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Review of etiopathogenesis and Early diagnosis of Mucormycosis

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Abstract:

Mucormycosis refers to invasive fungal disease whose causative organism are saprophytic fungi. Recently there has been an outbreak of mucor in post covid cases. It is a fatal disease until properly intervened.The literature regarding mucormycosis is scarce. There is urgent need to understand the aetiopathogeneis and clinical features of mucormycosis. This article helps to comprehensively understand the etiopathogenesis in patients having mucormycosis. This narrative review is an attempt to comprehend aetiopathogeneis and early diagnostic features in mucormycosis.Literature on various aspects of mucormycosis was collected from various search engines like pubmed, Google scholar. Various phrases used were mucormycosis, etiology, pathogenesis diagnosis.Various risk factors associated with this disease are use of corticosteroids, metabolic acidosis, diabetes mellitus, burns organ transplants, malignancies and various haematological disorders. Nososcomial infections mostly due to non sterility is proving to be a kajor risk factor has in mucormycosis. Immunocompromised patients are at a greater risk for acquiring infection however few reports of mucormycosis in immunocompetent patients have been published. Angioinvasion leads to thrombosis followed by necrosis of the tissue hampering drug delivery to the target tissue. Angio invasion is the single most important factor explaining pathology.Early diagnosis and intervention is the only key way to treat mucormycosis patients as early diagnosis will help in early treatment and that ultimately decrease angioinvasion and less extensive surgeries. Traditional cultural method can be used for early diagnosis but it has a higher possibility false negative results. Few clinical signs that may help in early diagnosis can be necrotic eschars in various mucosal surfaces, pleuritic pain, ophthalmic symptoms are important markers. Though these clinical signs help, they may present after long time. So imaging techniques like CT scan and MRI have a really important part in early diagnosis. MRI is more sensitive for tthe diagnosis of invasive fungal sinusitis than CT and it also gives less false negative results.

Keywords :Mucormycosis, etiology, pathogenesis, clinical diagnosis.

Introduction:

Mucormycosis refers to angioinvasive disease with causative organism as saprophytic fungi of order Mucorales. There are not many studies for prevalence of mucormycosis however it is estimated that preponderance of mucormycosis is nearly 70 folds greater in India compared to world.[1] The fungi of order mucorales and entomopthorales together are grouped in class zygomycetes causing infections called as zygomycoses. Entomopthoromycosis is infection caused by entomoptharales, mainly seen in immunocompetent hosts in developing countries.

Whereas mucorales are largely responsible for life threatening form of anion invasive mucormycosis uniformally affecting immunocompromised hosts all over world.[2]

Mucormycosis is mainly caused by Rhizopus and mucor species. Others that are involves include rhizomucor Syncephalastrum Cunninghamella, abertholletiae, Apophysomyces, Lichtheimia or Absidia and Saksenaea,There has been explosion of mucormycosis cases around the world following Covid 19 pandemic. What was a rare clinical entity before has achieved status of pandemic in pandemic. Majority of cases are turning up from countries like India. India alone reported 40285 cases till 28th June 2021.[4]

It is very hazardous infection with high death rate and morbidity unless treatment is instituted at the earliest.

Risk factors:

Metabolic acidosis particularly diabetic ketoacidosis, uncontrolled diabetes Mellitus, rampant use of high dose of steroids, various transplants, neutropenia, injury , haematological disorders appear to be risk factors. Haemolytic patients undergoing iron chelation therapy with desferioxime seem to be particularly vulnerable [1].

There is a discordant scenario regarding risk factors or underlying diseases in western countries and Asian countries. It is reportedly more common in diabetics in Asian countries whereas organ donation recipient and haemtatological malignancies were found to be major risk factors in European countries. Significant number of cutaneous mucormycosis in immunocompetent host is being reported. Health care associated mucormycosis as an enity is also being repoted in recent literature. [1]

Nearly half of patients with mucormycosis in India have diabetes as a predisposing factor whereas global data estimates it to be 36-40. Nearly 10% diabetic patients suffering from mucormycosis were reported to be in ketoacidiosis state . Nearly 10% patients presenting with diabetes were having diabetic ketoacidosis. The prevalence of mucormycosis in diabetics is reported to be 1.6 cases per 1000 diabetics[5]. Absence of regular follow up, non compliance with antidiabetic therapy my result in uncontrolled diabetes a situation quiet common in India .

Haematological malignancy is the most important predisposing factor for mucormycosis in Europe and USA accounting to almost 38% to 62%. Patients with AML, preleukemia , HSCT and ALL are greater danger of contracting mucormycosis along neutropenic stage.

A small proportion of patients with organ malignancies and SOT recipients also form major risk factors for mucormycosis. Different researches reported SOT as a predisposing factor in 2–15% mucormycosis patients. Patients suffering from Renal impairment, diabetes mellitus and voricaonazole or capsofungin antifungal prophylaxis for solid organ transplantation are more susceptible to develop mucormycosis.[6]

SOT Recipient receive high doses of steroid for purpose of immunosuppression making them susceptible for mucormycosis. Impairment of the macrophage and neutrophil function because of steroids may be the underlying mechanism. Autoimmune disorder another condition in which steroids are used for treatment is also reported as morbid condition in mucormycosis. From data gathered through all over the world registered 2 out of 100 patients suffering from mucormycosis presented with autoimmune disorder as a morbidity [7].

Iron overload and deferoxamine therapy has significant association with mucormycosis. Patients suffering from DKA, dialysis, acute renal failure and transfusion disorders receive deferoxamine for treatment or prevention of was iron/aluminium overload[8]. The Fe excreted through deferoxamine is collected by siderophores on *Rhizopus* species also it aids to flourish these fungi. Moreover, few new iron chelators like deferasirox and deferiprone adequately chelate iron and not exposing the patients to mucormycosis.

Antifungal prophylaxis: SOT recipients on antifungal Breakthrough antifungal prophylaxis with azoles or echinocandins suffer from mucormycosis as breakthrough infection. Other predisposing factors related to mucormycosis are HIV , IV drugs, low weight at birth, over or under nutrition, alcohol, liver disorders, chemotherapy and calcineurin inhibitors [7]

Surprisingly large number of cases of mucormycosis are being reported in immunocompetent host with no morbidity or predisposing factors. Two systematic reviews conducted at various times reported that approximately 19 out of 100 mucormycosis patients were immunocompetent hosts [5].

Healthcare Associated Mucormycosis

Healthcare related mucormycosis and cutaneous mucormycosis in particular are reported in immunocompetent hosts. Isolated cases of renal mucormycosis are also observed in India and China.[1] Various objects like infected umbilical catheter, dressings, tongue depressors, wooden sticks , and bandages are probably responsible for infection. Linen, corn starch are other reported sources of these infections. The skin, GIT, lungs, sinuses and brain were reported to be common sites of infection in descending order of frequency. Disseminated infection was seen in 2% of patients with hospital acquired infection.[1]

Pathogenesis

HOST DEFENSE AGAINST MUCORMYCOSIS

Severe Neutropenia secondary to underlying disease or as result of chemotherapy for malignancy therapy forms a significant risk factor developing mucormycosis in contrast to AIDS patients who do not have such risk. This may suggest lack of phagocytes or its impaired functions increasing risk of mucormycosis. Neutrophils, except T lymphocytes, are thought to be important for hindering fungal spore growth.

Mononuclear /polymorphonuclear phagocytes eliminate Mucorales by producing oxidative metabolites along with defensins. In the patients with diabetic ketoacidosis, phagocytes do not function normally , chemotaxis is hampered and these defences are damaged. However the exact mechanisms of impairment of phagocytic dysfunction in ketoacidosis, diabetes mellitus, and corticosteroids are still not found. These patients have higher incidence of mucormycosis but infection by other pathogens is not proportionately increased pointing to fact that only phagocyte improper functioning cannot help us understand the excessive prevalence of mucor in patients with Diabetic ketoacidosis. It is likely that Mucorales have distinctive virulence characteristics that help organism to use distinctive stage of immunosuppression and physiologic damage observed within these groups of patients[9]

Cutaneous mucormycosis typically seen in patients where skin barrier is disrupted as in burns, trauma and laceration The Mucorales seem to be unable of invading unflawed skin. This points to fact that skin barrier acts as host defence for cutaneous mucormycosis. Increased incidence of mucormycosis as seen after natural disasters like the tsunami in

Indonesia in 2004 and tornadoes occurred in Joplin, Missouri, in June 2011 point to fact that these Mucorales may arise from traumatic installation of polluted soil or water. Tainted surgical dressings, adhesive tape are also origin of primary cutaneous mucormycosis . Direct Introduction is also reported along with use of tainted tongue depressors for newborns / tainted wooden tools used for mixing drugs that are to be given to immunocompromised patients . The transfer of statistics in mucormycosis cases from community acquired to nosocomial infection in permitting host is alarming one [9].

IRON UPTAKE AND MUCORMYCOSIS PATHOGENESS

Iron is important for cell growth and development. Pathogens depend on host for obtaining it. Iron in mammalian host is generally sequestered to proteins such as transferrin, ferritin and lactoferrin making it unavailable for pathogens. This attribute of not having free iron acts as a host dense against many microbes.. in case of Mucorales it has been proved that they are dependent on free iron because *R. oryzae* doesn't grow well in normal serum until and unless iron from outside is added[10] . However patients suffering from diabetic ketoacidosis have increased level of free iron. The serum from DKA aids growth of R. Oryzae at PH less than 7.Addition of exogenous free iron along with maintenance of acidic PH allowed Oryzae to grow profusely. So, conditions with PH less than 7 decrease the iron—attaching capability of serum samples from healthy people , proposing acidosis deranges the capacity of transferrin to bind iron, possibly through proton-mediated shifting of ferric ions from transferrin[11].

The role of rhizoferin secreted by Rhizopus is also very important. It acts as a siderophore that supplies *Rhizopus* with iron. 13 possible siderophore permeases have been identified in genomic sequence of R. oryzae. They can act like receptors to siderophores, along with rhizoferin / deferoxamine. Rhizoferin is incapable in acquiring Fe from blood ; therefore, role of organism's internal siderophores as a virulence factor in that host specifically mammal is supposedly less. The deficiency of rhizoferin to get iron through serum is called attention by adaptation of fungus to utilize xenosiderophores, like deferoxamine, that maybe more efficient in getting Fe from host.[12]

One more method by which organism may extract Fe from host is by utilization of heme. *Rhizopus* genome project disclosed two equivalents for the heme oxygenase . *R. Oryzae equivalent* can help *R. oryzae* or extracting iron.[1]

Corticosteroids and Other Immunosuppressive Agents[14]

Long term use of steroids and immunosuppressive agents used to treat malignancies, transplantation and autoimmune diseases is evident predisposing factor for mucormycosis. Impairment of migration, ingestion and phagolysosome fusion in macrophages and drug induced diabetes might be the mechanism in patients on corticosteroids. Though mucormycosis is associated with long term use of steroid reports in patients with short courses of steroid are also present.

Autoimmune Diseases as underlying cause for mucormycosis has been reported in literature [7] Few patients of mucormycosis with SLE having high death rate are registered.

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Although very few such cases are found, it takes place mostly due to underdiagnoses, so mucormycosis needs to be added to list of differential diagnosis.

HOST-PATHOGEN INTERACTION

Considerable invasion resulting to blood vessel thrombosis and subsequent necrosis is hallmark for Mucormycosis infections. This has two important diverse effects. Firstly angioinvasion contributed to the capacity of haematogenous spread and secondly it prevents effective drug delivery to target tissue. How Mucorales achieve angioinvasion is not known but vandalization and piercing through endothelial cells or ECM proteins lining blood vessels is mostly an important step in the pathogenic strategy[15]. A previous research depicted *R. oryzae* may hold on to ECM laminin also to type IV collagen and attack cells by induced endocytosis. phagocytised *R. oryzae* destroys endothelial cells and aggregate multiplication of organism to result as endothelial cell destruction . Recently GRP78 was found to function as receptor that mediates penetration and damage of these cells by Mucorales . Inspire of its prime purpose as cellular chaperone protein few recent works show the translocation of part of GRP78 to surface of basic unit of life i.e a cell in variety of them. If mechanism of angioinvasion are identified new approaches or strategies for treatment and prevention can be established. [16]

- **MUCORMYCOSIS PULMONARY:**

- It's. the second most prevalent site of mucormycosis . To diagnose pulmonary mucormycosis is still a challenge.. Patients can come with clinical signs and symptoms like lung infiltration and consolidation, multiple nodules, pleural effusion, thickly walled cavities, lymphadenopathy, on imaging studies. Reverse halo sign which is considered ad characteristic feature of mucormycosis, was seen only in 9.8percent of the patients. Pulmonary mucormycosis may be generally unilateral, sometimes bilateral, very few times hilar / mediastinal. In unilateral disease, upper lobe is most common to be affected which is followed by lower and middle lobe of lungs. [17,18,19]

Early diagnosis

It is now known that survival rate of mucormycosis depends on early diagnosis and early diagnosis and early induction with amphotericin It is reported that this strategy will increase survival rate from approximately 40% to 80%. Mucormycosis carries high mortality in present scenario. Till the time there is paradigm shift in treatment methods the only hope of improving survival an outcome lies with early diagnosis. Identification pointing clinical evidence and proper use of laboratory methods is most efficient ways of improving early diagnosis. [22]

Initiation of treatment early in course of disease may decrease angioinvasion. All subsequent manifestations depend on secondary effects of thrombosis secondary to angioinvasions. So prompt diagnosis and treatment for invasive mucormycosis can reduce angioinvasion also prevent direct injury to tissue leading to less chances of disseminated disease. Early intervention also result in reduced need for extensive and debilitating surgery.

Identification of at risk patients

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High risk stratification is an important and accepted tool for assessment of probability of a patient's chance for developing invasive mucormycosis. The high risk factors include diabetic ketoacidosis and neutropenia. Other factors to be considered include type 2 diabetes, low birth weight, trauma, , organ transplantation , autoimmune disorders.

Clinical pointers[22]

Early recognition of some clinical pointers in patients with risk factors will aid in early diagnostic accuracy and predictive power. A necrotic eschar in maxillary, facial, or sino-orbital mucosal surfaces in an immunocompromised host may be an early marker of invasive mucormycosis. Pleuritic pain in a neutropenic host also may also indicate invasion.

In cases with Sino orbital mucormycosis diplopia secondary to involvement of extraoccular muscles should be considered as early marker of disease. Diabetics may have vision blurring. Hyperglycaemia in diabetics may have vision blurring , but no diplopia. Involvement and breaching of lamina leads to involvement of medial rectus creating diplopia.

Cutaneous mucormycosis occurs secondary to direct inoculation.. cutaneous lesions that are seen in immunocompromised hosts should be investigated aggressively.

Recognition of diagnostic imaging

Unfortunately by the time clincal evidence of mucormycosis like eschar appear the disease has been present for the long time leading to poor outcome. CT scans and MRI may help in early diagnosis .

Nodules, halo signs, reverse halo signs, cavities, wedge-shaped infiltrates and pleural effusions associated with pleuritic pain are pointers to pulmonary mucormycosis . Among these lesions, the reverse halo sign in the neutropenic patient has high predictive value for mucormycosis .Whether CT or MRI should be investigation of choice in rhinocerebral mucormycosis is debatable. Bony destruction, best evaluated with CT was considered hallmark feature for diagnosis. It however is feature of late disease. Development of soft tissue abnormalities outside sinus confines are being reported as more finer and earlier indications of the infiltrative nature of mucormycosis. Premaxillary and retromaxillary soft tissue abnormalities have high predictive values. These occur early in natural course of disease contributing to early diagnosis and early intervention.. Some people argue for early MRI scanning in patients with doubtful FIFS based on the possibility of underestimation of disease with CT. [23]

MRI offers better soft tissue contrast resolution thereby increasing chances of soft tissue abnormalities which are hallmark of early disease However, perfect imaging method to detect AFIFS in immunocompromised is still not established.[23]

MRI is more radical than CT in diagnosis of invasive fungal sinusitis, while their specificities are same. Also, MRI gives less false positive results.

A screening instrument should always have high sensitivity. Screening of all patients with sinus and facial signs with MRI as early as possible appears to be a better approach since missing a case is disastrous. It gives additional benefit of preventing ionising radiation exposure this is particularly important because these patient are likely to undergo serial

examinations. However the disease demands extensive and repeated surgical procedures where image guided surgery ma come into play. CT will be important form management point of view [23] . Using CT scan for planning treatment in patients having AFIFS by MRI would limit both exposure and radiation exposure. Focal areas of loss of contrast enhancement (LoCE) on MRI earlier described in other anatomic locations and pathologic conditions like myocardial and pancreatic ischemia and necrosis, is now supposed to correspond with sinus mucosal ischaemia. Early endoscopic signs of AFIFS include ischemic, dusky, or light mucosa blackening of posterior ends of turbinates; eschar being a late feature. Angioinvasion leading to ischaemia may be one of the reason for this early finding. Vascular invasion slowly leads to extrasinus involvement which is late finding in disease process but early finding in MRI.[23]

Culture of the Mucorales[22]

Identification of fungus till level of genus and species rely upon colonial and microscopic morphology also temperature. Majority medically important Mucorales are thermotolerant and grow faster at temperatures ≥37 °C. Microscopic features such as non-septate hyphae, rhizoids, columellae, sporangia and sporangiospores help to define genus and species within the order Mucorales.

Microbiology [20]

It is very important to accurately identify the infecting organism. To enhance recovery of Mucorales, clinical specimens need to be infused onto suitable media , incubated at room temp and at 37 °C. Tissue that is expected to be infected by Mucorales need to be crumbled with scalpel prior to infusion on media; grinding of specimens can demolish the fine hyphae resulting cultures negative. Mucorales Colonies generally show up within 1-2days until residual antifungal agents, that may suppress growth. Most species demonstrate a greyish white, aerial mycelium with a wooly texture and fill a culture dish within 3–5 days.

Fungal cultures have traditionally low yield there can be false negative reports of upto 50% . Homogenisation of samples, presence of genera needing specific culture, Proper sampling and handling of specimens prior to examination are a basic necessity to get a good yield. So, when a case is suspected , good communication also collaboration amongst clinicians and the microbiology laboratory is important to make sure that various steps for diagnosis will be done properly.[20]

Summary:

Mucormycosis is life threatening condition. Earl diagnosis and prompt treatment are essential for outcome. Having high risk strategy in place for effective screening is essential. Clinical and epidemiological high risk stratification followed by urgent MRI can be a good strategy. Fungal cultures though important in identifying genera and deciding pharmacologic treatment can not be used for early diagnosis as they take time for growth and reporting.

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