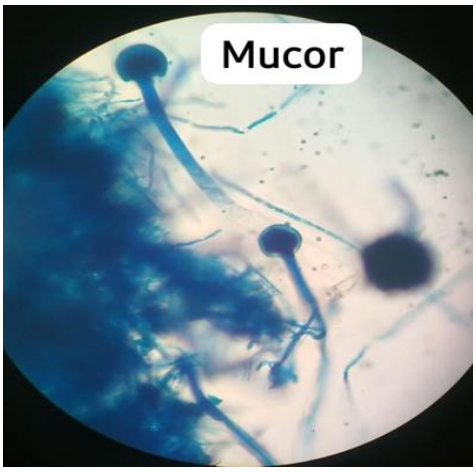
The microscopic appearance of some colonies in LPCB (Lactophenol Cotton Blue) mount consist of hyaline septate hyphae from which conidiophores arise, which end at vesicles. From the vesicles, finger -like projections of conidia producing cells arise called Phialides or Sterigmata. Conidia arise from the vesicles either on their entire surface or only on the upper half suggestive of Aspergillus species (Aspergillus niger, Aspergillus fumigatus & Aspergillus flavus).

Macroscopic and Microscopic appearance of Candida species

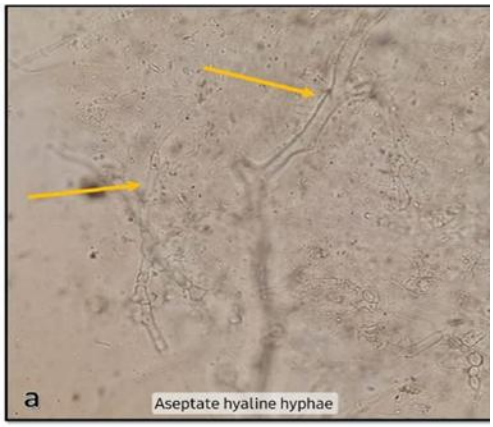


Macroscopic and Microscopic appearance of Rhizopus

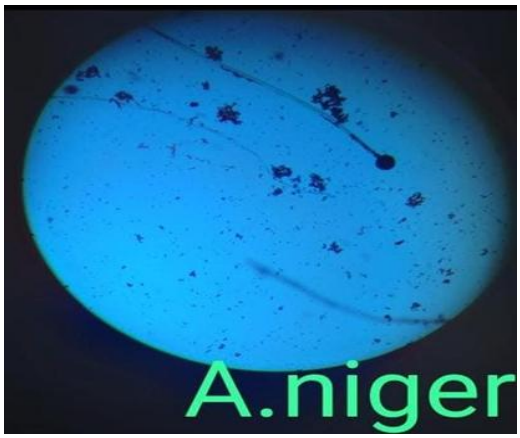
Macroscopic and Microscopic appearance of Mucor



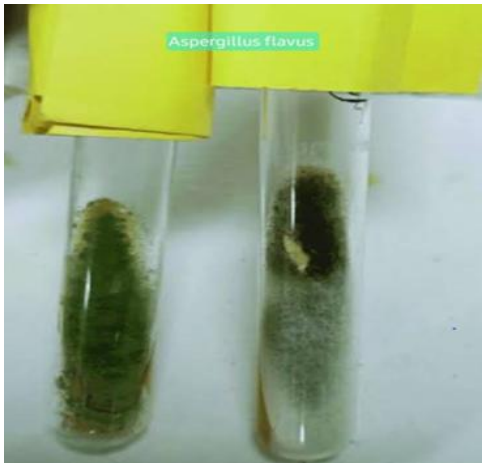
KOH Examination of Rhizopus and Mucor - Aseptate broad hyphae

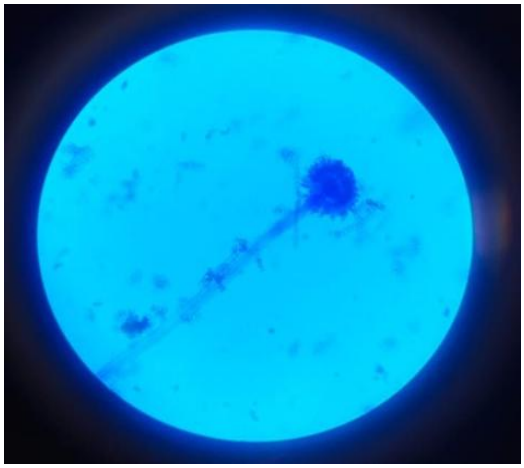


Macroscopic and Microscopic appearance of Aspergillus niger



Macroscopic and Microscopic appearance of Aspergillus flavus





Diagnostic and Treatment Algorithm of Mucormycosis in patients with COVID-19

COVID-19 with Mucormycosis patients

↓

Severely ill (Admission to ICU, requiring mechanical ventilation, long hospital stay)

↓

Risk factors – Diabetes mellitus, glucocorticoid use

↓

Diagnosis - 1. Direct Microscopy (10% KOH and Lactophenol cotton blue Mount)
2.Culture (Sabouraud's Dextrose Agar at two Temperature -2-4 weeks)
3. Molecular methods (PCR)

↓

Treatment /Management: 1. 1st line treatment: Liposomal Amphotericin B, Posaconazole
2. Surgical treatment (debridement), in needed cases

Table 1: Demographics, Risk factors and Covid-19 status of study population (n=77)

	No of cases (n=77)	***Percentage (%)***
Demographics		
Male	*44*	*57.14%*
Female	*33*	*42.85%*
Risk factors		
Diabetes Mellitus (DM)	*49*	*63.63%*
Hypertension (HTN)	*35*	*45.45%*
Sinusitis	*28*	*36.36%*
Ischaemic heart disease (IHD)	*37*	*48.05*
COVID-19status		
RT-PCR Positive	*46*	*59.8%*
RT-PCR Negative	*31*	*40.2*

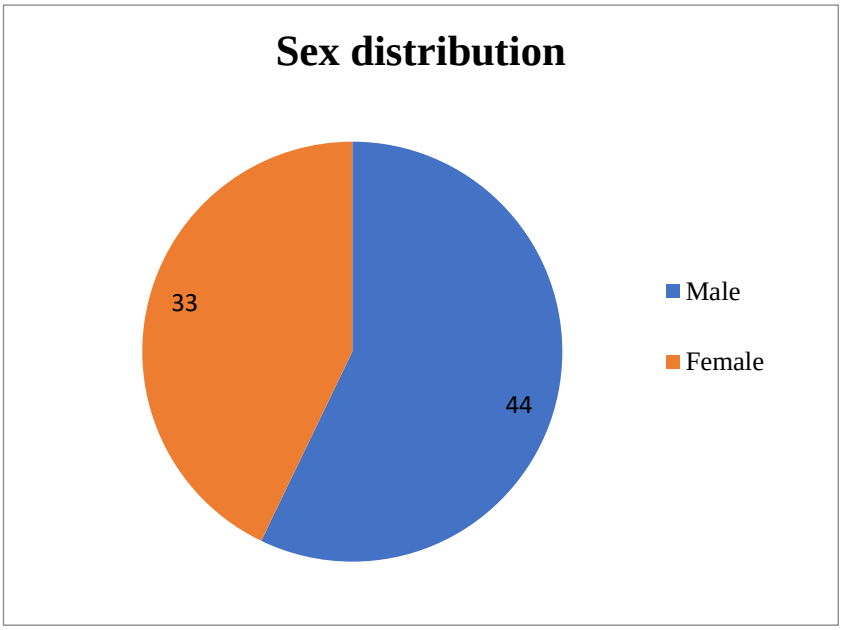


Chart 1: Sex distribution (n=77)

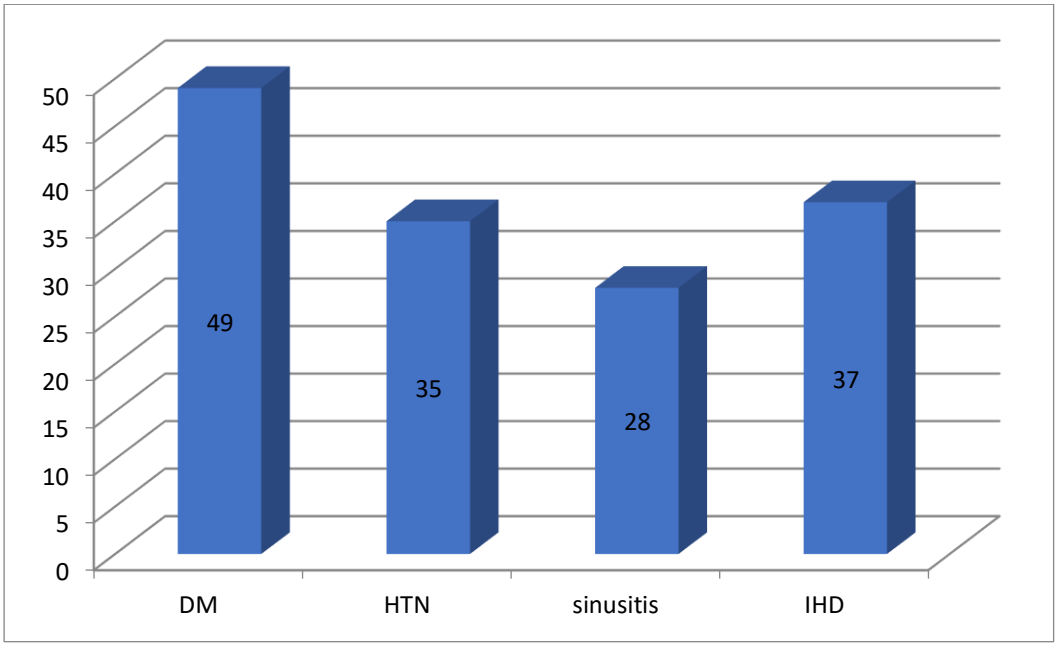


Chart 2: Risk factors distribution (n=77)

Table 2: Fungal Pathogens isolated among study population (n=77)

S. No	Name of Fungal pathogens	Fungal growth (n=77) 35	Percentage (n= 77) (45.4%)
1	Rhizopus	10	12.9%
2	Mucor	1	1.2%
3	Candida species	20	26.1%
4	Aspergillus flavus	2	22.6%
5	Aspergillus niger	2	2.6%
6	No fungal growth	42	54.5%

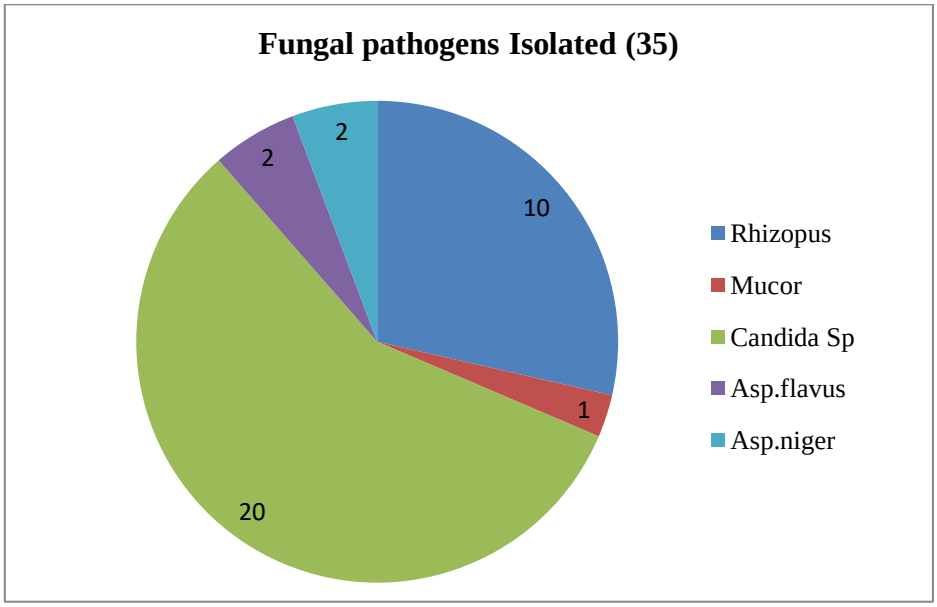


Chart 3: Fungal pathogens isolated among study population (n=77)

Discussion

This study was conducted in a tertiary care hospital, where 77 patients with clinically suggestive of COVID-19 were included to study the clinical and Radiological features of Mucormycosis. The Clinical and epidemiological data were analysed in correlation with isolated fungal pathogens. The Clinical outcome of the patients was analysed in correlation with COVID-19 status and risk factors association.

During the second wave of the COVID -19 pandemic, a sudden and rapid rise in Mucormycosis incidence was observed. It has been observed as deadly complication due to viral invasion to blood vessels by fungal elements leading to mycotic thrombosis, ischeamic infarction and ultimately necrosis of affective host tissues [5]. Globally, the incidence of mucormycosis has been described to range from 0.005 to 1.7 per million population, [6] whereas in India, the reported prevalence was 0.14 per 1000, approximately 80 times higher than that in developed countries, making it the country with

the higher burden of mucormycosis [7,8]. The disease peaked with a rapid clinical course with the worldwide mortality reaching 46%[5,6,8]. A delay in diagnosis of 6 days has been associated with doubling of the 30-day mortality rate from 35% to 66% [9].

The organisms commonly implicated in the disorder originate from the Mucorales order and include Mucor, Rhizopus, Rhizomucor, Absidia, Apophysomyces, saksenaea, Cunninghamella [4]. The fungus usually resides as a commensal in the nasal mocosa. Fungal spores gain entry via inhalation and subsequently enter the paranasal sinuses. Spores may also be acquired by the ingestion of contaminated food. Affected individuals usually present with acute sinusitis, fever, nasal congestion, purulent nasal discharge and headache [9]. if not treated early, contiguous spread to adjacent structures may occur, resulting in various clinical symptoms [8]. The orbital cavity is easily accessible through the ethmoid bone via the lamina papyracea etc. Contiguous intracranial extension can occur through the ethmoid cribriform plate, supraorbital fissure and perineural routes. Cavernous sinus or sagittal sinus thrombosis, carotid occlusion, cerebral infarction, intracranial aneurysm, intracranial haemorrhage and cerebral abscesses are potential sequelae.

In our study, the overall culture positivity, observed in this study was 45.4% and only from the FESS (Functional endoscopic sinus surgery) samples, fungi were isolated. This was lower when compared to the study done by Anupamasingh 4 *et al* and Ruchi 7*et al*[11], who showed a culture positivity of 86.6% and 64% respectively. The sensitivity of culture isolation depends on various factors like prior antibiotic exposure and collection of appropriate samples etc.

Like in many other studies, the predominant pathogen in the aetiology of Mucormycosis was determined to be Candida species (Budding yeast cells) 25.9% followed by Rhizopus and Mucor were isolated 12.9% and 1.2% respectively. Aspergillus flavus also 2.6% isolated among the study population. However, improper tissue handling and prior use of Antifungal therapy might provide false negative results in upto 50% of cases [4] (Sen *et al*, 2021a, Sen *et al*. 2021b).

Conclusion

Mucormycosisis an opportunistic and frequently emerging fulminant fungal infection caused by members of the family Mucoraceae, order Mucorales and Class Zygomycetes. Among these, Rhizopus, Mucor, Absidia and Cunninghamella Species are ubiquitous fungi. The Major predisposing factors for acquisition of mucormycosis are uncontrolled diabetes mellitus (DM) with ketoacidosis, malignancy, immunosuppression etc. The route of transmission is usually inhalation of spores. Mucormycosisis mostly manifests as rhino-cerebral, Rhino-orbital, pulmonary, cutaneous and disseminated form. Rhino-orbito-cerebral mucormycosis (ROCM) is the most serious and fatal form of disease with mortality rate of 70-80% if not treated adequately. 14 days course of intravenous Amphotericin B is the treatment of choice for this type of patients.

Mucormycosis is an increasingly emerging life-threatening infection particularly in uncontrolled diabetic patients. Uncontrolled Diabetes mellitus is an important predisposing factor for development of Rhino-orbito-cerebral mucormycosis (ROCM) [12].

The definitive management requires treatment of underlying cause (strict control of blood sugar levels), appropriate antifungal therapy and surgical debridement whenever possible (1&2) jung *et al* and Sachdeva. Though the mortality and morbidity are extremely high early intervention in diagnosis and prompt aggressive treatment can reduce the mortality.

List of abbreviations

SARS: CoV-2 - Severe acute respiratory syndrome coronavirus-2

COVID: 19: Corona virus disease

ROCM: Rhino-orbito-cerebral mucormycosis

DM: Diabetes mellitus

BOD: Biological oxygen Demand

RT-PCR: Real-Time Polymerase Chain Reaction

ENT: Department of Otorhinolaryngology

FESS: Functional endoscopic sinus

SDA: Sabouraud's dextrose agar

LPCB: Lactophenol Cotton Blue

10% KOH: 10% Potassium Hydroxide.

Declarations

Conflicts of Interest

We The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Funding Statement

The research and Publication of Article is only by self-funding.

Authors' contributions

The sample collection, processing, reporting and analysis of datas of this study done by Authors (Dr. S. Viji, Dr. C. Nithya, Dr. K. Shanmugam, Dr. P. Suganthi) combined work

References

[1] Jung SH, Kim SW, Song CE, Cho Jh, Lee JH, *et al*. Rhino cerebral Mucormycosis: consideration of prognostic factors and treatment modality. Auris Nasus Larynx. 2009:36 (3):274-79.

[2] Sachdev K. Rhino-ocular- cerebral mucormycosis with multiple cranial nerve palsies in diabetic patients; Review of six cases. Indian J Otolaryngology Head Neck Surg. 2013:65($)375-79.

[3] Gail Reid, Joseph P Lynch 3rd, Michael C Fishbein, Nina M Clark /pubmed

[4] COVID-19 - Associated Mucormycosis; An Opportunistic Fungal Infection. A Case series and review. Int J Inect Disea 2022 Aug: 203-210

[5] Mucormycosis and COVID-19; An epidemic within a pandemic in India, Lav S Elarka, Suktara Sharma *et al*, Mycoses/Volume 64, Issue 10/p.1253-1260

[6] Mucormycosisand Covid-19; An Epidemic turned pandemic in India. Jagadesh Chander, Medical Mycology Text Book, 4thEdition.

[7] Gonzalez CE, Rinald MG, Sugar AM. Zygomycosis. Infectious disease Clinics of North America, 2002;16;895-914

[8] Gamaletsou MN, Sipsas NV, Roilicles E, Walsh TJ, Rhino-orbital cerebral mucormycosis. Curr infect Dis Resp. 2012:14: 423-34.

[9] Prabhu RM, Patel R. Mucormycosis and Entomophthoramycosis; a review of the clinical manifestations, diagnosis and treatment. Clin Microbial infect.2004;10(Suppl 1); 31-47

[10] Essentials of Medical Microbiology, 4th Edition, Apurba S Sastry; page 564-567.

[11] Anupamasingh4 *et al* and Ruchi 7*et al*, Mucormycosis. 2004, Page 512-518.

[12] Rhino-orbital-Cerebral mucormycosis; Successful Medical Management in a Resource poor Setting; Pravit Kumar&, Sagar *et al*/NJLM/2016/18698;21;32