

Coronavirus (Covid-19) Associated Opportunistic Fungal Infections with a Special Reference to MucormycosisG.A.Praveen¹, P. Venkata Ramana², Siva V Kameswari Ramya³, Manda V Vijayasekhar⁴, P Kamala⁵¹Postgraduate, Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India²Associate Professor, Department of Microbiology Rangaraya Medical College, Kakinada, Andhra Pradesh, India³Post graduate, Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India⁴MCh Neurosurgery, Professor and HOD, Department of neurosurgery, Rangaraya Medical College, Kakinada, Andhra Pradesh, India⁵Professor and HOD, Andhra Medical College, Department of Microbiology, Visakhapatnam, Andhra Pradesh, India

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Conflict of interest: Nil

Abstract**Introduction:** The COVID-19 pandemic has posed an increased burden on the health care delivery system in India and has been associated with a wide range of opportunistic bacterial and fungal infections.**Amis and Objectives:** To know the prevalence of fungal infections among the post covid patients.**Material and Methods:** A total number of 482 samples from clinically suspected mucormycosis cases attending various departments and transported to department of Microbiology, All samples are processed according to standard Microbiological procedures.**Results:** In a total of 482 clinically suspected individuals, fungal culture and microscopy were used to evaluate mucormycosis. Out of 482 samples analysed in this study, 263 cases (54.5%) were found to have fungal elements with a 10% KOH mount, while 219 cases (45.5%) were found to be negative. Out of 482, 247 (51.2%) samples were positive for culture, and 235 (48.8%) samples were sterile. Of the 247 fungal isolates, 91 (36.8%) are Mucorales, of which 90 are Mucor species. But one of them was identified as Rhizopus, while the remaining 112 (45.4%) are Aspergillus species, 43 (17.4%) are Candida species, and 1 (0.4%) is a Penicillium species**Conclusion:** Despite the hype, mucormycosis cases were well handled by using simple microbiological methods for an early diagnosis and initiation of antifungal therapy in our hospital.**Keywords:** Covid-19, Opportunistic, mucormycosis, fungal, Diabetics.This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

The COVID-19 pandemic has posed an increased burden on the health care delivery system in India and has been associated with a wide range of opportunistic bacterial and fungal infections. [2] Given the sheer magnitude of the outbreak of mucormycosis, the Indian Health Ministry has advised all states to declare it an epidemic on 21 may 2021 [2].

The Mucorales are the largest and best studied order of Zygomycete fungi. Members of this order are sometimes called pin moulds [3]. The order includes 11 families, 56 genera, and approximately 300 species [3,5]. The most prevalent species are Rhizopus, Mucor, Lichthemelia (absidia), and

Rhizomucor. Other less frequent species are Cunninghamella, Apophysomyces, and Saksenaea. The public and media used the word "black fungus" colloquially to describe mucormycosis which is a misnomer probably because of the blackish discoloration of the muscle tissue due to thrombosis of the blood vessels [2]. The primary reasons that appear to be facilitating the growth of mucorales spores and other opportunistic fungi (Aspergillus, Candida and Penicillium) in COVID-19 patients are

1. uncontrolled diabetes mellitus (hyperglycaemia)
2. prolonged usage of high doses of steroids

3. high ferritin levels
4. Decreased phagocytic activity due to immunosuppression [2,3].

Aims and Objectives:

1. To know the prevalence of fungal infections among the post covid patients.
2. To isolate and identify fungal infections

Material and Methods:

A total number of 482 samples from clinically suspected mucormycosis cases attending ENT, Neurosurgery, Dermatology, venerology and Leprosy, Pulmonology, Medicine, Surgery departments were transported to department of Microbiology, Andhra Medical College-King Gorge Hospital for a period of 6 months (May 2021 to November 2021). All samples are processed according to standard Microbiological procedures.

Inclusion criteria:

1. Clinically suspected patients of mucormycosis.
2. Both genders and all age groups were included.

Exclusion Criteria:

Patients not willing to participate in the study.

Sample Collection:

All samples were collected under sterile aseptic conditions and transported in an appropriate sterile container 1. Sterile Nasal swabs with (Dacron coated), 2. Debrided tissues (endoscopically from sinuses in normal saline containers, 3. Swabs and skin tissue in suspected cutaneous mucormycosis in saline containers, 4. Sputum and 5. broncho alveolar lavage fluid (BAL) for pulmonary mucormycosis

Microbiological Examination

Direct Examination: by using 10% potassium hydroxide (KOH) mount: Hyphae are typically broad (6-16 m) ribbon like and irregularly shaped, aseptate or sparsely septate with branches often arising dichotomously at right angle suggestive of mucormycosis.

Culture: All samples were inoculated on Saborauds dextrose agar with gentamycin and incubated at 22°C in BOD. Slide culture was also done to know the undisturbed morphology of fungal isolate. Growth is observed everyday upto 7 days.

1. Fluffy, cottony, white colonies-Mucor.
2. Creamy white colonies on SDA: Candida species.
3. Black, yellow, green colored velvety colonies: Aspergillus species.

1. MUCOR



2. ASPERGILLUS



3. CANDIDA



Figure 1:

Species identification: Lacto Phenol Cotton Blue

Mount: Growth from SDA is put on a drop of LPCB and tease mount done for identification of species. Scotch tape method was also done for species identification.

Results

The 1st sample was received from suspected case of Rhinorbital mucormycosis on 21st May 2021 admitted in VIMS, Visakhapatnam. In a total of 482 clinically suspected individuals, fungal culture and microscopy were used to evaluate mucormycosis. The overall number of cases reached an abrupt peak with 225 (46.6%) cases in the month of June.

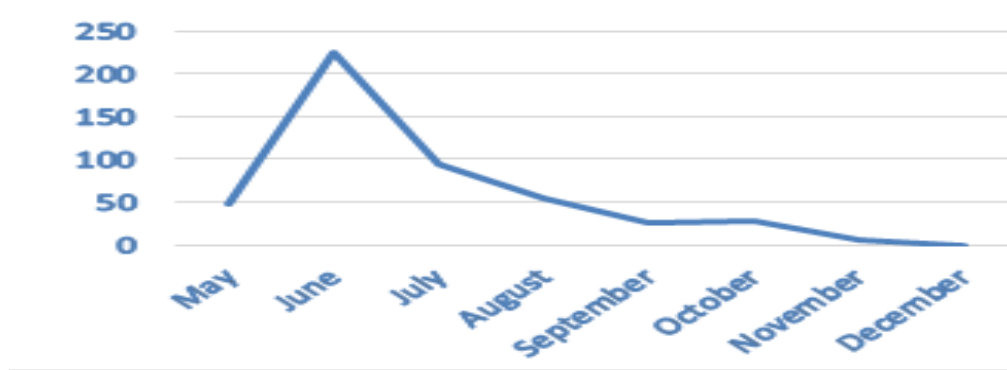


Figure 2:

Out of 482 samples, maximum number of samples was received from ENT followed by Neurosurgery and DVL.as shown below.

1. ENT 180 (37.34%).
2. Neurosurgery 166 (34.4%)
3. DVL 70(14.5%)
4. TBH 21(4.35%),
5. General Medicine 27(5.6%)
6. General Surgery 18 (3.73%)

▪ The fact that, 386 (80.1%) were found to be male and 96 (19.9%) female, made the male predominance obvious. The age range between 45 and 60 years is more represented in the current study, with a total of 228 (47.3%) cases.

• **Association of fungal infections with Diabetes:** In our study of 482 patients, 326 were known diabetics (67.6%), 126 non-diabetics with normal blood sugar levels, 30 non-diabetics whose blood sugar levels increased after receiving steroids.

• **Association of fungal infections with prolonged steroid usage:** 200 out of 482 cases received steroid medication in which 30 of these were non-diabetics who developed high blood glucose levels after therapy, 126 were known

diabetics remaining 44 were with normal blood glucose levels.

• **Association of fungal infections with oxygen therapy:** Mucormycosis and oxygen therapy (intra nasal industrial oxygen) are related. During COVID-19 treatment, 235 instances were discovered to have received oxygen therapy, while 247 did not.

• **Distribution of fungal positivity:** Out of 482 samples analysed in this study, 263 cases (54.5%) were found to have fungal elements with 10% KOH mount, while 219 cases (45.5%) were found to be negative.

• **Culture findings:** Out of 482, 247 (51.2%) samples were positive for culture and 235 (48.8%) samples were sterile.

Of the 247 fungal isolates, 91 (36.8%) are Mucorales, of which 90 are Mucor species. But one of them was identified as Rhizopus. While the remaining 112 (45.4%) are Aspergillus species, 43 (17.4%) are Candida species, and 1 (0.4%) is a Penicillium species. (Table 1). Eight samples showed polymicrobial development, with monomicrobial growth accounting for the rest. The table includes these combinations (Table 2).

Table 1: Distribution of fungal Isolates among various Departments

Department	Samples (n= 482)	Culture positive	Mucor/ Rhizopus	Aspergillus species	Others/ Penicillium	Candida species	No growth
Neurosurgery	166	115	56	34	00	25	51
ENT	180	73	24	41	1 (0.4%)	07	107
Dermatology venereology and Leprosy (DVL)	70	30	10	14	00	06	40
Pulmonology (TBH)	21	16	1	15	00	0	05
Medicine (GM)	27	3	0	3	00	0	25
Surgery(GS)	18	11	0	5	00	5	07
Total	482	247	91(36.8%)	112(45.4%)	1 (0.4%)	43(17.4%)	235



Figure 3:

Discussion

- In our study ,fungal infections are more in males compare to females
- Mostly seen age group between 45 and 60 years is more represented
- Most common isolate in our study was Aspergillus species followed by Mucor/ Rhizopus and candida species.
- Polymicrobial growth pattern was also observed in our study.
- Present study percentage of mucor isolated is compared with Namarata et al and Richa garg et.

Conclusion

Mucormycosis carries a very poor prognosis, severe infection was found despite active treatment. It is an invasive disease with a protracted clinical course, challenging treatment options, and a very high mortality rate. Typically, the disease has been found to be linked to the COVID-19 infection caused by the B.1617.2 delta variant, which has spread rapidly throughout the country.

Despite the hype, mucormycosis cases were well handled by using simple microscopy with KOH mount and culture on SDA (in microbiology lab) ,for a early diagnosis and initiation of antifungal therapy in our hospital In few of them surgical intervention was done as a life saving measure in severe cases.

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