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OPPORTUNISTIC INFECTION OF MUCORMYCOSIS DURING COVID-19 AND ITS CLINICO-MYCOLOGICAL PROFILE AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS.

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Abstract
A serious opportunistic fungal illness, zygomycosis has become more common during the COVID-19 pandemic, especially in immunocompromised people like those with diabetes mellitus. The current study's objective is to assess the clinico-mycological profile of mucormycosis and identify patterns of antifungal susceptibility, including the minimum inhibitory concentrations (MICs) of widely used antifungal medications. There were fifty clinical isolates that tested positive for mucormycosis. Samples were taken from lung and brain tissues, wounds, nasal swabs, bronchoalveolar lavage, bronchial wash, and paranasal sinuses. Gene sequencing, microscopy, and culture were used for identification. The CLSI M38-A2 broth microdilution method was used to test for antifungal susceptibility to itraconazole, posaconazole, voriconazole, and amphotericin B. The most common isolate (83%) was Rhizopus spp., followed by Apophysomyces variabilis (7%), Lichtheimia corymbifera (7%), and Cunninghamella bertholletiae (2%). Among the antifungal drugs under investigation, amphotericin B demonstrated the greatest efficacy, followed by posaconazole. Voriconazole and itraconazole were mainly unsuccessful. Mucormycosis remains a potentially fatal illness, particularly in individuals with diabetes and a history of COVID-19. The most frequent etiological agent is still Rhizopus spp. The antifungal drugs posaconazole and amphotericin B are thought to be the most likely to work, highlighting the significance of regular susceptibility testing for the best possible treatment results.

Introduction

During the COVID-19 pandemic, zygomycosis (also known as mucormycosis) has drawn a lot of attention, especially in India, where cases have increased at an unprecedented rate. The high incidence of uncontrolled diabetes mellitus, a major risk factor, and the extensive use of corticosteroids have both contributed to the rise in infections.(1-3) Patients with impaired immune systems, notably those recuperating from COVID-19, are more susceptible to invasive fungal infections like as mucormycosis.(2). Mucormycosis disproportionately affects diabetes individuals in India, the "Diabetic Capital of the World," creating a special burden.(4)There is a noticeable lack of thorough research assessing the clinico-mycological profiles and antifungal susceptibility patterns of zygomycetes in this context, despite the fact that numerous papers have shown the epidemiological connection between COVID-19, diabetes, and zygomycosis.(6). There is still a

dearth of thorough antifungal susceptibility profiling of zygomycetes in the Indian population, despite a large number of case reports during the COVID-19 pandemic. By assessing the clinico-mycological traits and patterns of antifungal resistance in patients with suspected zygomycosis, this study seeks to close that gap.

Materials and Methods

This prospective observational study was carried out from November 2022 to August 2023 at the microbiology department of a tertiary care facility. Fifty clinical samples were taken from patients who were thought to have Mucormycosis. Nasal swabs, lung tissue, paranasal sinus tissue, bronchoalveolar lavage (BAL), bronchial wash, infected wound tissue, and one brain biopsy were among the specimens. To find fungal elements, a 10% potassium hydroxide (KOH) mount was used for preliminary screening. Oatmeal agar slants were used to cultivate samples that displayed fungal hyphae on KOH mount. Tease mount and slide culture methods were used for the initial identification of the fungus. Gene sequencing was used to confirm the species. When accessible, histopathological reports were examined and incorporated into the analysis.

DNA extraction ,PCR amplification and sequencing

Every culture-positive isolate has its DNA extracted using an internal procedure. After suspending a loopful of fungal culture in 400µL of lysis buffer (10 mM TrispH8, 1 mMEDTApH 8, 3% SDS, 100 mM NaCl), the mixture was incubated for one minute at 100°C. After adding an equal volume of phenol:chloroform, the mixture was centrifuged for ten minutes at 10,000 rpm. DNA was precipitated using cold isopropanol after the aqueous phase was collected and cleaned with chloroform. The pellet was resuspended in 30 µL of TE buffer after being cleaned with 70% ethanol. Before being used, DNA was kept at -20°C. 25 µL of GeNei PCR master mix and 1 µL of ITS-1 and ITS-4 primers were used for PCR. Amplified products were sequenced via the Sanger technique on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA). The NCBI BLAST database and BioEdit software were used for sequence alignment and analysis.

Antifungal susceptibility testing

Susceptibility testing was conducted using the CLSI M38-A2 (2017) broth microdilution reference method for filamentous fungi. The antifungal agents tested were:

1. Amphotericin B
2. Posaconazole

MICs were read after 46–50 hours of incubation. The Minimum Inhibitory Concentration (MIC) was defined as the lowest drug concentration that completely inhibited visible fungal growth.

Results

Out of 50 samples:

37 (73.6%) were culture-positive
13 (26.3%) were KOH-positive but culture-negative
38 (75.4%) showed histopathological evidence of fungal infection

Among these, 33 patients (66.7%) had a history of COVID-19 infection, while 17 (33.3%) did not. 50 individuals with probable mucormycosis, ages 10 to 70, were included in the study; the age range of 50 to 60 had the highest incidence. 74% (37/50) were men and 26% (13/50) were women. 33 patients (66.7%) had a history of COVID-19, whereas 17 patients (34%) had never had the virus. Approximately 92% of our patients had COVID-19, and 84% of our patients (42/50) had diabetes. Of the 50 clinical specimens, 37 (73.7%) had positive cultures and 13 (26.3%) had positive KOH but negative cultures. 47 instances underwent histopathological evaluation, all of which verified the presence of fungal elements. Paranasal sinus tissue accounted for 36 cases (72%), with bronchoalveolar lavage/bronchial wash (6 cases; 11%), infected wound tissue (5 cases; 9%), nasal

swabs (2 cases; 4%), lung tissue (2 cases; 4%), and brain tissue (1 case; 0.5%). The most frequent presentation was rhino-orbitocerebral zygomycosis, which affected 39 patients (77%), followed by cutaneous zygomycosis (5 cases; 9%) and pulmonary zygomycosis (7 cases; 14%). Rhizopus spp. was the predominant fungal isolate, followed by other members of the order Mucorales.

All 37 culture-positive isolates underwent antifungal susceptibility testing against amphotericin B and posaconazole.The table 1 provides a summary of the MIC interpretative ranges for the tested antifungal drugs. With MICs between 0.5 and 2 μg/mL, amphotericin B is probably effective against the majority of isolates. Reduced susceptibility was suggested by isolates with MICs ≥4 μg/mL, whereas isolates with MICs ≤1 μg/mL were deemed likely to be susceptible. With MICs ranging from 0.125 to 4 μg/mL in the majority of isolates, posaconazole demonstrated good in vitro activity. MICs of less than 1 μg/mL were thought to be sensitive, whereas values more than 4 μg/mL indicated resistance. With MICs >8–16 μg/mL, itraconazole and voriconazole were ineffective against all isolates; these medicines have no susceptibility breakpoints, and MICs in this range are consistent with their intrinsic resistance against Mucorales.Table2

Table 1: Distribution of fungal species isolated and their MIC values for amphotericin B and posaconazole

Isolate	**Number (n = 37)**	**Amphotericin B MIC Range**	**Posaconazole MIC Range**
Rhizopus spp.	31	0.5–16 μg/mL	0.125–8 μg/mL
Apophysomyces variabilis	3	0.5–8 μg/mL	0.125–0.5 μg/mL
Cunninghamella bertholletiae	1	16 μg/mL	Not tested
Lichtheimia corymbifera	2	1 μg/mL and 16 μg/mL	0.12 and 0.5 μg/mL

Table 2: Identification of the isolates and their susceptibility pattern

Isolate	**Number**	**MIC value for amphotericin B and posaconazole**
Rhizopus *arrhizus*	16	0.5-16 μg /ml 0.125-8 μg /ml
Rhizopus *microsporus*	7	
Rhizopus *delmar*	8	
Apophysomyces *variabilis*	3	0.5 -8 μg /ml 0.125-0.5 μg /ml
Cunninghamella *bertholletiae*	1	16 μg /ml

Discussion

In India, zygomycosis has grown to be a common opportunistic infection, particularly during the COVID-19 epidemic. A high-risk population susceptible to invasive fungal infections has been

produced by the high frequency of uncontrolled diabetes mellitus and the careless use of corticosteroids for COVID-19 treatment.1, 4, 5 Since most zygomycosis cases were observed in individuals with a history of COVID-19 and diabetes, especially those with rhino-orbitocerebral involvement, our data supports this connection. The predominant isolate was Rhizopus spp., which is consistent with both domestic and international epidemiological trends.6, 7 However, the identification of less frequent species like Cunninghamella bertholletiae and Apophysomyces variabilis highlights the significance of molecular techniques for precise species-level identification, especially in unusual or resistant cases.(10)

Antifungal Mechanism

Overall, amphotericin B, a polyene antifungal, demonstrated the maximum activity; nevertheless, a small subset of isolates exhibited resistance (MIC ≥8 μg/mL). Changes in ergosterol biosynthesis or decreased ergosterol levels in the fungal cell membrane are commonly linked to resistance to amphotericin B, which impairs medication binding and effectiveness. 11 Serious clinical ramifications may result from this, particularly in cases of severe infections where poor or delayed treatment may be lethal.A second-generation triazole called posaconazole showed good effectiveness against the majority of isolates. Elevated MICs in a few instances, however, point to the potential for CYP51A gene mutations or efflux pump overexpression, which lower drug binding affinity. 10, 14 This emphasizes the significance of customized dosage plans and therapeutic medication monitoring, especially in salvage therapy.(12). Itraconazole's uneven activity was probably caused by its poor tissue penetration in certain anatomical areas and its restricted in vitro potency against Mucorales. Given that zygomycetes are inherently resistant to voriconazole due to a lack of target binding affinity, it is not surprising that voriconazole exhibited globally high MICs.2,13 Treatment failure and delayed management are concerns associated with the frequent empirical use of voriconazole, particularly in facilities with poor diagnostic capabilities. Antifungal resistance has serious therapeutic ramifications, including delayed response, tissue necrosis progression, increased surgical intervention rates, longer hospital stays, and higher death. These results also put a significant load on healthcare resources in high-burden environments.

Conclusion

This study demonstrates that Rhizopus spp. are the most common cause of mucormycosis in our patient population, especially in those with diabetes and recent COVID-19 infection. The requirement for accurate species-level identification was highlighted by the lower percentage of cases caused by non-Rhizopus Mucorales, which showed inconsistent antifungal susceptibility patterns. While posaconazole functioned as a dependable second-line or adjunct therapy that was likely to be beneficial, particularly in isolates exhibiting resistance or in patients with medication intolerance, amphotericin B remained the most likely antifungal to be effective.Crucially, regular testing for antifungal susceptibility in conjunction with molecular confirmation proved crucial for customizing the right course of treatment and enhancing patient care. The integration of these diagnostic and therapeutic strategies into routine clinical practice is strongly recommended to enhance treatment precision and outcomes in mucormycosis cases.

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