International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Breaking the Chain in Therapy of Mucormycosis

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Abstract: ***Background: Mucormycosis is a life-threatening fungal disease that occurs in immunocompromised patients. However, treating the disease without reaching the depth of its pathogenesis wavers one from establishing the effectiveness of said treatment. We present two cases of patients with maxillary mucormycosis with diabetes mellitus as comorbidity, where we studied the role of iron as a benefactor to the progression of the disease and was treated with aggressive surgical management and antifungal drug. Method: In both cases, patients were put on empirical antibiotics. Incisional biopsy was performed. Glycemic Control was achieved and later Surgical intervention in form of sequestromy (Case 1) and partial maxillectomy (Case 2) was performed. Result: For both cases healing occurred with neovascularization. Conclusion: High mortality rates despite current regimen of mucormycosis remains unacceptable. Focusing on eliminating predisposing factors seems more feasible than the foreboding nephrotoxic effect of antifungal therapy with its added resistance. This article aims to highlight the fact that therapy to control ketoacids and iron chelating agents, along with antifungal, should be used to augment the results.***

Keywords: Mucormycosis, Diabetes mellitus, Iron, immunocompromised host

1. Introduction

Mucormycosis is a lethal infection caused by a saprophytic fungus that belongs to the order Mucorales, family Mucoraceae, and class Zygomycetes the genera of Mucorales that are Rhizopus oryzae (R.oryzae), Absidia, Rhizomucor, and Mucor. It manifests in a rhinocerebral, pulmonary, gastrointestinal, cutaneous, or disseminated form. It's association with comorbidities like uncontrolled diabetes mellitus, is acidotic, and occurs in patients with hematologic malignant disease like leukemia or patients receiving immunosuppressive therapy. Symptoms involving the oral and craniofacial tissues account for about 60% of all cases [1]. In uncontrolled diabetes mellitus commonest signs within the head and neck region are maxillary and orbital cellulitis [2].

Intraorally, the hard palate is mostly affected because of its proximity to the infection of the nasal fossa. If left untreated, this condition will cause severe comorbidity with craniofacial spread and death [1]. It poses a diagnostic and therapeutic dilemma for people who are not acquainted with its clinical presentations.

We describe our clinical experience with two cases of mucormycosis of the maxilla related to uncontrolled diabetes mellitus managed at our centre. The series of cases was reported with subsequent aims:

1) To highlight the clinical presentation of mucormycosis.
2) To emphasize the requirement for early diagnosis, prompt and aggressive surgical management.
3) To form awareness that reduction of the predisposing factors results in hampering the proliferation of fungus.

Case 1

A 50 years old female patient reported to Maxillofacial department with pain in upper right back teeth region for 2 months. Patient was unaware of her medical condition. The patient gave history of removal of faulty prosthesis and extraction of mobile teeth in upper arch before 3 months.

On clinical examination facial symmetry was present and paraesthesia was evident over right cheek. Whitish yellow exposed bone observed in right maxillary arch extending from 13 to 16 region of approximately 3X2 cm in size (figure 1). Palatal defect in midline along with exposed bone was seen. No sign of nasal regurgitation.

Halitosis present with absence of pus discharge. Alveolar segment irt 13 to 16, tender on palpation with no segmental mobility.

An orthopantomogram (OPG) showed atrophic maxilla and mandible. However, computated tomography scan showed diffuse heterogeneous and sclerotic appearance of right maxillary sinus. Bone lysis and erosion involving right lateral half of anterior portion of hard palate and alveolar arch. Routine biochemical investigations showed elevated random blood sugar (RBS) levels 377mg/dl and Hba1c-10.7.

The patient was hospitalized and treated by cefotaxim 2000mg per day, metronidazole 1500mg per day and insulin actrapid. A provisional diagnosis of maxillary osteomyelitis

was made. Incisional biopsy of affected area was done under local anesthesia and sent for microbiological and histopathological examination (HPE). HPE report revealed broad, septate/pauci septate ribbon like mucorales hyphae.

The patient underwent surgery for maxillary sequestrectomy with antral curettage and complete removal of necrotic tissue was done leaving fresh oozing bleeding margins at wound site and fluconazole 150mg bd was started. Good postoperative healing was noted (figure 2). Patient was kept for follow up and obturator placement was done (figure 3) after a month with no recurrence within 1 year follow up.

Case 2

A 65 years old male patient reported to maxillofacial department with pain in upper right back teeth region since a week. Patient was hypertensive and diabetic for 5 years and was on medication for same with habit of bidi smoking 1 0-1 2 packet/day for 35 years.

On clinical examination facial symmetry was present. Whitish yellow exposed bone in anterior palate extending beyond midline covering right maxillary arch extending from 1 1 to 1 5 of approximately 3X3 cm in in size (figure 4). Sinus tract with purulent discharge was present in relation to (irt) 1 4,1 5 along with halitosis. Nasal regurgitation was absent. Alveolar segment irt 1 1 to 1 5 was tender on palpation. No segmental mobility was present.

OPG showed generalized horizontal bone loss. CT scan revealed mucosal thickening in bilateral maxillary, frontal, sphenoid, and ethmoid sinus. Routine biochemical investigations showed elevated RBS 402mg/dl, Hba1 c 8.2, PP2BS 322mg/dl.

The patient was hospitalized and treated by cefotaxim 2000mg per day, metronidazole 1 500mg per day and insulin actrapid as well as mixtard. A provisional diagnosis of acute suppurative osteomyelitis of maxilla was made. Incisional biopsy of affected area was done under local anesthesia and sent for microbiological and histopathological examination. HPE report showed broad, septate ribbon like fungal hyphae. Uncontrolled diabetes and hypertension were managed before planning any surgical procedure.

Once the values were acceptable, patient underwent for partial maxillectomy from 1 7 to 24 region with antral curettage and complete debridement were carried out, and the wound was left for healing with secondary intention (figure 5). Fluconazole 1 50mg bd was started. Improvement of healing and pus discharge was checked daily until better healing with neovascularization appeared. Then patient is kept on follow up.

2. Discussion

Mucormycosis is a life-threatening disease which requires multifactorial approach. The ultimate outcome of mucormycosis along with other factors depends on ability to reverse the predisposing conditions. In aforementioned cases, prompt correction of hyperglycemia and acidemia, followed by surgery was done. The stepwise approach taken up was starting patients on antibiotic regimen sans antifungal therapy which was later initiated after post-surgery as for socioeconomic reasons, patient couldn't afford high end antifungal drugs. Meanwhile predisposing factors were kept in line. Said predisposing factors which augment spread of mucormycosis include uncontrolled diabetes mellitus, raised serum iron levels. Role of these factors in disease progression is explained subsequently.

Role of Diabetic Ketoacidosis

Fungi grow better in an environment of increased glucose which causes excessive glycosylation of proteins such as ferritin and transferrin as well as low pH impairs their ability to chelate iron. Phagocytic effect of and neutrophils are affected by low serum pH (figure 6) [1, 2, 10, 13]. Martin showed that in diabetic patients polymorphonuclear leukocytes impair due to glycolytic process and produce less lactic acid than those from normal individuals. This reduction in bactericidal activity of the leukocyte is be reversed by insulin [9]. Angeliki et al suggested that Rhizopus changes physiological killing mechanisms of macrophages and establish prolonged intracellular dormancy via melanin induced phagosome maturation arrest. The inhibition of Rhizopus growth inside macrophages is a central host defense mechanism that depends on nutritional immunity via iron starvation [3].

Other factors which leads to the decreased resistance to infection in diabetes are as follows:

1) Reduced number of antibodies.
2) Lowered state of general cellular nutrition.
3) Decreased activity of complement system.
4) Reduced levels of properdin.
5) Vascular insufficiency [9]

The Role of Iron in Cell Growth of Fungus

Iron has necessary role within the life cycle of Mucorales and its utilization from the host could be a critical pathogenetic mechanism of mucormycosis. Iron is necessary for DNA synthesis and for oxygen transporters in mitochondria which activates the cyclin/cyclin-dependent kinase complexes, thus regulating the progression from the G1-phase to the S-phase of the cell cycle. Fungus, entering the S-phase of the cell cycle, up regulates transferrin receptor-1 expression to get iron from the extracellular environment (figure 7) [4].

The cyclin-dependent kinase inhibitor p21CIP1/WAF1 increases when intracellular Fe3+ decreases, thus delaying or inhibiting transition to S-phase. Hence Bcl2 is down regulated and Bax levels are increased, conditions that activate caspase-3, caspase-8, and caspase-9 which lead to apoptosis and cell death. Serum and other biological fluids act as fungistatic for fungi but pathogens require 10-6 to 10-7 M iron for growth [4].

Iron Uptake by Mucormycosis Pathogenesis

Iron is an important element for cell growth and development bounds to host carrier proteins, such as transferrin, ferritin, and lactoferrin. This degradation avoids lethal effect of free iron. When exogenous iron is added, Mucorales particularly R. oryzae grows extensively in normal serum. Patients with DKA have elevated levels of free iron in their serum which supports growth of R. oryzae

at acidic pH (7.3–6.88) but not at alkaline pH (7.78–8.38) (figure 7) [7, 20].

High-affinity iron permeases or low-molecular-weight iron chelators (siderophores) are used by fungi to obtain iron. The high-affinity iron permeases present in fungi contains redundant surface reductases that reduce ferric into the more soluble ferrous form. 3 ferric reductases, 6 copper oxidases, and 1 high-affinity iron permease captures reduced ferrous iron. The gene encoding high-affinity iron permease (FTR1) is expressed by R. oryzae and inhibition of FTR1 gene expression by RNA-I, reduction of FTR1 copy number by gene disruption reduces the virulence of the mucor [8].

Rhizopus secretes rhizoferrin, a siderophore (low-molecular-weight iron chelators) form rhizoferrin iron complex that supplies iron through a receptor-mediated, energy dependent process. Through use of heme, fungi can obtain iron from the host. The Rhizopus genome project revealed 2 homologues of the heme oxygenase. Which obtain iron from host hemoglobin. In R. oryzae facilitates intracellular heme uptake through use of FTR1, then releases ferric iron by degradation with hemeoxygenases intracellularly [6, 8].

Role of Deferoxamine

Deferoxamine is used in patients with iron overload strips ferric iron from transferrin and attaches itself on the mold through an inducible receptor, and act as xenosiderophore to Rhizopus by transporting it intracellular by an active reduction of the ferric form into the more soluble ferrous form (figure 7) [3, 15, 17].

The fungal proteins Fob1 and Fob2 act specifically as receptors for iron uptake from deferoxamine in patients with diabetic ketoacidosis, increases availability of free iron in the serum due to protonation of transferrin, is a critical pathogenetic event in the development of mucormycosis [3, 5].

Deferiprone (DFP) and deferasirox (DFX) do not act as xenosiderophores as the fungal iron uptake systems are incapable of detaching iron from them. DFP and DFX might form more stable chemical structures with iron that are not destabilized in the presence of fungal enzymes or siderophores due to inadequate molecular access, as they are smaller molecules than DFO, or higher affinity for iron [4, 19].

Host-Pathogen Interactions

Mucormycosis infections are characterized by extensive angioinvasion that results in vessel thrombosis and subsequent tissue necrosis which prevent delivery of leukocytes and antifungal agents to the foci of infection. Hematogenous dissemination to other target organs leads to angioinvasion. Rhizopus adheres to the extracellular matrix laminin and type IV collagen and invade cells by induced endocytosis.

Endocytosed rhizopus damages endothelial cells. Glucose-regulated protein (GRP78), a receptor that mediates penetration through and damage of endothelial cells by Mucorales [8, 11, 14, 16].

The fungal ligand binds to GRP78 during invasion of the endothelium belongs to the spore coating (CotH) protein family. Interaction between Mucorales CotH and endothelial cell GRP78 receptor and the ability of CotH, mediate invasive disease. More copies of CotH in Mucorales result invasive disease [5, 8, 18].

Rhizotoxin, a type of toxin produced directly from Mucorales; mediate the interaction between the pathogen and the host [5, 8].

Pathogenesis Driven Diagnosis & Treatment

Curbing a lethal disease such as mucormycosis has been subject of interest for years. Reaching its core pathogenesis and acting upon it can bring about change in treatment algorithm of current times. New antifungal agents with activity against mucorales and passive immunization such as iron acquisition through high affinity iron permease can be proven effective against its immunopathogenesis. CotH proteins and Mucorales-specific T cells may act as a potential diagnostic test for mucormycosis in future [1,12].

3. Conclusion

High mortality rates despite current regimen of mucormycosis remains unacceptable. Focusing on eliminating predisposing factors in this article seems more feasible than foreboding nephrotoxic effect of antifungal therapy with its added resistance. Mucorales already present in the wound can be stopped proliferating by altering the environment, favorable for their growth. This entails controlling the ketoacids (diabetic ketoacidosis) and iron uptake since Mucorales thrive on iron. Along with that aggressive therapy with antifungals and surgical debridement would prove to be exponentially helpful in improving the outcome of an otherwise dreaded disease.

This article aims to highlight the fact that therapy to control ketoacids and iron chelating agents, along with antifungal ,should be used to augment the results. Cases were treated at our place taking in to consideration the above facts which lead to dramatically encouraging results in our cases.

4. Conflict of Interest

Authors declare that they have no conflicts of interest.

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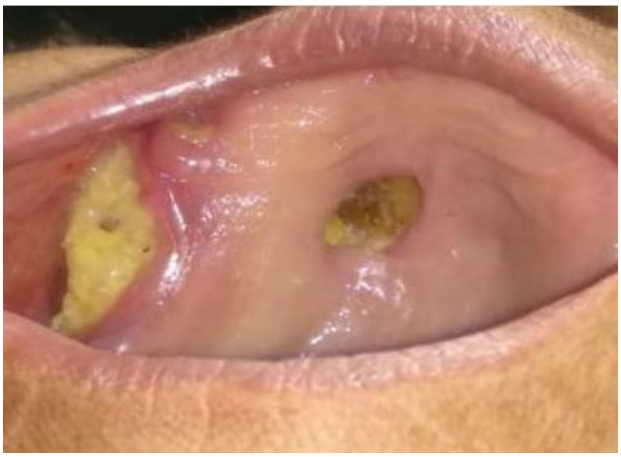

Figure 1: Exposed maxillary bone

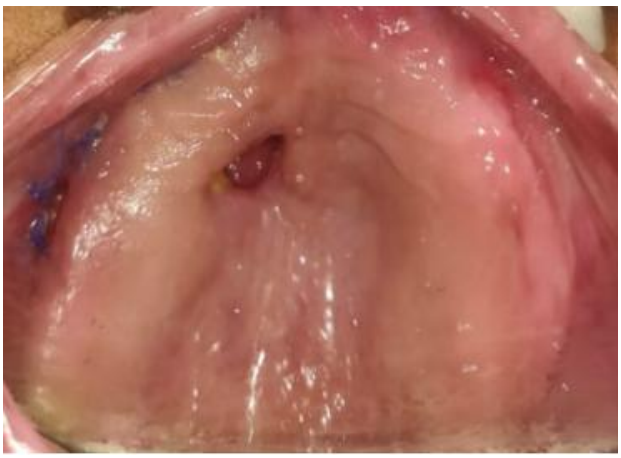

Figure 2: Postoperative healing after a month

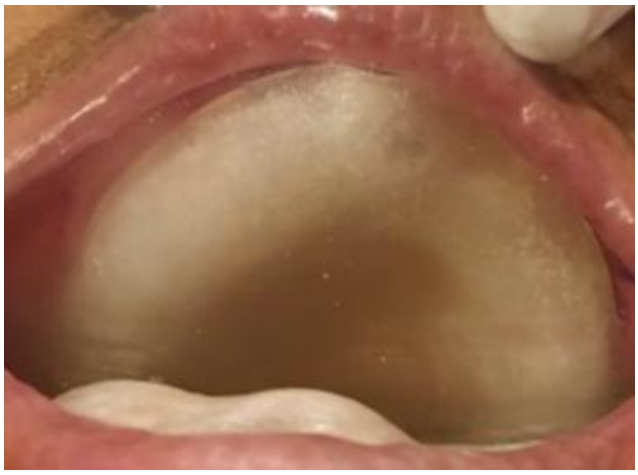

Figure 3: Obturator placement

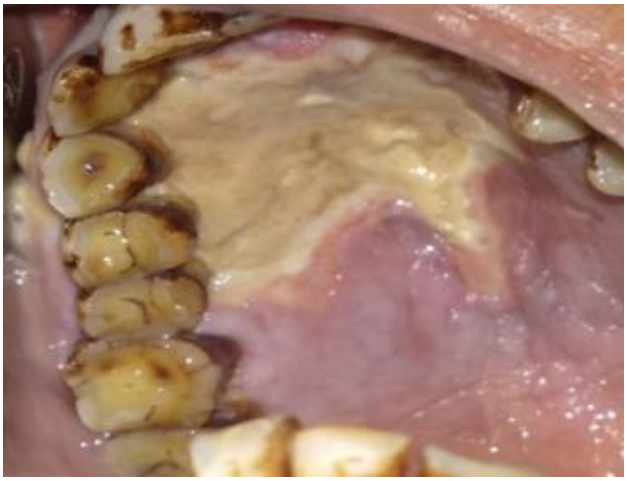

Figure 4: Exposed palatal bone

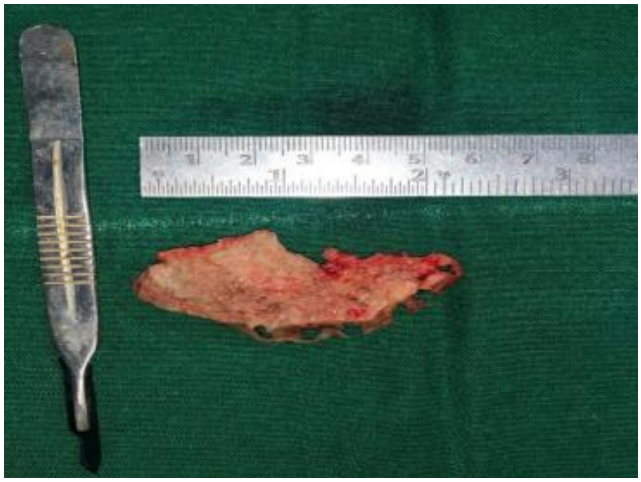

Figure 5: Specimen

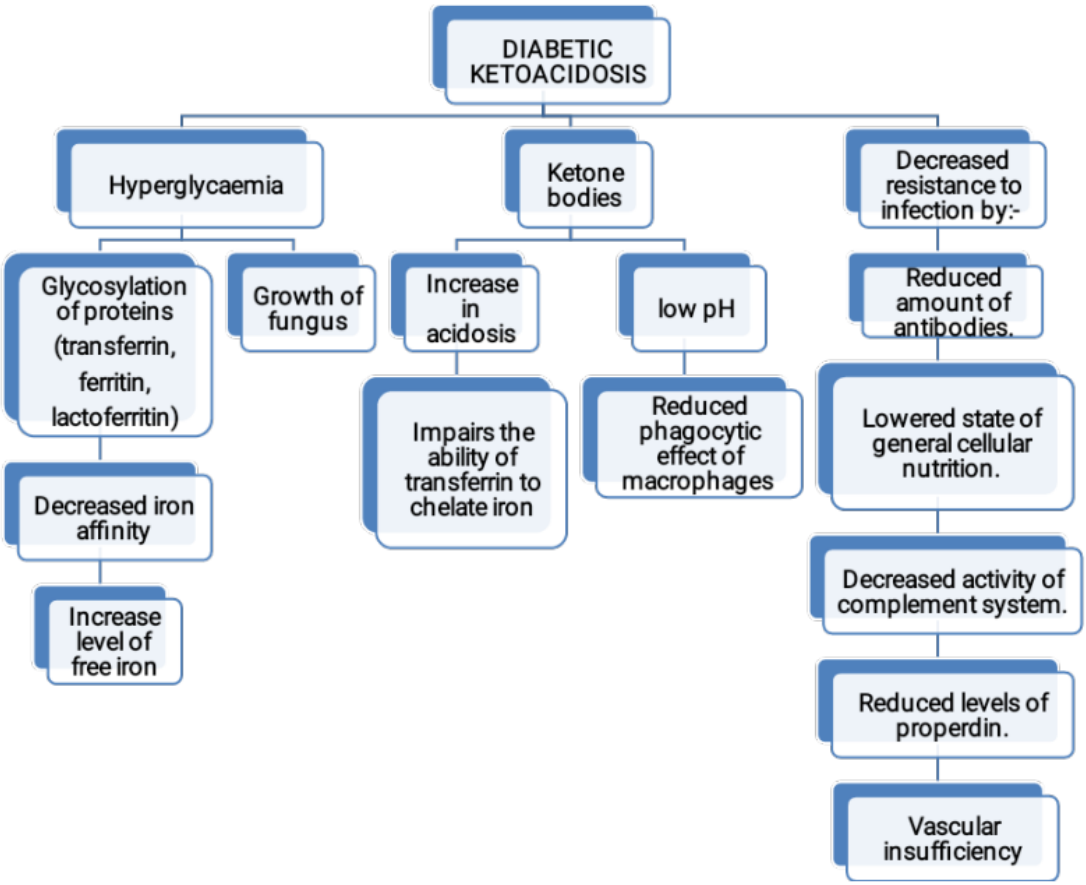

Figure 6: Role of Diabetes in Mucormycosis

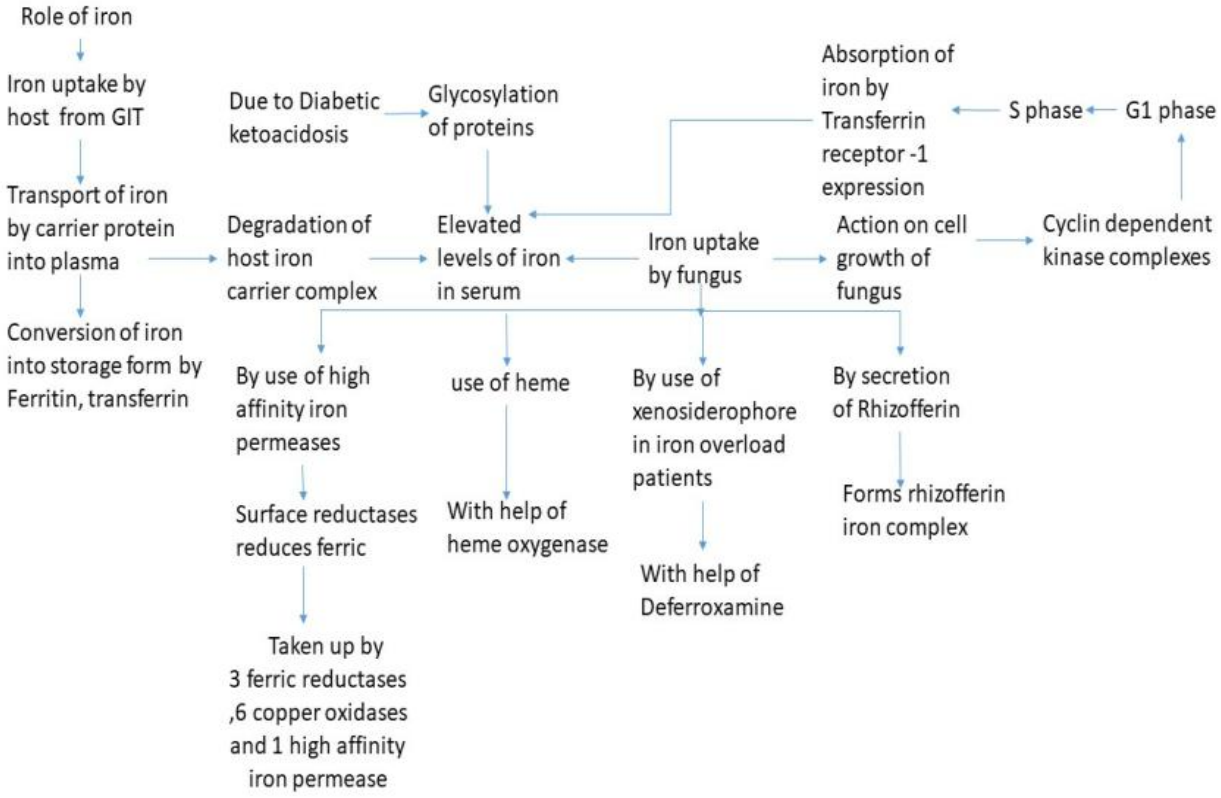

Figure 7: Role of iron in Mucormycosis