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Retrospective Study On Mucormycosis During COVID-19 At Tertiary Care Centre.

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ABSTRACT

Mucormycosis is an emerging aggressive angioinvasive fungal infection caused by the ubiquitous filamentous fungi of the Mucorales order of the class of Zygomycetes. Mucormycosis also called as black fungus and it is the third most common invasive mycosis after candidiasis and aspergillosis. To study the occurrence of mucorales in clinically suspected cases of Mucormycosis. A total of 50 samples were received with clinical suspicion of mucormycosis. Diagnosis was confirmed by KOH wet mount, and fungal culture with LPCB mount, with broad non-septate ribbon-like hyphae, with 90 degree-angled branching showing in specimen of tissue. Among the total 14 (28%) were positive for mucormycosis. Out of 14 positive cases, 9 (64.28%) were females and 5 (38%) were male. And the mean age of all patients was 40.43. Among risk factors, 78.57% had COVID-19 infection, 64% had diabetes mellitus, and 7% had received steroids. Majority of the cases (42.85%) had maxillary sinuses involvement. In all cases. *Mucor* and *Rhizopus* was the commonly identified species. Uncontrolled diabetes mellitus and use of steroids are two main factors aggravating the disease. Early diagnosis and appropriate and timely antifungal therapy with surgical treatment are the cornerstone of mucormycosis.

Keywords: Mucormycosis, Covid-19, Diabetes mellitus.

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INTRODUCTION

Mucormycosis or the deadly black fungus (also called zygomycosis) is a serious and life threatening fungal infection caused by a type of rare but opportunistic fungal pathogen belonging to order Mucorales. This infection is predominantly cause by Rhizopus, followed by Mucor ,Lichtheimia etc [1]. Mucormycosis is an angioinvasive disease that is characterized by tissue infarction and necrosis [2]. These fungi are ubiquitous with particularly higher prevalence in moist soil, decaying plants and foods, bird, animal feces, water and air around construction sites [3]. The main mode of infection of mucormycosis is through the inhalation of spores, consumption of contaminated food and inoculation of the fungi into abrasions or cuts on the skin [4].

In addition, outbreaks of mucormycosis have also been linked to contamination of medical devices, ventilation systems and hospital disposables like bandages, hospital linen etc [5]. Mucorales fungi are the most common fungi in hematological malignancies, hematopoietic stem cell transplantation and solid organ transplantation found after Aspergillus and candidiasis [6]. Mucormycetes are rare but opportunistic pathogens with high morbidity and mortality rates that primarily affect immunocompromised people or those who have metabolic problems. Their invasion in blood vessels and vasculotropism leads to tissue infarction [7]. The clinical presentations of mucormycosis are classified on the basis of anatomic localisation, such as rhino-orbital-cerebral mucormycosis (ROCM), pulmonary, gastrointestinal, cutaneous, renal, and disseminated mucormycosis [8]. Rhinoorbital mucormycosis is the most common clinical manifestation followed by rhino orbital-cerebral mucormycosis in the immunocompromised patients. both of which appear as secondary infections in post SARS CoV-2 exposure. The pathogens associated with mucormycosis varies considerably between India and developed countries. Globally, *Rhizopus arrhizus* is the commonest cause of mucormycosis [4]. Rhino-orbital-cerebral mucormycosis, is mainly caused by Rhizopus species [9]. Patients with diabetes mellitus, haematological malignancy, chemotherapy, haematopoietic stem cells, solid-organ transplant recipients, immunosuppressive therapy, with iron overload, on Peritoneal dialysis, extensive skin injury, human immunodeficiency virus (HIV) infection, and voriconazole therapy are at increased risk of acquiring mucormycosis [10]. Poorly controlled diabetes and steroid induced hyperglycaemia secondary to treatment of COVID-19 with steroids are a potent combination for creating a favourable environment for the growth of mucormycosis [11]. Undiagnosed and uncontrolled diabetes mellitus remains the leading cause of disease in developing countries. COVID-19 can also induce immune dysregulation (for example, by causing lymphopenia). It can therefore predispose to opportunistic infection, including mucormycosis, when coupled with steroid-induced immunosuppression [12].

MATERIAL AND METHODS

Study design

This study is retrospective observational study conducted at Department of Microbiology, Dr.BVP Rural Medical College, Pravara Institute of Medical Sciences, (PIMS-DU), Loni, Maharashtra, India, over a period of 8 months, from April 2021 to December 2021. A total of fifty clinical samples of suspected mucormycosis received in the Microbiology laboratory were included in this study. The present study was approved by the Institutional Ethics Committee. (IEC NO: PIMS/DR/RMC/2022/534.)

Samples were examined by Microscopy followed by fungal culture. For Microscopic study, KOH (10%) wet mount and Gram stain examination were performed. For establishing the diagnosis of mucormycosis, the demonstration of fungal elements on direct microscopy in the clinical sample is essential [13]. (Fig1) For culture, the clinical samples were inoculated on two sets of Sabouraud Dextrose Agar (SDA) one with antibiotics (with chloramphenicol and gentamicin) and second, without antibiotics. Each set was incubated at 22°C and at 37°C. Cultures were examined for growth daily for the first week and twice a week for the subsequent period. The fungal isolates were finally identified by conventional techniques like colony morphology and microscopy morphology. i.e. Lactophenol Cotton Blue (LPCB) mount of teased colonies [13] (Fig 2&3).

RESULTS

In present study, a total of 50 clinical samples were received with clinical suspicion of mucormycosis. Out of these, 14(28%) were reported as positive for mucormycosis and 36 (72%) were

reported as negative for mucormycosis. (Chart 1). In the present study, out of 14 positive cases, 9 (64.28%) were females and 5 (38%) were males. The mean age of incidence of mucormycosis was 40 years and age range of the patients was from 6 month to 73 years. (Table 1) Out of the 50 cases, KOH wet mount was positive for fungal elements in 30(60%) cases and negative for fungal elements in 20 (40%) cases, and in 13 (26%) cases gram stain was positive for fungal elements. And total 31(62%) cases were positive for fungal culture. Amongst which 14 (28%) were culture positive for mucorales, and remaining 17 (34%) showed growth of other fungi like 9 Aspergillus species, and 1 penicillium species & 7 Candida species. (Table 2)

Out of 14 culture isolates, *Mucor* was the most commonly identified in 13 cases (97%) ,and *Rhizopus* was found in only 1(3%) cases. (Table 3)

In the present study, out of 14 mucormycosis cases, Covid-19 disease was the most common risk factor seen in 11(78.57%) patients with mucormycosis, 9(64%) were known to have Diabetes mellitus. And only 1(7%) mucormycosis patient received steroid treatment for Covid-19. (Table 4) Out of 14, 6 (42%) of patients with mucormycosis had maxillary involvement. (Table 5)

Chart 1: Distribution of mucormycosis in clinical samples

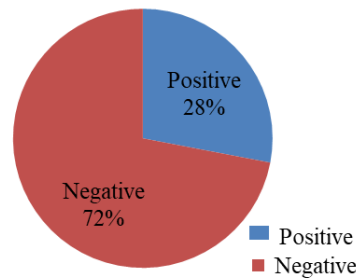


Table 1: Gender & Age wise distribution of mucormycosis cases in present study

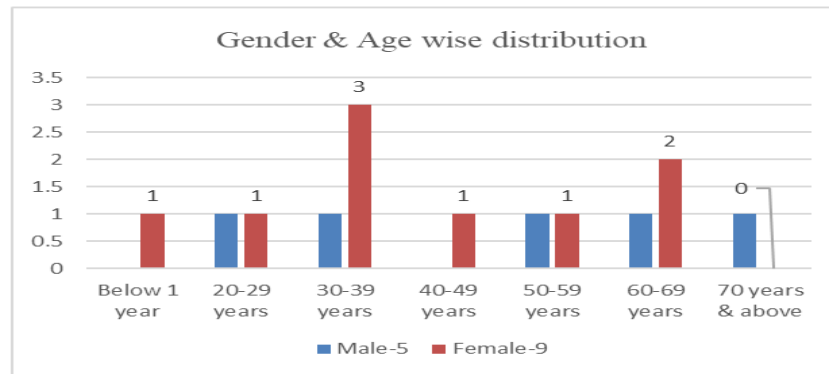


Table 2: Microscopy & culture positivity in clinical samples

Method	Number of samples
10% KOH positive for hyphae	30 (60%)
Gram stain positive for hyphae	13 (26%)
Total culture positive	31 (62%)
Culture positive for Mucorales (<i>Mucor</i> + <i>Rhizopus</i>)	14 (28%)
Culture positive for Other fungi	17 (34%)

Table 3: Distribution of mucormycetes isolates in culture-positive cases

Isolates	Total (n=14)
<i>Mucor</i>	13 (93%)
<i>Rhizopus</i>	01 (7%)

Table 4: Risk factors associated with mucormycosis

Risk factors	Number of patients
History of Covid-19- Present	11 (78.57%)
History of Diabetes mellitus	09 (64%)
Use of steroids for Covid-19	01 (7%)

Table 5: Site wise distribution of the samples

Site	Total number (n=14)
Maxillary sinus	06 (42.85%)
Nasal cavity/septa	03 (21.42%)
Middle meatus	02 (14.28%)
Rhino-orbital (infra-orbital, medial canthus)	02 (14.28%)
Paranasal sinus	01 (7.14%)

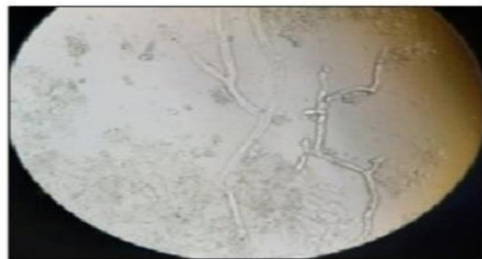


Fig.1 :- 10% KOH mount showing thick Ribbon like aseptate branching hyphae

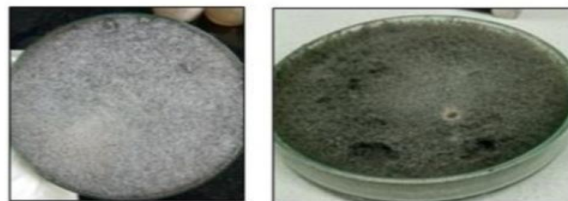


Fig.2 :- Black cottony fluffy colonies of mucor on SDA

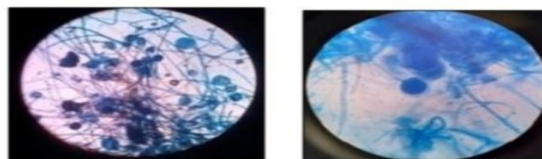


Fig. 3 :- LPCB Mount of black cottony colonies showing mucor with aseptate hyphae with sporangia filled with spores

DISCUSSION

Mucormycosis is an aggressive angioinvasive fungal infection caused by ubiquitous filamentous fungi of the order mucorales. In the second wave of the COVID-19 pandemic, Covid associated mucormycosis (CAM) has been identified as a serious complication [14]. In the present study, 14(28%) patients were reported as positive for mucormycosis (Table no.1). A study by *Rajkumar et al.*, reported, a incidence of mucormycosis were 52 (55.3%) [15].

According to *Moorthy et al.*, the incidence of mucormycosis was not depending on age or gender [16]. In present study, disease was observed predominantly in females 9 (64.28%) as compared to males 5 (38%) and the mean age was 40.43 years with 6 months being the youngest and 73 years being the

eldest. In a study conducted by Roden *et al.*, the mean age was 38.8 years, and the disease was more common in males (65%) as compared to females (35%) [17]. And a study by Priyanka and Jignasa showed that 72% were males and 28% were females [18]. A definitive diagnosis of mucormycosis is mainly based upon the demonstration of fungal elements by direct microscopy (KOH wet mount) and isolation of fungus in culture [13]. In the present study the positivity by microscopy with 10%KOH wet mount was 30 (60%) as compared to total culture positivity 31 (62%) amongst which 14(28%) were culture positive for mucormycosis. The remaining 17 samples were positive in KOH wet mount for fungal elements but culture negative for mucorales, and showed growth of other fungi, such as *Aspergillus*, *Penicillium*, and *Candida* were not included in this study. Several risk factors are affiliated with the development of mucormycosis. According to Mehta *et al.*, Covid-19 is a major risk factor for mucormycosis [19]. A study conducted by Patel *et al.*, Covid-19 was the single disease in 32.6% of Covid Associated Mucormycosis [20]. In present study showed 11(78.57%) of mucormycosis patients were Covid-19 positive which is comparable with our findings. According to Patel *et al.*, diabetes mellitus (62.7%) was seen as a most common underlying disease for mucormycosis [20]. In the present study, diabetes mellitus was seen in 9(64%) of mucormycosis patients. A Case series on Covid associated mucormycosis by Sharma *et al.*, reported Diabetes Mellitus as a risk factor in 91% cases of which 52% had uncontrolled Diabetes Mellitus [21]. Our study showed, one case of Post Natal Care (PNC) mother with no history of Covid-19 disease presented with mucormycosis. In present study, only 7% of mucormycosis patients had received steroids for the treatment of covid-19. A study by Chavan *et al.*, 44% of mucormycosis patients had received steroids [22]. In present study, the history of corticosteroid intake was seen in less number compared to other study. Thus, COVID-19 disease, Diabetes Mellitus and the use of steroids were the main risk factors contributing to the development of mucormycosis in our patients. Prakash *et al.*, identified *Rhizopus* as the commonest species as a cause of CAM,4 however, in our study *Mucor* (97%) was the predominant species grown. Mucormycosis is mainly acquired by inhalation of fungal spores. The spores from the nasal mucosa spreads to turbinates, paranasal sinuses, orbit, palate and brain. A typical characteristic feature of the disease is its rapid progression into tissue necrosis due to invasion of blood vessels [13]. Amongst the clinical presentation of mucormycosis, Rhino-orbital cerebral Mucormycosis (ROCM) is the commonest clinical presentation. If the disease is left untreated, ROCM results in fatal complications in a few days. In our study, majority of the cases (42.85%) had maxillary sinuses involvement, according to Agrawal *et al.*, the maxillary sinus (55%) being the commonest site of involvement which is similar to present study [23]. And only 2 cases (14.28%) presented with rhino-orbital lesion. A recent study conducted by Muthu *et al.*, revealed, rhino-orbital mucormycosis (ROM) and rhino-orbito- cerebral (ROCM) mucormycosis were the most common clinical presentations of CAM (89%) [24]. The case fatality rate of cases reported from India was significantly less than the globally reported cases (36.5% vs. 61.9%, respectively) [24]. In our study, case fatality rate was 15.38% and all the other patients recovered and are on follow up.

CONCLUSION

Our study supports Diabetes mellitus and irrational use of steroids as the major risk factors contributing to Covid associated mucormycosis. To reduce the burden of mucormycosis, strict control of blood glucose level and judicious use of steroids in covid-19 patients is recommended. Appropriate and timely antifungal therapy with surgical treatment is the cornerstone in mucormycosis.

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