MUCOR MYCOSIS AND OTHER FUNGAL INFECTIONS IN COVID-19 PATIENTS DURING SECOND WAVE OF PANDEMIC AT A TERTIARY CARE HOSPITAL

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INTRODUCTION
The disease pattern of COVID-19 can range from mild to life-threatening pneumonia with associated bacterial and fungal co-infections. Due to the associated co-morbidities like Diabetes mellitus (DM), chronic obstructive pulmonary disease, immunocompromised conditions [1], corticosteroid therapy, ventilation, intensive care unit (ICU) stay [2,3], these patients are prone to develop severe opportunistic infections, such as ophthalmic canaliculitis, pneumocystis jiroveci pneumonia, pulmonary aspergillosis, bloodstream candida infections, and rhino-orbital Mucor mycosis [4]. Mucor mycosis, a newly emerging malignancy associated with the Coronavirus (COVID-19) infection, had infected at least 7,250 people in India by the 3rd week of May 2021 [5].

Fungi mostly cause Mucor mycosis are Rhizopus spp., Mucor spp., Rhizomucor spp., Syncephalastrum spp., Cunninghamella bertholletia, Lichtheimia (formerly Abisidia) [6-8] Cokeromyces, Actinomucor [1], and Apophysomyces elegans [9].

Mucor mycosis, commonly known as “Black Fungus” which was then a rare fungal infection, has suddenly come to light post the COVID-19 pandemic, more so during the second wave in India. Individuals who lack phagocytes or have impaired phagocytic function like severely neutropenic patients are at higher risk of Mucor mycosis [10]. Mucor mycosis infections are characterized by extensive angioinvasion that leads to increased mortality. It thus becomes important not only for the medical fraternity but also for the general population [11] to build awareness about the same during second wave when Mucor mycosis cases in COVID-19 patients created an alarm in the country. Mucor mycosis has been declared an epidemic in several Indian states and has been classified as a notifiable disease [12].

METHODS
Samples that were received from suspected Mucor mycosis cases of COVID-19-positive patients, from Covid ward, Government General Hospital, Srikakulam were processed under strict aseptic conditions at our laboratory. A history of co-morbidities was taken. Samples (maxillary wash, Nasal wash, nasal scrapings, nasal crusts, sputum) were placed on a clean glass slide, and about 5–10 ul potassium hydroxide (KOH) solution is poured. The KOH solution completely dissolves non-fungal components while fungal hyphae and yeast cells are exposed to visualization under a microscope [13]. All samples were inoculated on Sabouraud’s dextrose agar (SDA) with antibiotic gentamicin alone and incubated at 25°C in BOD for 5 days. SDA bottles were examined for growth once daily for 5 days and those showing growth were identified by, the color and appearance of colonies, lactophenol cotton blue teasing mount, and slide culture [14]. Samples were recollected from patients whose samples were negative in KOH mount, but positive for fungal culture, to exclude contamination during the process.

RESULTS
A total of 116 samples were received and four samples were rejected due to leaked samples and improper labeling. Samples were collected from 7 to 84 years age group. 31–60 years of group constituted 81.25% of total cases. 83 samples were collected from males (74.10%) and 29 were from females (25.89%) as shown in Table 1.

Samples received were nasal wash (42), maxillary wash (37), nasal swab (9), sputum (9), crusts from nasal cavity (8), and biopsy and scrapings from nasal cavity (7). DM was the most common risk factor (51.78%) followed by steroid therapy. Direct smear, i.e., KOH mount for fungal elements was positive for 37 samples (33%). Among these 37 direct smear-positive
Type of fungal isolates, their number and percentage were given in Table 3. From the 45 samples that were culture positive for fungi, 47 isolates were isolated. Other fungi isolated were, Aspergillus, Candida, Syncephalastrum, Rhyzopus, Penicillium, and Fusarium as shown in Table 3.

The type of sample and their positive rate regarding fungal isolates and mucor isolates was represented in Table 4.

### DISCUSSION

The 2019 novel coronavirus or severe acute respiratory syndrome coronavirus 2 started in China and soon spread over the world, becoming a pandemic. On December 1, 2020, the first report of the U.S. Centers for Disease Control and Prevention on COVID-19-associated fungal infections was released, highlighting COVID-19-associated mucosal infections. The recommended treatment strategies primarily included surgical debridement and antifungal therapy using Amphotericin B and selected azoles [15].

Some attributed the higher incidence of Mucor mycosis in India than in other countries due to macro-environmental factors such as: masks, improperly discarded Ryle’s tube, Foley catheter and nebulization instruments, improperly sterilized or autoclaved reusable instruments, the central air conditioning systems in many hospitals that are breeding grounds for fungi, and all these combined with lack of cross-ventilation and humid weather, that are ideal for fungal infections [16].

Devang et al. studied 70 cases of Covid-19 patients with Mucor mycosis admitted in their hospital and among them 47 were males (67%) and 23 were females (33%) [17] whereas as in our study males were 74.10% and females were 25.89%. Another study reported 101 cases of Mucor mycosis where males predominate females (78.9%), almost similar to our study 74.10%. Mucor mycosis involving nose and sinuses (88.9%) was most common followed by rhino-orbital (56.7%) [18]. Sangeetha Kandasamy et al. reported 58 cases of Mucor mycosis and found mixed fungal infections (Aspergillus sp. and Candida sp.) in eight (14%) cases, which was higher than the present study (4.4%) [19] In the study of Anuja et al., KOH mount was positive for fungal elements in 62 patients and fungi isolated in 37 patients, out of total 90 patients of invasive fungal sinusitis. Aspergillus group and Mucorales contribute equally being found in 17 samples each [20], whereas Mucorales exceeded Aspergillus group in the present study.

In one study, they reported only one case of Mucor mycosis in an incidentally diagnosed Covid 19 positive case and isolated Rhizopus from sample obtained after sinus debridement in FESS [21], whereas 4 strains of Rhizopus were isolated out of 45 positive cases in this study. Another study also reported only one case report of rhino cerebral Mucor mycosis concurrent with COVID-19 pneumonia in a 41-year-old man with a history of type 1 DM [22].

DM was the common risk factor in most of the studies [4,23] in addition to IV dexamethasone [9,24,25], as in the present study, whereas Stroke is the major complication of Mucor mycosis in one multicentric study [26]. Sinuses were the most common site of Mucor mycosis among COVID-19 patients at 79.4% with maxillary sinus (47.4%) being most common followed by maxillary/ethmoid sinus (24.4%) and frontal sinus (21.4%).

<table>
<thead>
<tr>
<th>Name of the Isolate</th>
<th>Number of Isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucor</td>
<td>23</td>
<td>48.93</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>7</td>
<td>14.89</td>
</tr>
<tr>
<td>Candida</td>
<td>6</td>
<td>12.76</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>4</td>
<td>8.51</td>
</tr>
<tr>
<td>Syncephalastrum</td>
<td>3</td>
<td>6.38</td>
</tr>
<tr>
<td>Penicillium</td>
<td>2</td>
<td>4.25</td>
</tr>
<tr>
<td>Fusarium</td>
<td>2</td>
<td>4.25</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>100</td>
</tr>
</tbody>
</table>

While India faces tough times during the second wave of COVID-19, adding more burden to such a challenging situation, Mucor mycosis, an invasive fungal infection, has seen a sudden surge in patients with COVID-19. One systematic review observed that CAM constitutes 0.3% of COVID-19 coinfections. As a result, Govt. of India has declared it as an epidemic [15]. Steroids, monoclonal antibodies, and broad-spectrum antibiotics used to treat COVID-19 disease have been shown to increase the risk of a new fungal infection or intensify an existing one. Furthermore, COVID-19 patients receiving oxygen therapy in an ICU with a humidifier are susceptible to fungal infection due to moisture exposure [9].
commonly infected in one review [23]. 31.11% of isolates in our study were from maxillary wash, at a lesser rate than the above observation. Mucor mycosis affecting the nasal sinuses was the commonest (44%) in one multicentric study [25], we isolated 26 fungal strains (13 were Mucor among them) from 66 nasal samples such as nasal wash, nasal swab, nasal crusts, biopsy and scrapings from nasal cavity, constitutes 57.77% out of total 47 fungal isolates.

CAM was observed in 58.1% of the pandemic cases in one study [27]. Whereas only 1.8% subjects were diagnosed with Mucor mycosis in COVID-19 patients in another multi centric study [28] In our study, it was 26.78% (30 Mucorales spp. were isolated from 112 samples). We diagnosed Mucor mycosis by KOH mount and culture on SDA unlike some study who used histopathological examination for diagnosis [29].

Diagnosis of CAM is challenging as the clinical and radiological features of pulmonary and disseminated Mucor mycosis are non-specific and could overlap with findings thought to be associated with COVID-19, which results in missed or late diagnosis. CAM can also be mistaken for other Angio invasive fungal infections, particularly with COVID-19-associated pulmonary aspergillosis being the predominant mould disease in COVID-19-associated acute respiratory distress syndrome [3]. In clinically suspected patients, presence of fungal hyphae, characteristic of Mucorales fungi, by direct examination in 10% KOH from sinus wash, scrapings, biopsy etc. was used for diagnosis. Mucor mycosis was subsequently proven based on microbiological culture or specific histological features from biopsy specimen [28]. Owing to the high mortality, high index of suspicion is required to ensure timely diagnosis and appropriate treatment in high-risk populations.

CONCLUSION

DM, steroid therapy and ICU admission were the common risk factors for the development of Mucor mycosis in covid-19 patients. Mucorales were the most common species isolated (63.82%) followed by Aspergillus species. High suspicion and early diagnosis are the crucial steps in reducing the mortality by Mucor mycosis in covid-19 patients.

AVAILABILITY OF DATA AND MATERIAL

Available with all authors.

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AUTHORS’ CONTRIBUTIONS

1st Author: The conception and design of the study, analysis and interpretation of data and drafting the article. 2nd Author: The conception and design of the study and acquisition of data. 3rd Author: The conception and design of the study and acquisition of data.

CONFLICT OF INTEREST

Not applicable (No conflict of Interest for any author).

FUNDING SOURCES

Not applicable (Nil source).

REFERENCES


Table 4: Type of sample and percentage of positive samples and number of Mucor isolates

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number of samples</th>
<th>Number of Positive samples and percentage</th>
<th>Percentage of positivity</th>
<th>Number of Mucor isolates</th>
<th>Percentage among positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Wash</td>
<td>42</td>
<td>11 (24.44)</td>
<td>26.19</td>
<td>8</td>
<td>72.72</td>
</tr>
<tr>
<td>Maxillary Wash</td>
<td>37</td>
<td>14 (31.11)</td>
<td>37.83</td>
<td>8</td>
<td>57.14</td>
</tr>
<tr>
<td>Nasal Swab</td>
<td>9</td>
<td>4 (8.88)</td>
<td>44.44</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Sputum</td>
<td>9</td>
<td>5 (11.11)</td>
<td>55.55</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Nasal Crusts</td>
<td>8</td>
<td>7 (15.55)</td>
<td>87.5</td>
<td>2</td>
<td>28.57</td>
</tr>
<tr>
<td>Biopsy and Scrapings from nasal cavity</td>
<td>7</td>
<td>4 (8.88)</td>
<td>57.14</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>112</strong></td>
<td><strong>45</strong></td>
<td></td>
<td><strong>23</strong></td>
<td></td>
</tr>
</tbody>
</table>


