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Mucormycosis: Allegory of a Neo Plague

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Abstract

The commonly known black fungus also referred to as mucormycosis, though rare but is a potentially dangerous fungal infection.The infection affects the sinuses and further affecting other parts of the body. As a primary co-infection with COVID-19, mucormycosis primarily affects immunocompromised persons. The most typical kind of mucormycosis observed in people with compromised immunity is Rhino-Orbital-Cerebral Mucormycosis (ROCM) is discussed in this review article. The asexual spores enter the respiratory tract and spread the infection. Although this fungal infection is difficult to diagnose, it can be treated if diagnosed in the early stages of infection. With initial diagnosis of mucormycosis infection, the physicians advise for relevant imaging and surgical intervention. The first-line of treatment for mucormycosis symptoms includes high doses of liposomal amphotericin-B, posaconazole, and in combination that can be used in salvage therapy, and adjunctive therapy, for better effectiveness. Surgical treatment increases the survival rate; nutraceutical-based therapy is an effective therapy in the early stages of infection. Due to its severity and expensive treatment this infection is one of the major concerns amongst patients.

Key words: Rhino-orbital-cerebral mucormycosis, immune deficiency, mucormycosis, mucormycetes, and Mucorales.

Introduction

When Palatuf first articulated mucormycosis in 1885, it was either distinguished as zygomycosis or phycomycosis[1]. Mucormycosis though rare but conceivably fatal fungal infection that is caused by a class of moulds termed mucormycetes[2]. Mucoralesare found in an array of habitats, which includes soil, deteriorating vegetation, baked goods, and particulates of dust in the air [3]. Ingestion of contaminated food items or through open wounds and injured are few of the potential routes of spore entry. When inhaled, its

filamentous fungi induce mucormycosis. Mucormycosis is a disease that is uncommon in people in good health but is on the rise in people with weak immune system. In patients with immunosuppressed conditions in particular, (hyphal form) fungus [4]those who have diabetes mellitus are more vulnerable to infections. Spores usually enter the respiratory system and affect the paranasal sinuses and lungs. Additionally, they can enter through the gastrointestinal tract or by injury to the skin [5].There are several ways that it can appear, such as superficial, scattered, gastrointestinal in nature, respiratory, rhino-orbito-cerebral, and so on[6]. *Mucorales* can enter deep tissues by injection through contaminated needles or by eating or inhalation of spores. They must engage in combat with the body's first line of defence after they have penetrated a healthy person's deep tissues. Using oxidative metabolites and cationic peptides, the immune system of a healthy host can eliminate the spores [7]. An inadequate health care practice increases the risk of persistent fungal infections among individuals with impaired immunity[8], [9].

Mycobacterium tuberculosis coinfection, acquired immunodeficiency syndrome (AIDS), diabetes mellitus, excess iron content, malignancy, organ transplantation, renal failure, and immunosuppressive medication have all been linked to mucormycosis [10] [11]. Rhino-orbito-cerebral-mucormycosis (ROCM) is the most typical and aggressive clinical type that affects the sinuses or para-sinuses and occurs in individuals who are unable to function because of its tendency to expand contiguously [12]. Nosocomial mucormycosis has been linked to medical interventions and equipments used in hospitals such asinfected wound dressing, contaminated oxygen tubing, air filters, catheters, humidifiers, tongue depressors, cutaneous nitrate patches, and even allopurinol tablets[13]. Although it rarely affects otherwise healthy individuals, people of any generation, sex, or ethnicity can experience ROCM[14]. The infection also affects the mucosa of the nose in addition to the palate, throat, and paranasal sinuses. Usually, the ethmoid and maxillary sinuses are responsible in subsequent invasion into the retro-orbital area through the paranasal sinuses[15]. Mucormycosis includes chromoblastomycosis, mycetomas, sinusitis, and superficial (Tinea nigra), beneath the, cutaneous, and systemic phaeohyphomycosis, according to the disease's clinical spectrum and epidemiological features[16]. After rhino-orbital-cerebral mucormycosis, infections of the skin and lungs are the next most common cases in India (which may or may not have cerebral participation) [17].

Fungus Morphology

Mucorales constitute a collection of primitive fungi distinguished by numerous distinct and exceptional characteristics [18]. Within the order *Mucorales*, these microorganisms serve as enduring inhabitants of the human environment, acting as pioneers on various moist organic substrates [19]. A number of these species possess the capability to induce life-threatening infections, notably mucormycosis, which primarily affects individuals with compromised immune systems [20]. Mucoralean fungi exhibit both sexual and asexual modes of reproduction (Fig 1). Asexual sporangiospores are produced within spherical structures known as sporangia, located at the apex of sporangiophores. These sporangiospores disperse and germinate to form a complex mycelium when the right conditions are met. The majority of infectious Mucorales species are heterothallic, which means that in order to reproduce sexually, two different mating types' hyphae (designated as (-) and (+)) must sense one another and fuse to produce zygospores. After germinating to create a sporangium at the tip, these zygospores eventually produce sexual meiospores. Two compatible mating types are necessary for the formation of zygospores, and the germination of zygospores usually takes a considerable amount of time [21]. *Mucorales* produce their sporangiospores, which are single-celled asexual spores, inside of specialized cells. The development of sporangiospore cell walls occurs independently, independent of pre-existing cell walls, in contrast to conidia generation. The term "sporangia" refers to these cells that are responsible for spore formation. globose cells in sporangiola have one to a countable concentration of sporangiospores, and elongated cells in merosporangia have one to a countable concentration of sporangiospores. The two adjacent cell walls that identify this structure are the outer wall, which is representative of the sporangiolum, and the inner wall, which corresponds to the sporangiospore [22]. One of the most important elements for fungal survival is iron. However, because free iron is bound by particular host mechanisms—ferritin and lactoferrin being the most notable examples—it is usually unavailable in tissue fluids like plasma[23].

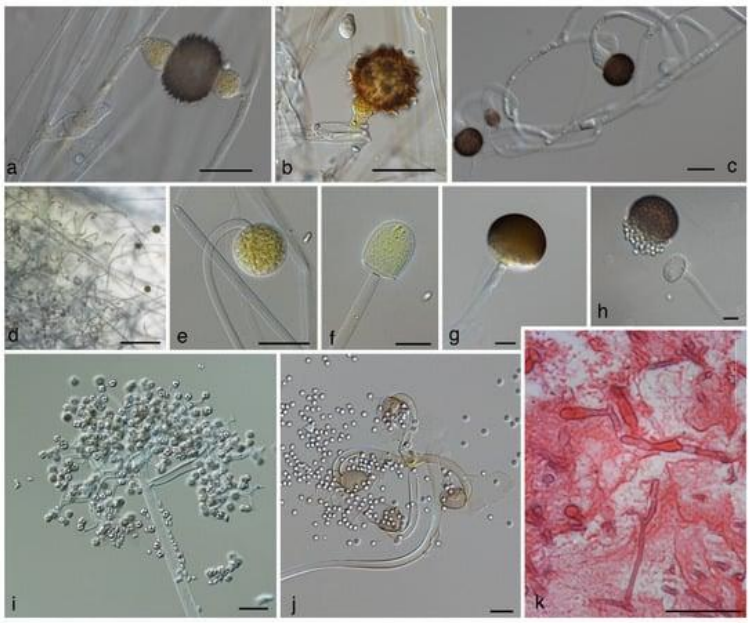

Figure 1. *Morphology of the Mucorales. (**a**) Zygospore with equal suspensors of Mucor endophyticus CBS 385.95; (**b**) azygospore of Mucor bainieri CBS 293.63; (**c**) zygospores with unequal suspensors of Mucor multiplex (syn. Zygorhynchus multiplex) CBS 110662; (**d**) top view on a young mycelium with transitorily recurved sporangiophores; (**e**) recurved sporangiophore and (**f**) columella of Backusellarecurva CBS 318.52; (**g**) sporangium with circumscissile zone of dehiscence and (**h**) discharged sporangium of Pilairaanomla CBS 699.71; (**i**) sporangiola-bearing complex sporophore of Thamnidium elegans CBS 341.55; (**j**) circinate sporangiophore branches with columellae and detached sporangiospores of Circinellaumbellata; (**k**) hyphae of Rhizopus microsporus in human lung tissue. Scale bars = 50 µm except of d = 500 µm. (*Source:Waltheret al, 2019.)

Exposure to mucormould, which is frequently found in soil, manure, plants, decomposing organic matter, airborne particles, and even in the nasal mucus of people without underlying medical conditions, can cause mucormycosis, an uncommon fungal infection. This infection impacts the sinuses, brain, and lungs, posing a significant risk to individuals with diabetes or compromised immune systems, potentially leading to life-threatening complications. [4]. Fungal spores are naturally occurring in surroundings and in healthy individuals with a strong immune response, the body's defenses usually keep them from getting infected. But these fungal spores can easily penetrate the body's defenses and cause serious systemic infections in immune-compromised patients. These infections have a high mortality rate, which can be anywhere from 45% to 80% [4]. People who have both diabetes and hyperglycemia often have a state of inflammation that leads to ongoing localization and stimulation of antibodies, such as neutrophils and macrophages. These cells cause the tissues they infect to remain in a

state of chronic inflammation by releasing proinflammatory cytokines [24]. Immunosuppressive conditions, such as hyperglycemia and glucocorticoid therapy, impair the host's defense mechanisms. This reduces phagocytosis and promotes fungal proliferation, aggravating the course of the disease. The acidic serum pH of diabetic ketoacidosis (DKA) releases iron from proteins that are sequestered, which encourages the growth of pathogens. Furthermore, *Mucorales* have the ability to inhibit host defense genes, which aids in immune evasion[25]. The urgency of intervention is paramount in suspected cases of mucormycosis due to its frequently rapid progression and destructive impact [26]. Delayed onset of therapy is linked to heightened mortality rates [27]. The best chance of survival depends on timely diagnosis and treatment, which means that a multidisciplinary team with experience in medicine, surgery, radiology, and laboratory work must be brought in right away[28].

Rhino-Orbito-Cerebral Mucormycosis

Rhino-orbital-cerebral mucormycosis (ROCM) is one the most common type of mucromycosis. It is caused by inhalation of sporangiospores that enters the nasal mucosa (Figure 2) and the infection progresses to sinuses to the brain [15]. Mucormycosis restricted to the orbits and brain is rare; just a few instances with unilateral or bilateral orbital involvement have been reported [29]. In most cases, the involvement is typically unilateral [14]. Common non-ocular symptoms include nerve paralysis, fever, headaches, sinusitis, hemiplegia, nasal discharge, nasal ulceration, eschar (black necrotic tissues), and face numbness and pain[30]. Early indications of sinusitis are frequently confused with bacterial sinusitis [31]. Ocular symptoms include pain in the eyes, abnormal vision, proptosis, chemosis, ptosis, ophthalmoplegia, orbital cellulitis, and periorbital discoloration, which is frequently accompanied by necrosis. Imaging tests typically reveal thicker mucosal lining, variable degrees of sinusitis, and erosion of the maxillary, orbital, and septal bones of the nose. In more severe cases, there may be soft tissue infiltration, orbital cellulitis, ocular neuritis, bone rarefaction, infarctions, intracranial abscesses, and erosion of the skull bones. The ethmoid-sphenoid sinus, superior orbital fissure, sinonasal, or retro-orbital regions are initially impacted by the infection (Fig. 3). After that, it may proceed to the brain through the cribriform plate or the perineural pathway[30].The mortality rate significantly rises following cranial invasion [31]. Fundus examination plays a crucial role in diagnosing Rhino-Orbital-Cerebral Mucormycosis (ROCM) [32].

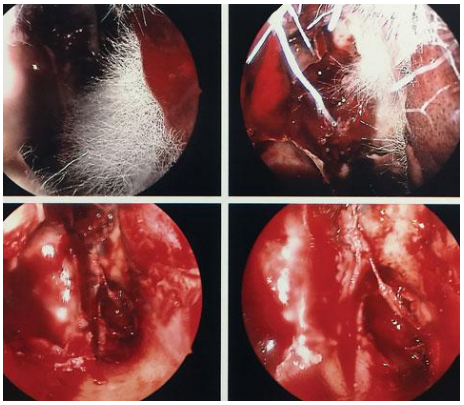

Figure 2. *These nasal sinus endoscopy images showing Mucorales hyphae*.

(Source: Nicholas, K. and Andrew G. (201,). Orbital Mucormycosis on the attack., https://www.reviewofoptometry.com/article/orbital-mucormycosis-on-the-attack*)*

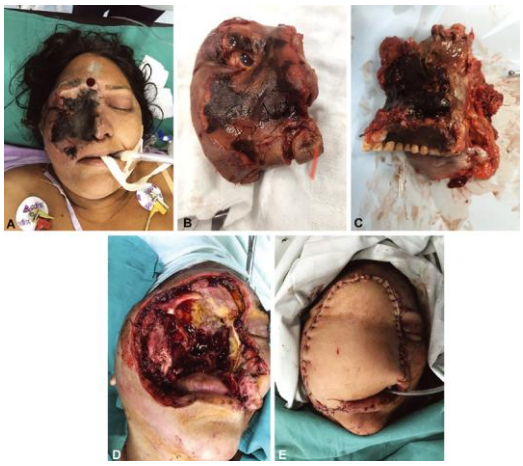

Figure 3. *A patient who was operated using free ALT flap.* ***(A)*** *Preoperative picture of invasive ROCM.* ***(B)*** *Anterior aspect of excised segment.* ***(C)*** *Posterior aspect of excised segment.* ***(D)*** *Residual defect created.* ***(E)*** *Flap inset. ALT, anterolateral thigh*.(Source: Gupta, et al., 2022)

Effect on Different Organs

Mucormycosis can spread to various bodily organs. Organs that could get infected include the skin, gastrointestinal tract, paranasal sinuses, lung, and central nervous system[29]. The brain and oral cavity are the main areas of the body that are affected by mucormycosis, though it can also affect the epidermis, gastrointestinal tract, and different organ systems. The maxilla may also be affected in rare cases [30]. Usually, the infection starts in the mouth or nose and travels through the eyes to the central nervous system. Headaches, nasal congestion, facial swelling, and the appearance of "black lesions" on the nose or upper mouth are some of the symptoms. There may also be systemic symptoms like eye swelling and fever. Hemoptysis, coughing, fever, bulging eyes, chest pain, and breathing difficulties can all be signs of

pulmonary involvement. Symptoms of a gastrointestinal infection can include vomiting, nausea, abdominal pain, and gastrointestinal bleeding [31], [32], [33].

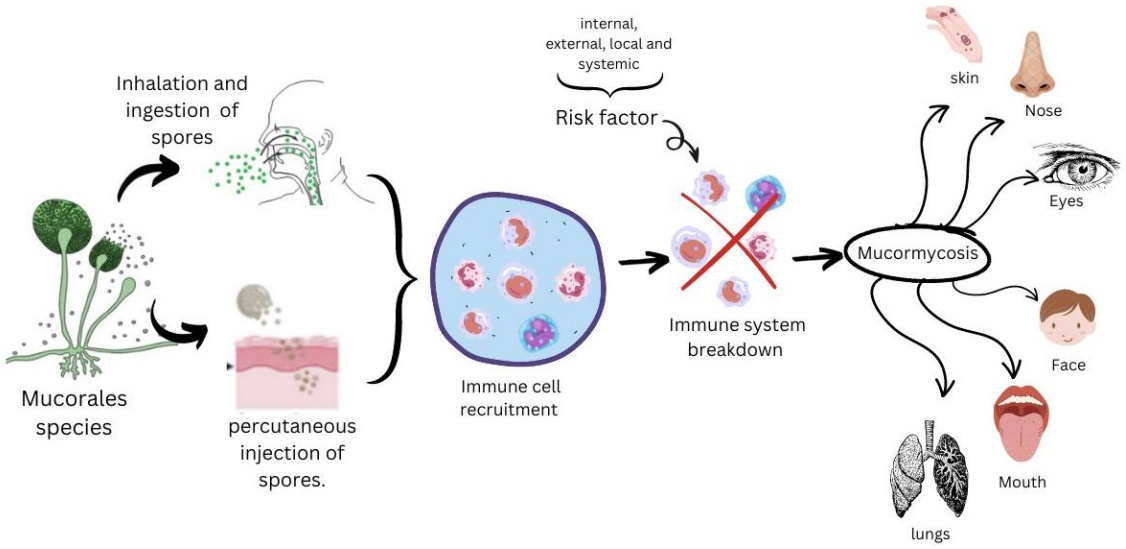

Figure 4. Proposed scheme for cause of mucormycosis in both immunocompromised and immunocompetent patients. This figure depicts the overall Mucorales species (approximately 27 different types) that are known to cause the infection. Initially the spores released by Mucorales enter the body deep into the tissues either by ingestion, inhalation or percutaneous injection of spores. Soon after the infection, the immune cells are recruited to fight against the foreign agents. However, due to influence of various risk factors (internal, external, local and systemic) as shown in the figure, immune system fails to fight against the organism and result in immune disregulation or immune system breakdown. This result in a disease called 'Mucormycosis'. Mucormycosis can affect various vital body organs including skin, nose, eyes, face, mouth and lungs. They cause various symptoms which can vary from minor amputations to major symptoms including depletion of organs and even death. This disease can be prevented or treated by timely diagnosis and effective treatment methods.

Infection

Fungal spores can get into the body via the gastrointestinal tract, inhalation, or direct penetration into damaged skin [38]. After entering, the spores develop into hyphae, which can cause angioinvasion and spread to various organs. *Mucorales* possess essential virulence factors, such as high-affinity iron permease (FTR1), which aids in surviving in environments with low iron levels[39], [40].Furthermore, *Mucorales*surface spore coat protein (CotH) impairs host immune responses [41], and their growth and morphology are influenced by the ADP-ribosylation factor (Arf)[42]. To fully comprehend the effects of these and other

virulence factors on fungal survival and host invasion, more research is required. Furthermore, the dynamics of an infection can be greatly influenced by exogenous variables in addition to those inherent to the fungus and the host. There is conjecture that some of the toxins that cause endothelial disruption might not come from the fungus itself, but rather from bacterial endosymbiosis, which would increase the virulence of the fungus[43], [44]. Research has demonstrated that *Mucorales* can grow and become more virulent when exposed to voriconazole, even in situations where selection is not present. However, the exact mechanisms underlying this phenomenon are still unknown[45], [46]. Even in patients with previously healthy immune systems, severe viral infections such as influenza and COVID-19, which are known to cause acute respiratory distress syndrome (ARDS), increase a patient's susceptibility to mould infections[47], [48]. Elevated mortality rates are associated with COVID-19-related mucormycosis (CAM) and pulmonary aspergillosis (CAPA)[49], [50].

Diagnosis

One of the features of mucormycosis is tissue necrosis due to angio-invasion. Nonetheless, *Aspergillus*, *Fusarium*, *Lomentospora* infections can also lead to tissue necrosis [51]. In these instances, a collaborative effort involving clinicians, histopathologists, microbiologists, and radiologists is essential. Detecting mucormycosis promptly in patients with multiple predisposing risk factors necessitates a vigilant level of suspicion [52]. Endoscopic examination of nasal cavity provides initial indication andadditionally, imaging methods like Magnetic Resonance Imaging (MRI) and non-contrast computed tomography (NCCT) of the orbit and paranasal sinuses are useful diagnostic instruments. These scans could show thickened, hyper inflamed sinus mucosa along with evidence of bone erosion, possibly involving the periosteum and suggesting mucormycosis[53]. These imaging modalities are essential for figuring out how big the lesion is. A strong suspicion of mucormycosis is raised by the combined clinical and radiological features; confirmation is usually obtained by microbiological and histopathological analysis. High-resolution computed tomography (HRCT) of the thorax may reveal characteristic observations in cases of respiratory mucormycosis, such as the reverse halo sign (RHS), the presence of multiple (≥10) nodules, pleural effusion indications, and the air-crescent sign[54],[55],[56]

Routine Laboratory Diagnosis

In clinical settings, wet mount examination, culture, and histopathology are routinely used in the laboratory diagnosis of mucormycosis.

Histopathology

In cases of pulmonary mucormycosis, a final diagnosis depends on the presence of distinct fungal hyphae consistent with mucormycetes in biopsies taken from the affected tissues or bronchoalveolar lavage (BAL) samples. In order to distinguish the fungus from contaminants in the culture, histopathology must first confirm that the fungus is present in the specimen as a pathogenic agent.Moreover, histopathological examination is essential for determining whether there is invasion of blood vessels [57]. Furthermore, histopathology has the capability to reveal any concomitant infections involving other types of molds.Mucorales genera typically produce non-pigmented, ribbon-like, wide (5–20 μm), thin-walled hyphae that are characterized by right-angle branching and sparse or absent septations (pauciseptate)[58]. *Mucorales* hyphae have unique characteristics, which are usually 3–5 μm wide, septate, and show acute-angle branching. Regular staining with hematoxylin and eosin (H&E) may occasionally show severely degenerated hyphae, or it may only show the fungal cell wall with no visible internal structures. The staining techniques like Grocott methenamine-silver (GMS) and periodic acid-Schiff (PAS) stainsare routinely used for highlighting fungal cell wall [57].

Direct Microscopy

For mucormycosis observation, direct microscopy of potassium hydroxide (KOH) wet mounts can be a quick and reliable diagnostic technique. All specimens submitted to the clinical laboratory can be processed using this method, which ideally includes the use of KOH and fluorescent brighteners such as blankophor and calcofluor white. These additives make the characteristic fungal hyphae more visible, which makes observation with a fluorescent microscope necessary [59]. During intraoperative procedures, direct microscopy of fresh specimens is a cost-effective yet invaluable technique for quickly determining precise surgical margins and providing an assumption diagnosis for invasive fungal infections[60]. A different strategy uses monoclonal antibodies to target *R. arrhizus*that has proven useful in differentiating between aspergillosis and mucormycosis (with a sensitivity and specificity of 100% for mucormycosis), and it can aid in diagnosis in cases where cultures produce negative results[61], [61], [63].

Culture

Just like any other microbial identification, culture samples are essential that helps in genus and species level identification. Most *Mucorales* that are important for medicine are thermotolerant, growing quickly at 37°C. They can develop on a variety of substrates that

contain carbohydrates, and colonies usually form in 24 to 48 hours [59]. For the identification of cultured Mucorales, Matrix-Assisted Laser Desorption Ionization-Time OfFlight Mass Spectrometry (MALDI-TOF MS) offers a promising method especially in diagnostic labs [64]. A positive culture from a site free of contamination is necessary for confirmation of the diagnosis; conversely, a positive culture from a site prone to contamination could indicate the existence of non-pathogenic microorganisms, which would need to be evaluated in conjunction with clinical and radiological data to establish a probable diagnosis [26].

The main issue with culture observation is its restricted ability to detect, which could result in incorrect negative findings in nearly half of mucormycosis instances [59], [65]. This is due to a number of factors, one of which is the homogenization or grinding of tissue specimens, which can harm the fragile hyphae of mucormycetes. Moreover, inexperience can also affect sensitivity[59], [66]. To get the best diagnostic results, specimen handling and adequate sampling are crucial. Therefore, in order to guarantee that all diagnostic procedures are performed accurately when there is a suspicion of a case, clinicians and the microbiology laboratory must work closely together and communicate effectively.

Treatment

It is imperative to take immediate action in order to treat mucormycosis, as it requires both medical and surgical intervention. Upon suspicion of ROCM, prompt consideration of empirical antifungal therapy and surgical debridement should be given due consideration, due to its risk factors, clinical presentation, and imaging findings[26]. A tripartite strategy involving reversal of immunosuppression, antifungal treatment, and thorough surgical debridement is commonly employed.

First Line Therapy

Liposomal amphotericin-B at high doses is used as first-line treatment. For better results, the best time to start liposomal amphotericin-B treatment is from the initial stages of diagnosis. In the study involving 80 clinical isolates of Mucormycetes, amphotericin B, posaconazole, itraconazole, and isavuconazole were found to be the most effective antifungal drugs. Liposomal amphotericin B has been shown to be effective in treating mucormycosis in a variety of organ involvement scenarios by numerous case series [66], [67], [68], [69], [70], [71], [72], [73], [74],[69],[75]. However, patients administered 10 mg/kg per day experienced notable, albeit mostly reversible, increases in serum creatinine levels [69],[71]. There was no observed increase in blood concentrations with doses exceeding 10 mg/kg per day [76].

Adjunctive Therapy

As a result of a severe lack of research with reliable data, adjunctive therapy is not recommended to treat mucormycosis symptoms. A few scientists used Deferasirox and hyperbaric oxygen to test*Mucorales*. Only patients with type 2 diabetes had higher survival rates when exposed to hyperbaric oxygen; all other patients had very low survival rates. Case studies have shown that patients with mucormycosis who received recombinant interferon-γ, granulocyte macrophage colony-stimulating factor, and recombinant granulocyte colony-stimulating factor in addition to liposomal amphotericin B (LFAB) survived [77], [77], [78], [80], [81]. Granulocytes are better equipped to combat mucormycosis pathogens when they are exposed to inflammatory cytokines like interferon-γ and granulocyte macrophage colony-stimulating factor. However, it's unclear exactly what role recombinant cytokines play in the first stages of treating mucormycosis [82].

Surgical Treatment

The major task would be to remove the necrotic tissues surgically in curing mucormycosis. When paired with appropriate systemic antifungal medication, surgical intervention has been demonstrated to curecompared to antifungal drugs[83]. Reduced penetration and delivery of systemic antifungal medications to the infection site is caused by mucormycosis, which is caused by blood vessel thrombosis and subsequent tissue necrosis. In most cases of rhino-orbital-cerebral mucormycosis (ROCM), therefore, successful management often depends on surgical excision of necrotic tissues. When it comes to the diagnosis and treatment of mucormycosis, the Mycoses Study Group Education and Research Consortium (MSGERC) and the European Confederation of Medical Mycology (ECMM) global guidelines strongly support the use of first-line systemic antifungal therapy in conjunction with prompt, comprehensive surgical intervention whenever possible[26]. A thorough review analysis revealed that patients who had surgery had a 78% survival rate as opposed to 22% for those who did not[84]. The entire orbit, including the eye, surrounding tissues, and structures behind the eye, must be removed during orbital exenteration. When there is an invasion of the orbit, ROCM is typically treated with this major surgical procedure. The most challenging aspect of managing ROCM is deciding whether to perform exenteration. While exenteration may be necessary to save a patient's life, it comes with the significant consequence of permanent facial disfigurement and lifelong impact on appearance. In the absence of clearly defined guidelines, the criteria and optimal timing for performing exenteration remain uncertain. The available evidence from literature is insufficient to offer physicians enough information to confidently determine when exenteration should be pursued [85]. A common

indication for exenteration is an orbit that is actively infiltrated and contains a blind, painful, immobile globe. Even in situations where the infection has progressed to the brain, the treatment of mucormycosis may be made simpler by eliminating the afflicted orbit, which frequently lowers the total fungal burden. An extensive review of invasive fungal sinusitis states that situations involving intraocular invasion, orbital apex syndrome, or central retinal artery thrombosis may require orbital exenteration more than others[86].

The prevalence of *Mucorales* in the region, hospitals, susceptible hosts with immune compromised or diabetic, negligence contribute to occurrence of mucromycosis. The early diagnosis prevents disease prognosis and if not diagnosed, mucormycosis can progress rapidly that could worsen the fatality.Although,advancement instrumentation in diagnosis, antifungal drugs and surgical procedures are used as a part of diagnosis and treatment. Additional studies are required to determine drug combination, dosage, duration for standardizing the therapies become important factors for managing RCOM. With a kind of advancement in diagnosis and treatment creates a less painful and ray of relief for patients.

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