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An overview on the veracity of Mucormycosis associated with covid-19

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ABSTRACT

To spread awareness, veracity, and affiliated information regarding rare and fatal fungal disease "Mucormycosis associated with COVID-19". We have conducted a descriptive review of all related papers known to author for this topic. Mucormycosis is one of the most rare and fatal fungal disease. To solve the complication related to this disease some diagnostic methods are used to detect it in earlier stages such as total blood count, biopsy, PCR, CT scan and LIFA, however due to the lack of surgical therapy it can be treated by some antifungal agents such as Amphotericin-B, Liposomal and liquid complex Amphotericin-B, Posaconazole and Isavuconazole. Now a day, Amphotericin-B is used on large scale to treat the Mucormycosis disease. The main mode of action of Amphotericin-B is to bind with ergosterol which is a component of fungal cell membrane and cause subsequent fungal cell death. We have reviewed the mechanism of disease progression in the body as well as the method of diagnosis regarding to the disease. The therapeutic agent and assumed treatments also have been reviewed.

KEYWORD: COVID-19, Amphotericin-B, Mucormycosis, Biopsy.

I. INTRODUCTION

[1][2][3]. Corona virus disease 2019 (COVID-19) continues to spread, but some medications, therapies, such as glucocorticoid evaluated the treatment and has shown improved survival rate. But exorbitant use of it leads to fungal infection, namely mucormycosis. Mucormycosis is a rare but severe fungal infection caused by fungi mucorale Rhizopus oryzae species. The frequently identified types of these species in patients are Rhizopus (delemar and oryzae) species, Lichtheimia species and other species belongs to mucoraceae family followed by rhizomucor species, canninghamella species, apophysomyces species, saksenaea species. It is a filamentous heterothallic micro fungus saprotroph in soil, water, dung and rotting vegetation. R. oryzae species require iron mineral and acidic pH (6.88-7.3) for growth, whereas alkaline pH (7.78-8.38) is not supportive for the growth of this species. All the patients identified with corona induced fungal infection had a similar aspect such as diabetic-ketoacidosis, diabetes mellitus, neutropenia, fluctuated serum iron level, solid organ transplantation, haematopoetic stem cell transplantation, chemotherapy, cancer immunotherapy. As shown in Figure 1, mucormycosis can be classified into 6 types based anatomical localization:

Patients who were diagnosed with COVID-19 triggered mucromycosis mainly developed symptoms of Rhino-cerebral-orbital mucormycosis and pulmonary mucormycosis[1-3.] Hence, medical practitioners are in suspicion and doubtful whether all the types of mucormycosis can be triggered in patient? Why only Rhino-cerebral and Pulmonary part became more sensitive on initial basis? What is the exact role of COVID-19 with this fatal fungal disease? Here, we have collected data from respective published review and research articles and have tried to show the relation between COVID-19 and fatal fungal species.

II. PATHOPHYSIOLOGY

[4][5]. A collective clinic data and reference shows that the patient suffering from an impaired immune function, specifically malfunction of neutrophils are at top of the index to be infectious or induce mucormycosis and causes severity in association with COVID-19. When neutrophils get exposed to R. oryzae species they enhance the number of Toll-like receptor 2 and cause up-regulation of Toll-like receptor 2, which

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allows more Rhizopus species to bind and initiate their activity.

[4][7]. The host cell's basement membrane contains extracellular protein formed by laminin and collagen IV. From the underlying stroma, this basement membrane separates the epithelial or endothelial cells. Due to diabetes or chemotherapy or cancer immune therapy or for any reason this epithelial cell damages and that extracellular protein come in direct contact with inhaled sporangiospores of R. oryzae. In endothelial cell, there is a specific recognition site, namely glucose regulator protein 78 receptor which allow Rhizopus to adhere and cell-mediated endocytosis occurs which causes cell death[4-6]. Short hairpin RNA (sh-RNA) is kind of artificial RNA which helps to either suppress or block the function of R. delemar by AntiGRP78 antibodies that resulting to reduce host endothelial cell injury. R. delemar is one of most important and commonly identified species responsible for mucormycosis. A mouse with diabetes ketoacidosis is protected against mucormycosis by antiGRP78 antibodies. The ligand of fungal that binds specifically with GRP78 belongs to the spore coating protein (cotH) family. In mice it has been observed that blocking the site of cotH protein present on fungal species by anticotH antibody or by attenuating cotH protein expression on fungal species, the potency of fungal species decreases. Most often, isolated species from infectious patients are Rhizopus, Lichtheimia, and other species belongs to mucoraceae family found with 3-7 copies of the cotH protein. However occasionally infection causing species are Apophysomyces, Cunninghamella, Saksenaea and Syncephalastrum found with only 1-2 copies of cotH protein. But there is an exception exists which is a taxonomically identified mucorale specie known as "THE ENTOMOPTHORALES" which is non invasive as-well-as non disease-causing due to lack of cotH protein. This data justifies here that somewhere distinctive interaction occurs between endothelial cells GRP78 (glucose regulator protein) of host and cotH protein of fungal species. So by this collective valued data we've to find out the rate of frequency and fatalness caused by interaction between GRP78 and cotH protein.

MECHANISM OF IRON INTAKE BY MUCORMYCOSIS CAUSING FUNGAL SPECIES

[8][10]. Iron is an essential mineral in the human body as it carries oxygen in the haemoglobin of RBC(red blood cell) throughout the body so that body can produce energy. The species responsible for mucormycosis can acquire iron from the host, in the presence or free increased iron serum level stimulates the growth of R. oryzae. So study and analysis shows that fungal species hold certain mechanisms and abilities by which can acquire iron from the host cell. There is a universal host defense mechanism against microbes. Mammalian host cell contains a carrier protein-like transferrin, ferritin, and lactoferrin which bound together with iron which avoids toxicity due to increased iron level. R. oryzae grows poorly in normal iron serum level until extra iron support is not available. Patients diagnosed with diabetic ketoacidosis (DKA) have changed the level of iron which decreases the capacity of transferrin to bind iron causing acidic pH of blood which results in growth of R. oryzae. At (7.3-6.88) pH a fungal species R. oryzae grows well, but at alkaline pH at (7.78-8.38) it can't grow so patients with DKA (diabetic ketoacidosis) have distinctive growth support. Mice present with diabetic ketoacidosis were successfully saved by infection of R .oryzae on administration with iron chelators like deferiprone, deferasirox. Different information started to unlock step by step by study and analysis is going on study shows that every species is not susceptible to iron chelators such as Cunninghamella, Bertholletia and other species belongs to mucoraceae family shows higher inhibitory concentration than Rhizopous species do. Here some clinical reports reported some other complications such as patients who're taking dialysis and treated with iron chelator deferoxamine are at higher risk of Rhizopus infection. The iron of transferrin striped by Deferoxamine and attach itself to a receptor present on mould through which iron start to transport by a reduction mechanism in which iron ferric form converts into ferrous which is in more soluble form. An animal study already performed on guinea pig in which guinea pig infected with Rhizopus and administration of deferoxamine worsen the survival of guinea pig. So after a long discussion on the iron presence and fungal growth shows a distinctive relation between iron and fungal species causing mucormycosis. Fungal species can take iron from the host by special enzyme high-affinity iron permeases or by low molecular weight iron chelator. In fungi as a part of the reductive system, presence of high-affinity iron permease, which contains a surface reductase enzyme that is responsible for the conversion of the ferric form of iron to the ferrous form of iron. This

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ferrous iron form attaches to a protein complex that contains multicopper oxidase and iron permease. High-affinity iron permease enzyme is seen in species R. oryzae during murine infection. The animal model shows that inhibition of this high-affinity iron permease (FTR1) by RNA1 decreases the ability of species to attack host cell or causes an infection. Mice with diabetic ketoacidosis and infected with R. oryzae was protected by administered immunotherapy with anti FTR1p immune serum. Here this animal data justifies that anti-FTr1p immune serum is a very high improvable treatment in mucormycosis.
[8][10]. The fungal species also have been identified with the third mechanism by which it takes iron from the host cell's heme which is the iron source. The Rhizopus species genome project 2 homologues heme oxygenase allows Rhizopus to catch iron from the host cell. This investigated data shows the angioinvasive nature of Rhizopus. So overall what happened is FTR1 at Rhizopus probably work as a cytoplasmic membrane permease that allows heme to uptake intracellularly by Rhizopus. By this reductive system heme if transported to the plasma of Rhizopus where the special enzyme heme oxygenase catalyzes of conversion from heme to ferric ion. So data were collected and analyzed from respective review and research articles by an acceptable mechanism that help our medical practitioners and health care advisors to predict or assume the treatment of the patient upon patients data.

MUCORALE INTERACTION WITH IMMUNE SYSTEM

Whenever our body suffers from a disease caused by a microorganism then immunology is one of the important studies to be considered, because it unlocks the information about microorganism that how a species interact to our protective system and where our protective system become weaken towards the fight against disease-causing species. So here in this section is given the details about the mucorale species and how species interact with the immune system.

THE BRONCHIAL ALVEOLAR MACROPHAGES

[10][11]. The bronchial macrophages are an important attribute of the innate immune system by providing first-line defence. R. oryzae shows high mortality index in diabetic mice than A. fumigatus but it didn't have any effect in non-diabetic, healthy mice. Spore germination seen enhanced in diabetic mice whereas in non-diabetic mice spore germination was inhibited by bronchial alveolar macrophages. Immune suppressed mice alveolar macrophage could neither inhibit the germination nor kill the spore of both R. oryzae and A. fumigatus. The oxidation of L-arginine to nitrite found the essential and important conversion in inhibition of germination of R. oryzae spores in murine alveolar macrophages; on the other hand, gamma interferon and endotoxin were essential and important that prevents germination of R. oryzae spores in human macrophages. Opsonization of spores enhanced phagocytosis of L. corymbifera spores by cell culture murine alveolar macrophages compared with resting and swollen spores. The spore wall's compositions appear to determine recognition and intracellular killing by phagocytes. The Rhizopus species inhibit phagosome maturation of mice alveolar macrophages by melanin presence on the surface of spore and by iron metabolism plays a key role in regulating immune defence.

EPITHELIAL CELLS

[10][11]. Epithelial cells are the first line of contact with fungal pathogens because they surround the outer surface of the skin and alveoli. Mucorales damage the epithelial cells. When transcriptomic analysis interaction of species L. corymbifera, R. oryzae, R. delemar and G. bertholletiae were performed the result obtained was that when this species cultured with human epithelial cells (A-549) that showed that platelet-derived growth factor receptor B(PDGFRB) signalling was the main response of A-549 during infection with mucorale. Blocking of this receptor (PDGFRB) reduced the damage of A-549 were caused by Mucorales fungi. The in-vitro studies performed on polymeric material reveal that mucorale attached to extracellular matrices equally like how laminin or type IV collagen under depletion and supplementation of carbohydrate, but this attachment failed when adding fibronectin or glycosaminoglycans. These results have proven that lectin does not play important role in the interaction of mucorale with immune cells and suggested adherence of the spore to the epithelial basement membrane. The transcriptomic analysis shows that epidermal growth factor receptor (EGFR) is up regulated during the interaction of R. arrhizusw var. delemar with the epithelial cell of the lung. Within infection, with spores, EGFR was phosphorylated on the surface of the epithelial cells and found to be co-localized with spore surface.

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The EGFR inhibitors such as cetuximab or gefitinib diminished (in-vitro) the capability to invade and cause damage to epithelial cells, abolished the fungal burden and attenuated the virulence of R. arrhizus in vivo.

POLYMORPHONUCLEAR LEUKOCYTE (PMNS) OR NEUTROPHIL GRANULOCYTES

[10][11]. Neutrophils are an important part of the innate immune system and they participate in the regulation of the adaptive immune system. It plays a key role in combating the pathogen by producing cytokines and formulation of neutrophil extracellular traps (NETs).The chemotactic factors affect the inflammatory response of neutrophils to mucorale pathogens. These factors were decreased during ketoacidosis and hyperglycemia leading to reduced killing hyphae of R. oryzae by human neutrophils. Swollen spores enhance the neutrophils to produce chemotaxis but not resting (dormant) spores and the hyphae reduced the production of neutrophils chemotaxis. Intracellular and extracellular production of total neutrophils of superoxide anion (O_2-) was increased when contact with hyphae of mucorale species occur but it was less compared to A. fumigatus. The Rhizopus species's hyphae activate the Toll-like receptor (TLR2) and pro-inflammatory genes as IL-1B and tumor necrosis factor-alpha (TNFalpha or TNFA) in neutrophils. Only voriconazole is available for prophylaxis in the patient who suffers from neutropenia. Investigation of TLRs in combination with liposomal amphotericin-B in the interaction of neutrophils with spores showed that neutrophils reduced pro-inflammatory response by turning TLR signalling pathways from TLR-2 to TLR_4 which highlights the capability of TLR to increase the microbicidal activity of neutrophils without cytotoxicity effect. Interferon-gamma and granulocyte-macrophage-colony-stimulating factor alone or in combination stimulated the neutrophils to release the TNF-alpha leading to hyphal damage and eradication of mucormycosis. IFN-gama reduce the release of IL-8 by neutrophils during their contact with Mucorales fungi.

T-CELLS

[10][11]. T cell is part of the adaptive immune system. Mucorales-specific T-cells were found in the patient who suffers from mucormycosis but not in another patient that produces interleukin (IL-4, IL-10, and IL-17) and interferon-gamma. The cytokines damage the hyphae of morale promptly. Treating the T-inactivated cells with IL-2, IL-7, or both cytokines improves the production of mucorale-specific T cells and their cytokines IL05, IL-10, IL-13 and also stimulates the production of CD4+ T cells that are specific mucorale antigens. This is a new method that promises the increase of the response of T cells against mucormycosis.

NATURAL KILLER (NK) CELLS

[10][11]. Natural killer cell is lymphocyte cells contribute to the immune defence against infected pathogens by reducing its semination. Natural killer cell expresses. The receptors that can recognize infected cells and inhibit major histocompatibility complex (MHC) class I molecules that inhibit activation of the receptor. The hyphae damaged by stimulated or non-stimulated natural killer cells, but these natural killer cells don't cause any damage to resting (dormant) spores. The protein produced by natural killer cells namely known as perforin is damage by the hyphae of mucorale. The damage of fungal hyphae discussed here depends on the growth and appears to be more effective in the early stage of infection.

PLATELETS

[10][11]. It plays an important role not only in homeostasis but also in recognition and killing of pathogen. Platelet adheres to spores and hyphae of mucorale but causes damage only to the hyphal structure. The hyphal growth diminished when it come in contact with platelets.

ENDOTHELIAL CELLS

[10][11]. Endothelial cells playing the important role in the internal layer of blood vessels and possess various important roles in pathogen recognition and maintaining physiological function. It can phagocytose and damage the spore of Mucorales fungi. The glucose-regulated protein 78(GRP78) is a receptor present on the surface of endothelial cells and can specifically recognize species belongs to mucoraceae family and not another fungal pathogen such as A. fumigatus. Furthermore, GRP78 mediates invasion and damage of endothelial cell by Mucorales fungi. Increasing the concentration of iron and glucose in diabetic ketoacidosis mice resulted in enhancement of expression in brain, lung, and sinus compared with normal mice. Immunochemistry investigation of the ethmoidal sinus tissue infected with mucorale fungi revealed that GRP78 was highly

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expressed on endothelial cells and was significantly accessible on the surface of macrophages on necrotic tissue.

DENDRITIC CELLS (DCS)

[10][11]. It is considered as a link between innate immunity and adaptive immunity as they can be found between the epithelium and the interstitium. Dendritic cells usually move to the site of infection in response to the release of microbial antigens that enhance the immune response. The resting (dormant) spores and germ tubes of mucorale stimulate the maturation of dendritic cells; in contrast, resting spores of A. fumigatus do not influence the maturation of dendritic cells.

III. DIAGNOSIS

[12][13]. The severe COVID-19 virus started to show its other phase like an association of invasive infection mucormycosis, at this time early diagnosis becomes important for the early management of infection. Diagnosis depends on the case of the patient, because the patient may not present with same type of mucormycosis in association with COVID-19. From when it starts to appear, it is mostly seen with Rhino-cerebral/Rhino-orbital mucormycosis and pulmonary mucormycosis. Still, this fatal fungal infection does not unlock its other remain type in correlation with COVID-19. So concerning general mucormycosis diagnosis, treatment and management first step that should be taken by the clinician is to identify the type of fungal infection upon its geographical dependency. Diagnosis depends on instrumentation sent to be identified or to confirm the type of fungal infection. By multiple diagnostic instrumentation and methods like Laboratory tests, Radiological studies, Biopsy and Histological features clinician get the exact collective report about patient and management may become straight forward.

LABORATORY TESTS

When we are talking about infection firstly microorganism takes apart to be a prior source of the cause. So there are prior lab tests that already exist to help in diagnosing infections caused by microorganisms. Such tests like a total blood are performed to examine the NEUTROPHILS which guide the clinician about patient's body immunity and risk factor. A decrease in NEUTROPHILS shows patient is presenting with a condition of immunosuppression which means decreased count of NEUTROPHILS. Chemistry of the blood such as medical blood glucose, bicarbonates and electrolyte studies shows the correlation of acidosis inpatient. In diabetic ketoacidosis, patient present with abnormal blood pH which is a supportive medium for mucormycosis fungal infection. Furthermore, iron count indicates the activity of fungal organisms. Iron is one of most important risk factor that plays a major role in the growth of R. oryzae. So decreased level of iron from the host shows that active iron uptake occurs by R. oryzae. Below given is the summary of overall tests which are promisingly performed in the diagnosis of mucormycosis.

1) Histopathology

[12][15]. This method is based on biopsies of affected tissue or bronchoalveolar lavage (BAL) which demonstrate fungal hyphae. In patients with pulmonary mucormycosis, for them the histopathology is a very important diagnostic tool. It also can reveal other co-infection with other models. Mucorales genera produce non-pigmented, wide(5-20 µm), thin-walled, ribbon-like hyphae with few or no septation and right-angle branching, in total opposite Aspergillus piece or other hyaline molds, which are typically 3-5µm wide, septate and form acute angle branching. So the patient with pulmonary mucormycosis can be diagnosed by the histopathological method much before.

2) Microscopic examination and culture

[12][15]. Direct microscopy is rapid, most commonly and accurate method to presumptively diagnose mucormycosis. For improved diagnosis, essential first linear tests are done by investigation of specimen obtained from the patient by its media culture and tomographical studies and per cutaneous lung biopsies. Hyphae of mucrorales are varied in width it's of 6-25 µm and are nonseptate or sparsely septate; it's shaping is irregular, ribbon-like appearance. Some culture media such as sabouraud agar and potato dextrose agar boost up the growth of Mucorales at 25-30°C. Microaerophilic environment improves the culture yield for species associated with genera Cunninghamella and Rhizopus, this condition mimics the environment in infarcted tissue. Some species of a group of mucormycosis such as A. elegans and S. vasiform fail to form spore on routine media. Sometimes identification becomes difficult because the strains collected from the patients who receive antifungal sometimes show morphological abnormalities.[12,13] For the identification of species require characteristic structures such as sporangiospores which produce either in sporangia (multiplied), in merosporangia

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(rows of spores) or sporangia (single or few spores). Some other essential characteristics are the shape of columella, presence or absence of an apophysis, branching and organization of stolons, presence or absence of rhizoids, and shape, structure, size of zygospores (sexual stage) including suspensory cells. For sufficient identification keys and subsequent information guide of fungal, mycologist provide more towards it.

3) Serology

[12][15] There is a time when no commercial antigen marker was present to detect mucorale, as galactomannan (GM) for Aspergillus. But further research shows new targets for serological tests, Burnham-Maurish et al evaluated a monoclonal antibody (2DA6) in sandwich ELISA and found it to be highly reactive with purified glucomannan of Mucor species. They constructed lateral flow immunoassay(LFIA) to detect Mucorales cell wall glucomannan in a clinical sample and expressed that lateral flow immunoassay(LFIA) was more commodious to use than ELISA (Enzyme linked immunosorbent assay) and potential to use as a point of care test on BAL (Bronchoalveolar Lavage), serum, urine and tissue. This test is able to identify R. delemar, L. corymbifera, M .cicinelloides and C. bertholletiae, early after infection (in 3-4 days of infection) in murine models.

4) Molecular based techniques

[12][15]. The confirmation and identification of the strain of species involved in infection can be done by molecular methods. For investigation in tissue, methods developed such as PCR(Polymerase chain reaction) based techniques like a nested PCR, RT or real-time PCR (qPCR), nested PCR combined with RFLP(Restriction fragment length polymorphism), PCR coupled with electrospray ionization mass spectroscopy(PCR/ESI-MS) and PCR high-resolution melt analysis(HRMA). From above mentioned methods, many methods reported were successfully applied and performed better on fresh or deep-frozen samples than on paraffin-embedded tissue. For the success of the method, selection of a key determinant is a target for PCR. Mostly used test targets are ITS genomic region, 18s ribosomal RNA genes, 28s rDNA, cytochrome b gene or morale-specific CotH gene. A new pan-Mucorales real-time (qPCR) commercial kit appeared to be a fast diagnostic test with sensitivity 75% tested on infected patient's blood sample. By qPCR assay targeted 18s rDNA in pulmonary samples that allowed detection of 4 main clinically relevant genera i.e. Mucor-Rhizopus spp., Lichtheimia spp., Rhizomucor spp. And Cunninghamella spp. To detect fungal DNA commercial assay was user friendly and all qPCR assays used in the study were superior to conventional methods. Sample containing hyphae of mucorale upon histopathological examination and application of molecular methods confirms the diagnosis and it's recommended highly. For the patient who is Immunocompromised and developed with mucormycosis in them serum mucorale PCR shows a highly reliable tool for diagnosis. In a study using three qPCR for Absidia corymbifera (Lichtheimia) Mucor/Rhizopus and Rhizomucor targeted 18S ribosomal, RNA genes on sera of mucormycosis patient showed this method to very high sensitivity and can detect infection in 3 to 68 days earlier than conventional methods. Spore coating protein homolog COtH genes which is unique for mucorale has shown encouraged results in a mouse model, with urine sample compare to plasma or BAL. This model had 90% sensitivity and was 100% specific.

Radiological studies

[16][17]. Radiological studies are one of the diagnostic approaches in mucormycosis. Because there are may subclinical diseases present. Here history, physical examination and computed tomography of brain, sinuses, chest and abdomen are performed. Depending on geographical localization and the manifestation of infection further procedure of radiological studies is performed.

1) **Rhino-cerebral infection**

[18][19]. Rhino-cerebral associated mucormycosis radiological plain film results may show involvement of sinus with mucosal thickening, air-fluid level and bony erosions. On initial basis the patient should be preferred with head and facial computed tomography (CT scan) that show sinusitis of ethmoid and sphenoid within orbital and intracranial extensions. Infection may spread to the brain or orbits and bony erosion may occur when the disease progress further. A chance of thromboses of the cavernous sinus or internal carotid artery may occur because of vascular involvement of mucormycosis organism. Brain and sinus magnetic resonance imaging is superior to estimate tissue invasion and the need for surgery.

2) **Pulmonary disease**

[18][19]. Radiography's sensitivity and specificity for mucormycosis are low therefore non enhanced high-resolution computed tomography scanning imaging is the modality of choice. In

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scanned imaging film, the most common findings are pleural effusion, nodules, consolidation and ground-glass opacities, consolidation can be multilobar when the disease progress. The strong promising suggestion of pulmonary mucormycosis is concluded by the sign of reverse halo sign (i.e., nodule with central ground-glass opacity and ring of peripheral consolidation); it's rarely observed in invasive aspergillosis. When observation of halo sign means it represents the lung infarction surrounded by alveolar haemorrhage and disassociated with invasive mold infections, but that can be present in bacterial or viral infections and non-infectious lung disease such as Wegener granulomatosis, sarcoidosis, malignancy. When disease further progress in late-stage further findings of air crescent sign and hypodense sign can be observed but it's less specific.

3) **Gastrointestinal disease**

[18][19]. When infection further affects the other organs and system or occur in a specific organ or system such as the gastrointestinal tract or relate to this system initially the abdominal computed tomography may show infection associated with the gastrointestinal tract. With aid of EGD (Esophagogastroduodenoscopy) as well as biopsy the medical practitioner and physician are able to observe the areas of tissue necrosis.

4) **Central nervous system**

[18][19]. When magnetic resonance imaging (MRI) or computed tomography (CT) is performed for the central nervous then it may reveal abscesses or extension of rhino-cerebral disease into the brain. Findings in scanned film obtained were cavernous and less common sagittal sinus thrombosis. The radiological examination of the affected area reveals the signs and confirmation of disease to that particular area and also shows its efficacy to progress and affected neighbor system, tissue or organ. So radiological studies or examination of infected area are initially selected based on affected area, its geographical localization and its physical appearance.

BIOPSY AND HISTOLOGIC FEATURES

[20]. When mucormycosis is under the diagnostic phase the biopsy is one of the modalities of choice. The involved tissue is the most critical mean to establish mucormycosis. A rapid histologic assessment of the frozen tissue section should be performed to promptly institute surgical and medical management for the infection.

BIOPSY OF NECROTIC TISSUE

[20]. The sample of necrotic tissue or specimen of the patient is obtained by nasal, palatine, lung, cutaneous, gastrointestinal or abscess wall site. Fix the tissue after taking a sample and stain it with hematoxylin and eosin or by special fungal stains such as Grocottmethenamine-silver or periodic acid-Schiff (PAS) stains. After staining, in results it shows the appearance of pathognomonic broad (6-25 µm diameter), irregular, ribbonlike, nonseptate or sparsely septate) hyphae with irregular branch occur at 45-90º. Thus, neutrophil infiltration, vessel invasion and tissue infraction are observed. There may be granulomatous reaction also. For the determination of mucorale species culture of biopsy sample are required. Non-septate hyphae are prone to damage so carefully handle it. Don't crush or grind the specimen. It grows usually in 2-3 days. After growth genus and species are determined by examining fungal morphology (i.e. presence and location of rhizoids). For rapid and acute identification of species-level, the Matrix-assisted lasers desorption/ionization time-of-flight (MALDI-TOF) can be used. But it requires a reference database. 18s ribosomal RNA (rRNA) sequencing provides genus-level identification. If tissue damage precludes the fungal growth.

IV. TREATMENT

[21][26]. The treatment of this severe infection in association with COVID-19 depends on the individual patient and patient's factors. So first of all examine the patient that which type of mucormycosis is associated with COIVD-19 depending on its geographical localization. This is because in general mucormycosis anti-fungal therapies are available that can use against the infection but when mucormycosis is associated with COVID-19 a medical professional should be aware about its rapid steps to be taken. Some medical management include diabetic ketoacidosis factor in infection require insulin and volume repletion with intravenous fluids, avoid glucocorticosteroids and other immunosuppressive drugs, inhibit or interrupt deferoxamine therapy and should give its alternative such as hydroxyproline, in neutropenic associated with haematological malignancy, should use colony-stimulating factors. This is a very rare disease induced by COVID-19 and because of this no primary treatment has been performed. But according to the cases seen from September 2020 to January 2021 among mucormycosis patient treated by amphotericin-B and isavuconazole, they

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are an ideal choice of drug in mucormycosis. Table 1 shows first line anti-fungal therapy. For primary treatment these 2 drugs are licensed by us food and drug administration (FDA). Before the antifungal therapy, collection of previous antifungal treatment and related toxicity should be studied. Because the antifungal drug primarily provide to the patient may contain several toxicities, so of that abnormal dose all pre-existing factor present, patient condition worsen the case. The antifungal drug which are primarily provide to the patient that contain several toxicities, so of that abnormal dose or pre-existing present patient condition worsen the case. Example: Patient with present renal impairment or any other condition where patient's renal not able to filter out drug, if in this condition Amphotericin-B formulation administer to patient then the condition of patient become worsen. Like this there is also another factors related to therapy and condition of patient with pre-existing abnormalities.

1) Amphotericin-B

[21][26]. Amphotericin-B is a drug of class polyene. Amphotericin-B has a several formulation such as amphotericin-B deoxycholate (d-AMB or AMB-d), liposomal amphotericin-B (L-AMB), amphotericin-B lipid complex (ABLC), and amphotericin-B colloidal dispersion (ABCD). In many cases liposomal amphotericin-B perfectly cured the mucormycosis within various involvements of organs patterns. Its range of daily dose is 1 mg/kg per day to 10 mg/kg per day. Response rate can be increased by increase the dose. If the CNS is involve in disease then 10mg/kg per day of liposomal amphotericin-B is seen supportive. If the CNS is absence in disease then amphotericin-B lipid complex 5 mg/kg per day seen supportive. In patient with kidney transplantation should be given dose 10mg/kg per day of amphotericin-B lipid complex. Amphotericin-B deoxycholate is drug of choice, but its use limited due to its substantial toxicity. For all pattern of organ involvement the strongly supportive first-line treatment is 5-10 mg/kg per day of liposomal amphotericin-B, if patient develops the substantial renal toxicity then reduced the dose as necessary.

2) Isavuconazole

[21][26]. Isavuconazole has similar efficacy as amphotericin-B in the use of first-line antifungal. It licensed by USA for first-line treatment of mucormycosis. In comparison of other mould active azoles, isavuconazole is less hepatotoxic but it can result into the shortening of the QTc interval. Within the moderate strength isavuconazole is recommended as a first-line treatment of mucormycosis. The dose regimen of isavuconazole is either intravenous or per oral administration is about 3*200 mg day 1-2, 1*200 mg per day from day 3.

3) Posaconazole

[21][26]. In the first line treatment. Posaconazole oral suspension has been use successfully. It is delayed release tablet develop after concern of its oral bioavailability and an intravenous infusion formulation. The dose regimen of posaconazole oral suspension is about 4*200 mg per day. The dose regimen of delayed release tablet is about 1*300 mg day 1 and 1*300 mg per day from day 2.

B) Antifungal combination therapy

[21][26]. Due to lack of beneficial data regarding combination and enhanced toxicity there are no definitive data to guide the utilization of antifungal combination therapy. By the collective data and available information, class polyenes and azoles or polyenes plus echinocandins can be used as a combination.

C) Antifungal salvage treatment

[21][26]. The meaning of salvage treatment is to switch on other class due to intolerance or toxicity of first-line treatment, pre-existing organ damage or refractory mucormycosis. Isavuconazole was the only salvage treatment licenced by Europe for mucormycosis. It was successful in both clinical scenarios, refractory disease and intolerance or toxicity. In the class of polyene drugs liposomal amphotericin-B was effective as salvage treatment. In the category of azoles, posaconazole delayed release tablet or posaconazole infusion are potentially supports salvage treatment.

V. OUTCOME

The mucormycosis is rare fungal disease, but now when the world is facing the COVID-19 there are some triggering factors which can induced the fungal infection and worsen the health of patient after recover from COVID-19. But it seen that the patient who recovered with corticosteroids and if they have diabetic problem are under high risk of fungal associated disease. We have read respected published review and research articles, case studies of patient who are with general mucormycosis and mucormycosis associated with COVID-19. So the outcome of our review paper is that we here given the types or mucormycosis,

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pathophysiology, pathogenicity, multiple diagnostic methods, and patient condition based treatment. Each and every patient present with different conditions and factors, which can change the vision of initiation of treatment. So it is not possible to decide the perfect initiation of therapy, but by the proper examination of patient or after patient's proper examination it's possible to initiate the antifungal therapy. Each antifungal therapy has its own pros and cons which are necessary to know first otherwise it can worsen the condition of patient. So here we classified the different diagnostic methods by which patient can be examined properly plus we have put multiple information of antifungal therapy, which will help the medical practitioner when they practice the antifungal therapy on a specific conditional patient. So here we overcome the trouble and tried to solve the confusion which will may show progress in diagnosis and management of mucormycosis induced by COVID-19.

VI. CONCLUSION

The main purpose behind this article is to spread awareness, veracity and affiliated information regarding to the rare fatal fungal disease 'Mucormycosis'. We reached at the conclusion that the mucormycosis are of so many types but only few types are associated with COVID-19. Rhino-orbital Mucormycosis and pulmonary mucormycosis are the only types mostly seen in patients suffering from Mucormycosis associated with COVID-19. There are so many species identified as etiological agents of mucormycosis though only few species are responsible behind occurring of mucormycosis associated with COVID-19 such as R oryzae, R. delemar and other genus belongs to mucoraceae family. All patients infected with corona virus induced Mucormycosis had a similar aspect such as diabetes ketoacidosis, diabetes mellitus, neutropenia, hematopoietic stem cell transplantation, fluctuated serum iron level and solid organ transplant. For the effective treatment it has to be diagnosed in earlier stages using various diagnostic tests such as histopathology (total blood count), microscopic examination (biopsy), serology (LFIA), molecular based technique (PCR), and radiological study (CT scan). After successfully diagnosed a patient can be treated via some antifungal therapy. There is no primary treatment available for this disease so some antifungal agents are used to control this disease like AMPHOTERICIN-B, LIPOSOMAL AND LIPID COMPLEX AMPHOTERICIN-B, ISAVUCONAZOLE and POSACONAZOLE.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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TABLE 1: FIRST LINE ANTIFUNGAL DRUGS

SL. NO	CLASS	DRUGS
1	Polyenes	Amphotericin-B deoxycholate(AMB-d) Liposomal amphotericin-B(L-AMB) Amphotericin-B lipid complex(ABLC) Amphotericin-B colloidal dispersion(ABCD)
2	Azoles	Posaconazole Isavuconazonium sulfate(isavuconazole)
3	Echinocandins	Micafungin

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		Capsofungin

TABLE: 2 VARIOUS MEDICATION WITH THEIR MECHANISM OF ACTION, ADMINISTRATION, ADVERSE DRUG REACTIONS, CONTRAINDICATION AND MONITORING[26]

NO	FDA APPROVED DRUGS	MECHANISM OF ACTION	ADMINISTRATION	ADVERSE DRUG REACTION	CONTRAINDICATIONS	MONITORING
1	Polyene antifungals: Eg;- 1)Amphotericin-B deoxycholate(AMB-d) 2)Liposomal amphotericin-B (L-AMB) 3)Amphotericin-B lipid complex(ABLC)	Polyene binds to ergosterol and makes a polyene-ergosterol complex, which create pore in the fungal cell membrane, which leads to leakage of electrolyte, cell- lysis and cell death.	i)Amphotericin-B formulations such as AMB-d, L-AMB and ABLC can be administered intravenously ii) AMB-d can also administer intraventricularly	AMB-d Hypotension, chills, headache, hypokalamia, hypomagnesaemia, anemia, renal insufficiency, renal function abnormalities, injection site pain, nausea, vomiting, rigors, and fever. ii) L-AMB Nephrotoxicity, hypertension, hypotension, tachycardia, localized phlebitis, chills, headache, skin rash, electrolyte abnormalities, hyperglycemia and abnormal liver function tests. iii) ABLC Nephrotoxicity, increase serum creatinine, rigors and chills.	All formulations of amphotericin-B (AMB-d, L-AMB, ABLC) are contraindicated in patient with hypersensitive to amphotericin-B or any component of AMB-d, L-AMB, ABLC formulations. i) AMB-d should use for invasive, potentially life-threatening mycoses, avoid use in patient with renal impairment and electrolyte abnormalities. ii) L-AMB, ABLC, ABCD (amphotericin-B colloidal dispersion) are require caution in renal impairment.	All patients receiving any formulation of amphotericin-B should have Bun (blood urea nitrogen) and creatinine assessed at baseline then frequently after; additionally, CBC, electrolytes, and LFTs (liver function test) require monitoring.

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2	Azoles: Eg,:- 1)Posaconazole, 2)Isavuconazonium sulfate (isavuconazole)	It is non-competitive inhibitors of the fungal enzyme lanosterol 14 alpha demethylase. This action destablilizes the fungalcell membrane, causing cell content leakage, cell-lysis and eventual death.	The available preparation for systemic azoles antifungal includes tablets, capsules, oral Solution and I.V solutions. Azole drugs for local or topical use include use powders, creams, ointments, gels, shampoos and lozenges	I) Posaconazole: - thrombophlebitis, hypertension, hypotension, headache, rash, hypokalemia, and thrombocytopenia. Rarely prolongation of QTc interval was also seen. ii)Isavuconazole:-Severe gastrointestinal side effects than most of other azoles, headache, hypokalemia, dyspnea, cough and peripheral edema	i) Posaconazole:- Contraindicated in pregnancy, caution in patients with electrolyte abnormalities, renal insufficiency, cardiomyopathy, medical history, family history, congenital prolonged QTc interval. ii) Isavuconazole:- Contraindicated in patients with familial short QTc syndrome and should be used with caution in patients with hematological malignancies.	i)Posaconazole:- Crearinine, electrolyte, (magnesium and calcium) and LFTs should be checked at baseline, then frequently during treatment. ii) Isavuconazonium sulfate (Isavuconazole): Require checking LFTs at baseline, then periodically during treatment.
3	Echinocandins Eg:-i) Micafungin ii) Caspofungin	It inhibit the fungal beta-(1,3)-D-Glucan synthase, the enzyme which is responsible for synthesizing beta-(1,3)-D-Glucan, a key component of fungal cell walls. Losing this cell wall	Intravenously administer as a reconstituted solution.	i) Micafungin: -it can cause phlebitis, anemia, transaminitis, hyperbilirubinemia, renal failure and fever. ii) Caspofungin: -it can cause hypotension,	All of the echinocandins are contraindicated in patients with hypersensitivities to any of the echinocandin drugs, or dosage form components, caspofungin	Patient which on echinocandin therapy should be regular monitor for hepatotoxicity via hepatic aminotransferases (AST, ALT), with the additional consideration of alkaline phosphatase..

		component leads to osmotic instability and cell death.		peripheral edema, tachycardia, chills, headache, rash, anemia, localized phlebitis, respiratory failure, and infusion-related reactions.	should be used with caution in hepatic impairment.	