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A REVIEW ON BLACK FUNGI CLINICAL AND PATHOGENIC AND POST COVID COMPLICATIOND

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ABSTRACT

Data are presented on the clinically relevant black yeasts and their relatives, i.e., members of the Ascomycete order Chaetothyriales. In order to understand the pathology of these fungi it is essential to know their natural ecological niche. From a relatively low degree of molecular variability of the black yeast Exophiala dermati- tidis, potential agent of brain infections in patients from East Asia, it is concluded that this species is an emerging pathogen, currently going through a process of active speciation. It is found to be an oligotrophic fungus in hot, moist environments, such as steambaths. Cladophialophora-, Fonsecaea- and Ramichloridium-like strains, known in humans as agents of chromoblastomycosis, are frequently found on rotten plant material, but the fungal molecular diversity in the environment is much higher than that on the human patient, so that it is difficult to trace the etiological agents of the disease with precision. This approach has been successful with Cladophialophora carrionii, of which cells resembling muriform cells, the tissue form of chromoblastomycosis, were found to occur in drying spines of cacti. Phagocytosis assays provide a method to distinguish between pathogens and non-pathogens, as the killing rates of strict saprobes proved to be consistently higher than of those species frequently known as agents of disease. The therapeutic possibilities for patients with chromoblastomycosis are reviewed.

KEYWORDS: Antifungal therapy, Black yeasts, Chromoblastomycosis, Phagocytosis.

INTRODUCTION

Black yeasts have been known since the end of the 19th century, but they still are among the most difficult fungal groups to identify and therefore the knowledge on this group is still only fragmentary. The diagnostic confusion in the past is not surprising, since the taxonomy of black yeasts is now known to be much more complicated than was anticipated. With the application of molecular criteria a great number of undescribed species is encountered. This number is expected to increase even more when detailed studies in biodiversity are performed. Apparently undescribed taxa from the environment and even from human patients are regularly found, and their number is likely to augment exponentially when less commonly explored sources are sampled. It seems probable that within a few years from now the number of taxa known in black yeasts and their relatives will multiply tenfold. Revealing further teleomorph/ anamorph relationships will be key issues in the study of these organisms.

Tracing the source and route of infection of neurotropic black yeasts

The black yeast Exophiala dermatitidis is known from the environment, but also from systemic mycoses in humans. In Southeast Asia fatal cerebral infections are noted in patients which are otherwise in good health. However, the preponderant clinical picture in Europe is subclinical colonization of the lungs of patients with cystic fibrosis (CF); the rare systemic cases in this part of the world are mild and occur in immunocompromised patients only. The two clinical pictures are partly caused by members of a single population, as has been determined by random amplified polymorphic DNA (RAPD). The question is whether E. dermatitidis is a contaminant: opportunistic fungus only, as might be concluded from its European occurrence, or whether it should be regarded as a systemic pathogen, as seems apparent from its behaviour in Southeast Asia.

To address this question, E. dermatitidis was compared with Pseudallescheria boydii, an environmental species showing neurotropism after temporary coma and aspira- tion of contaminated water. The taxon displays a re- markable degree of variability in ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences and polymerase chain reaction (PCR)-fingerprint data.

Within the species, several nuclear DNA homology groups are known, but identical strains (i.e., with 80% homology) vary by 10% in their ITS sequences. P. boydii easily forms a teleomorph in culture and thus it is likely to show abundant meiotic recombination. These

data indicate that the taxon inhabits a permissive ecological niche (namely polluted, nitrogen-rich, water), where many genotypes that emerge in the course of evolution are able to survive and can occur next to each other. Due to its high degree of recombination the tree shows poor resolution.

E. dermatitidis is much less variable; no sexuality is known. This may indicate that the species is in an active process of adaptation to a new niche. The species was proven to be oligotrophic and thermophilic. These conditions are met, for example, in steambaths, which are hot and moist, and have slightly osmotic wall surfaces. This ecology explains the prevalence of the species in the lungs of CF patients. Bathing facilities in Europe were proven to contain several more Exophiala species, each inhabit- ing slightly different microniches determined by tempera- ture relationships. Apparently, oligotrophism is an ecological mainstay. Neurotropism is also a plesiomorph characteristic in relatives of Exophiala, such as Cladophialophora bantiana and Ramichloridium mackenziei. Hence, combining the two ecological tendencies, E. dermatitidis is likely to be predisposed to adapt as a neurotropic pathogen. Its molecular structure seems to indicate that this event has happened only recently.

Comparison of phagocytosis, oxidative burst and killing of black yeasts

Phylogenetic analysis of black yeasts and their relatives revealed that all type strains of the genus Exophiala clustered as a monophyletic group together with members of the Herpotrichiellaceae (order Chaetothyriales), indicating a close relationship.[1] Therefore, it may be expected that they share virulence factors resulting in comparable pathogenicity. The presence of melanin has been considered as an important virulence factor and it was recently shown that this leads to lower killing rates in E. dermatitidis when comparing melanized strains with a respective albino mutant in a bioassay using whole human blood.[2] Surprisingly, melanized species considered virulent were found at a phylogenetically short distance to melanized, but virtually non-virulent species, e.g. E. spinifera and Phaeococcomyces exophialae.[3] Since the most important defense system of the human organism against fungal infections are professional phagocytes (i.e., macrophages and neutrophils releasing reactive oxidative intermediates [ROI] that have been described to be able to kill yeasts and filamentous fungi).

The killing of the non-pigmented yeasts C. albicans and S. cereisiae was comparable in degree to that seen with the non-melanized E. dermatitidis strain. The de- posited melanin in the cell wall of black fungi is known to absorb light and heat energy due to numerous free

carboxyl groups. This accounts for many of the protective, as well as the photosensitizing, properties of melanin.[4] In the case of plant pathogens, it is well known that melanin increases cell wall rigidity and thus it might render killing more difficult.[5] In the case of ascomycetous black yeasts, dihydroxynaphthalene (DHN) melanin is formed by oxidative polymerization of phenolic compounds.[6] It can be speculated that the presence of melanin confers a higher capacity to neutralize oxidants, resulting in survival during the evoked oxidative burst in the phagolysosome of neutrophils. Thus, for all melanized yeasts analyzed in the present study a comparable survival rate would be expected, especially since the degree of phagocytosis and evoked oxidative burst was comparable in all strains studied. Intracellular location of the yeast cells associated with the neutrophils was ensured by microscopic evaluation of the phagocytosis process.[2]

Despite our working hypothesis that due to their close phylogenetic relationship the same type of melanin should be present in all the black yeasts studied, the degree of killing after 5 h differed significantly between the melanized strains studied. The black yeasts that were killed to a degree comparable to that seen in non-melanized strains (i.e., C. albicans, E. dermatitidis mel3¼ , S. cere×isiae) are mainly isolated from mild human infections, whereas strains killed to a lesser extent are well-known for their potential to cause severe infections, with the exception of E. mesophila. In the latter species its reduced growth at 37 °C might prevent inva- sion of the human host.[7]

Invasiveness of fungal pathogens has often been linked to defects in cell-mediated immunity, but the results of the present study clearly show that neutrophils of healthy donors killed pathogenic melanized species to a lesser extent than other species. Since neutrophils are still considered to be the most important effector cells, low killing rates of the respective species most probably reflect their high virulence. Therefore, the striking differences in killing rates of melanized species strongly indicate that melaninization of the cell wall alone is insufficient to confer the killing resistance.

If all black yeasts tested possess the same type and structure of melanin the difference in killing might be attributable to the expression of an additional virulence factor. Due to the close phylogenetic relationship of Exophiala species,[1] acquisition of novel virulence factors is unlikely. Therefore, one can speculate whether expression of such a plesiomorphic virulence factor depends upon ecological stress factors. Another explanation is that due to the complex composition of melanin, i.e., monomers usually complexed with proteins and

carbohydrates,[4] differences in final polymerization could result in different linkage patterns of monomers with different a capacity for scavenging radicals which may contribute to the observed differences. Survival in the phagolysosome might subsequently result in its pen- etration and invasion of the surrounding tissue, since melanized hyphae exert larger turgor-derived forces at their apices than non-melanized cells.[8] Definitive proof of the involvement of melanin in the virulence of black yeasts awaits further experiments by specifically altering DHN-melanin biosynthesis pathway by, for ex- ample, gene disruption. Due to the establishment of genetic transformation, gene disruption protocols and a gene expression system,[9] such experiments could be feasible for E. dermatitidis in the near future.

Molecular identification of dematiaceous environmental versus patient strains

An attempt was made to find agents of human chromoblastomycosis in the environment, on the assumption that the infection is initiated by traumatic inoculation and thus that the aetiological agents are likely to be saprobes. In a phylogenetic tree derived from sequences of ITS1, ITS2 and 5•8S rDNA we included all known agents of chromoblastomycosis, supplemented with morphologically similar environmental strains and other potentially pathogenic members of the Her- potrichiellaceae. Approximately 10 groups can be recog- nized.

Species of Phialophora are in a subgroup exclusively comprising agents of subcutaneous mycoses. The monophyletic character of this group is underlined by the presence of phialides with collarettes in all strains. Strains of Phialophora ×errucosa, one of the classical agents of chromoblastomycosis, formed a distinct clade with representatives able to form muriform cells, as observed in Fonsecaea. This fact supports the suggestion that the muriform cells may indeed be a main virulence factor in the development of the disease, representing an adaptation to the conditions prevailing in host tissue.

A complex containing the type strain of Cladophialophora arxii comprised a number of environmental strains, and C. de×riesii (CBS 834.96). Gerrits van den Ende & De Hoog[10] found a relatively close kinship between C. bantiana and non-neurotropic Clado- phialophora species. In Cladophialophora, the presence of introns in the 18S rDNA subunit may be strictly related to the specialization of the neurotropism of C. bantiana.[10]

Saprobic species with Ramichloridium-like morphology were found all over the tree. Strain F11PLA was found in group III with species of Cladophialophora that are agents of

chromoblastomycosis. The same holds true for Exophialalike anamorphs. Different levels of adaptation seem to occur in these groups. Group IV was the only group with teleomorph relationships, confirming earlier reports.[11,14] The species E. spinifera and E. jeanselmei formed a well-defined monophyletic branch.[15] Based on ITS sequences, the same studies found close affinity between E. spinifera and Capronia munkii. Haase et al.[16] observed Capronia teleomorphs all along an SSU phylogenetic tree of the Herpotrichiellaceae.

Using RAPD, the majority of F. pedrosoi strains were located in group I, with specific subgroups presenting bootstrap values above 85%, which was partly explained by saprobic and pathogenic strains being isolated from the same geographic region. Similar results were obtained with ITS rDNA sequence analysis and with nutritional physiology. Isolate FP8D, which is morphologically Cladosporium-like, was found to cluster with isolates from clinical cases, based on RAPD marker analysis. In the recent phylogeny-based taxonomy, Cladosporium contains only saprobes which are classified in the order Dothideales, family Mycosphaerellaceae, whereas the pathogenic genus Cladophialophora belongs to the Herpotrichiellaceae, order Chaetothyriales. Apparently the two genera with very different clinical significance are sometimes morphologically difficult to distinguish from each other.

C. Carrionii in cacti at the chromomycosis semi-arid endemic zone in Venezuela

Chromomycosis is a chronic subcutaneous granulomatous disease caused by several melanized dimorphic fungi reported predominantly from tropical countries. In Venezuela, the first case was described by O'Daly in 1938; in 1943 he also reported for the first time an aetiological agent now known as C. carrionii. The endemic area is in the Northwest of Venezuela. Since 1959, Borelli noted that patients infected by Fonsecaea pedrosoi came mainly from humid climates, whereas C. carrionii seemed to occur in semi-arid zones. Keeping goats is one of the main agricultural activities in the latter area and over the years labourers repeatedly traumatize themselves with cacti thorns. An increase in persons susceptible to the development of chromoblastomycosis is thus observed. Chromoblastomycosis is considered to be a multi- factorial disease, involving genetic as well as environmental factors. A prevalence of 16:1000 cases of chromoblastomycosis should be explained by the coincidence in the same geographical area of a homogeneous genetically susceptible population and facility of exposure to the natural source of infection. Since 1983, several studies were carried out at Francisco de Miranda University in order to confirm the presence of C. carrionii in the endemic zone.[17,20]

Samples were collected in the vicinity close to the patients' houses: fragments of cacti, spines, decaying wood and fence bark fragments. Brown erosive lesions in cactus stems were studied. Thin sections of vegetative tissue, spines and wood were carefully examined to search for brown muriform cells. Positive samples were covered with a thin layer of glycerin:yeast, peptone and glucose liquid medium (1 volume), placed on a slide upon a bent glass rod into a Petri dish with 5 ml of sterile water to maintain humidity and incubated at room temperature with daily examination. Proteolytic activity and thermo- tolerance tests were carried out to confirm strain identification.

Several isolates of C. carrionii, one of Sporothrix schenckii, and a number of unidentified fungal species, were repeatedly observed to produce similar spherical, thick-walled cells growing by isotropic enlargement. C. carrionii was detected in 11 localities in association with common xerophytes: Prosopis juliflora, Aloe ×era, and the Cactaceae Opuntia caribaea, O. caracasana, Stenocereus griseus and Cereus lanuginosus. Brown muriform cells were observed in the stems of living Cactaceae, in the medullar tissue, and in the spines.

It is postulated that the fungi survive in a very dry, hostile tropical environment inside living cactus tissues. The saprobic filamentous form rapidly expands under favorable conditions on the decaying wood surface, or intro. After accidental implantation of the pathogen into the human skin, a localized subcutaneous chronic granulomatous lesion may then occur.

C. carrionii is ubiquitous in the semi-arid part of the endemic area, where decaying wood and spines of xero- phytes, predominantly Cactaceae, have been implicated as a source of infection by the rural population and could be considered as the natural reservoir of the fungus. It is important to clarify the mechanisms of infection and pathogenicity of this fungus in humans and in Cactaceae.

Chromoblastomycosis: A therapeutic challenge

Chromoblastomycosis (CBM) is a chronic, subcutaneous fungal infection, caused by the transcutaneous implanta- tion of several species of dematiaceous fungi. The disease is more frequent in tropical and subtropical regions among rural workers. After traumatic implantation, the initial lesion can evolve into pleomorphic lesions, leading to dense dermal fibrosis and oedema.[21,22] CBM le- sions are recalcitrant and extremely difficult to eradicate. In this manner, patients with CBM are a true therapeutic challenge for clinicians. During the last few decades, several treatment regimens have been employed.[23,28] In the early stages,

the lesions respond to surgical resec- tion but later, as the severity increases, better results are achieved with chemotherapy. Therapeutic success can be related to the aetiological agent (C. carrionii is more sensitive than F. pedrosoi,[29]) to the severity of the disease (oedema and dermal fibrosis can reduce antifun- gal tissue levels) and obviously, to the choice of the antifungal drug.[30] There are no comparative trials in CBM. In most of the clinical trials, the lesions are not graded according to severity and standardized criteria of cure are not used by the different authors dealing with this mycosis. Currently, itraconazole (ITZ) alone or com- bined with flucytosine or topical liquid nitrogen (cryotherapy) appears to be the best treatment for CBM.[28,32]

The study of 71 patients with chromoblastomycosis in the State of Parana´, South Region of Brazil, between 1985 and 1996, accumulated information relating to the aetiology, epidemiology, clinical course and treatment of the disease. F. pedrosoi was the primary aetiological agent, and was isolated in 94•3% of the cases. However, unusual agents such as F. compacta, E. jeanselmei and E. castellanii were detected in the lesions of three patients that presented with typically muriform cells upon histo- pathological examination. The research of the epidemio- logical pathways of autochthonous cases revealed that in the State of Parana´, transmission of the disease is mainly occupational, affecting the inhabitants of the State's up- lands. In 48 patients, a non-comparative clinical trial with itraconazole was carried out to evaluate its efficacy and toxicity. Eighteen patients were considered unevalu- able because they failed to return for their control visits or because of non-continuous therapy. The CBM lesions were classified according to morphology and severity. A mild form was defined as a solitary plaque or nodule measuring less than 5 cm in diameter. A moderate form was taken to be solitary or multiple lesions (nodular, verruciform or plaque types), existing alone or in combi- nation, covering one or two adjacent cutaneous regions, and measuring less than 15 cm in diameter. The severe form consisted of any type of lesion, alone or in combi- nation, covering extensive cutaneous regions, whether adjacent or non- adjacent.[30] All subjects received itra- conazole at $200-400$ mg day^{¼1} until the established crite ria of cure were achieved. Clinical criteria included: disappearance of pain and itching, and complete healing of all lesions with scarring. Mycological criteria were the absence of pathogens on direct microscopic examination and no fungal isolation on culture. Histological criteria included absence of pathogens, atrophy of the epidermis, disappearance of microabscesses and granulomas, re- placement of granulomatous infiltrate by chronic inflam- mation and fibrosis. The persistence of all these findings had to continue for three

consecutive monthly biopsies.[33] Clinical, mycological, histopathological and labora- tory evaluations were performed before, during and after therapy. In order to establish whether the chronic itra- conazole therapy could interfere in human steroidogene- sis and androgenesis, the adrenal response to cortico- tropin and testosterone was evaluated in 15 patients by radioimmunoassay.

This report presents the results obtained with 30 CBM patients treated with itraconazole (Table 1). Nine patients (30%) presented mild CBM lesions with a median of 7•5 (range 1 – 19) years of duration. Four patients (44%) in this group had been treated previously. In 12 patients (40%), the lesions were moderate and had been present for a median time of 20 (range 6 – 50) years. In this group, five patients (42%) referred earlier treatments with anti-fungal drugs. Finally, lesions were typed as severe in nine patients (30%) and were of long duration, median 24 (range 18 – 40) years. Sixteen patients (53%) had been treated previously. Final assessment showed that eight patients (89%) with mild forms achieved clinical and mycological cure after 10•9 (range 7 – 17•6) months of therapy. No relapses were observed in this group after the mean time of 31•2 (range 12 – 72) months. Similar responses were observed in 11 of the 12 patients (91%).

Table 1: Clinical and Demographic characteristics of 30 patients wih chromoblastomycosis treated with itraconazole.

Clinical form	Clinical and mycological cure *n* (%)	Duration of treatment (months (median))	Improvement *n* (%)
Mild	8 (89%)	10·9 (7–17·6)	1 (11%)
Moderate	11 (91%)	12·9 (5–31)	1 (9%)
Severe	4 (44%)	30 (10–51)	5 (56%)
Total	23 (76%)	18	7 (24%)

With moderate forms, after an average of 12•9 (range 5 – 31) months of continuous treatment. In this group, one patient relapsed after 6•3 months of follow-up while the remaining patients did not relapse (12 – 60 months follow-up). Among the nine patients with severe CBM lesions, four (44%) had clinical and mycological response after a mean of 30 (range 10 – 51) months of treatment, and the remaining patients had improved significantly. One relapse was observed during the follow-up (after 35 months), but the patient improved again after a new course of therapy. No significant changes in the values of hematological and biochemical tests were observed.

Mean cortisol and testosterone concentrations at base- line were 12•4 mg dl¼ 1 and 454 ng dl¼ 1, respectively, and after 12•495•2 months of treatment with itraconazole were 15•4 mg dl¼ 1 and 480 ng dl¼ 1, respectively. There was no clinical or laboratory evidence of steroidogenic or androgenic impairment.[34]

These results show that the therapy with itraconazole can achieve long lasting clinical and mycological cures in most of the patients having mild to moderate forms of CBM, after prolonged periods of treatment. On the other hand, only 44% of the severe cases were cured clinically and mycologically. The clinical outcome observed in those patients presenting severe lesions of CBM, could be related to decreased itraconazole tissue concentrations. Local fibrosis, oedema and bacterial co-infection are common associated factors that can decrease local itra- conazole concentration, especially in the subcutaneous tissues, which in severe lesions are replaced by dense fibrosis.

Other therapeutic strategies available include the com- bination of itraconazole with flucytosine and:or the asso- ciation of local liquid nitrogen.[31,32] Both methods may reduce the duration of itraconazole treatment. Ac- cording to preliminary data, terbinafine at a daily dose of 500 mg for 6 – 12 months also seems to be effective in CBM (efficacy 85%). However, the results presented by Esterre et al.[35] cannot be compared with our results because different assessment criteria were employed in both trials.[30,35]

In the future, the new antifungal drugs under develop- ment may play an important role in the treatment of CBM. In ×itro dematiaceous fungi are very sensitive to the new triazoles voriconazole and posaconazole and also to MK-0991, an echinocandin.[36,37] The results pub- lished to date suggest that these new agents have broad- spectrum activities in ×itro; however, their effectiveness in the treatment of human mycoses remains to be determined.

Fungal infection after Covid-19

To put into fact, for fungal infection, Chen et al. performed this infection test in China on 99 patients out of which 5/99 were found having Aspergillusflavus and one case of Candida glabrata, and three cases of C. Albicans. After extensive search and study on mucormycosis and with little or no result of journals on this deadly fungus, one search result lead to another researcher, Yang et.al found a little higher percentage of people affected by this disease.[38] Many patients were treated with anti-fungal medicine but were in vain. Another German study associated with COVID-19 found 6 out of 19 patients infected with black fungal. In the

Netherlands, there were fresh new cases of black fungus, infecting 7 with A.fumigatus. In France, there were 5 patients infected with A. flavus by tracheal aspirates culture.[39]

Many incidences in that period have found covid with fungal infection increase from 16-27%, with severely ill patients dying. Most percentage of them with mucormycosis has died since the beginning of this fungal incidence began. The below figure shows penicillin in mucormycosis.[40]

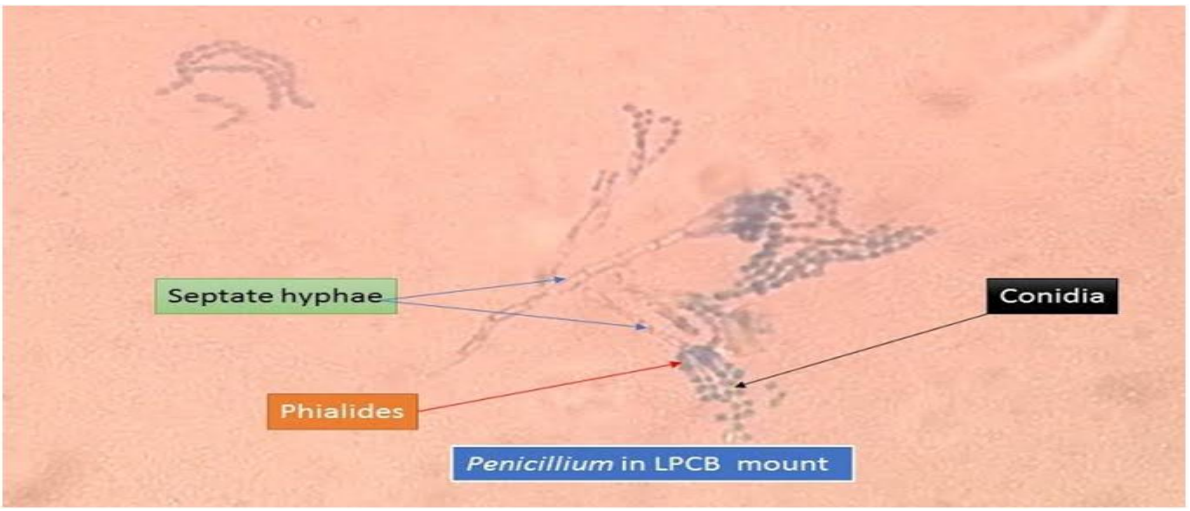

Figure 1: Figure shows penicillin in mucormycosis.

One report also suggested that despite IPA on fungal infection the mortality rate has increased from 23% to 51%. Clinically many patients are subjected to fungal testing attributing to severe respiratory symptoms. Some diagnosis like antibiotics has delayed this fungus. It is critical to pay attention to this mucormycosis in current COVID-19 patients. Below is the figure of Aspergillusflavus.[41]

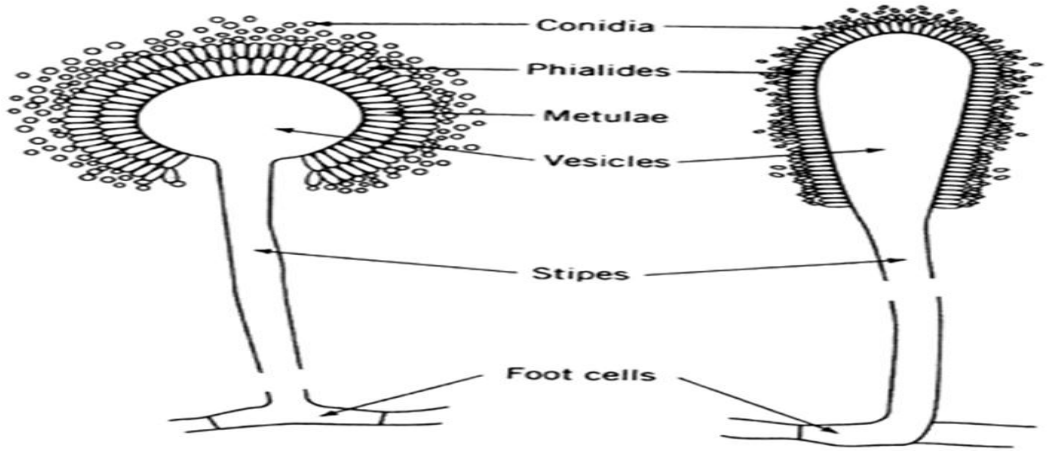

Figure 2: Figure of aspergillusflavus.

Discussion for diagnosis

It has been understood that a black fungus called mucormycosis is complicating the treatment and recovery of COVID-19 patients. There were several reports of patients with invasive aspergillosis and positive growth of Aspergillusfumigatus and Aspergillus-ag in endotracheal aspirate.[42] Many patients tested has a two-week history of COVID-19 before and after admission in ICU, a CT scan a valuable tool for corona patients revealed, slight mold infections in the chest region, but slight reversible halo, ground-glass opacities also observed in some. Patients with severe influenza or halo or reversible halo show pulmonary mold leading to mucormycosis.[43] To take a step further on discussion, a diagnostic setup is suggested. The discrimination between colonization and infection was put to test. With Aspergillus-specific LFD and certified point assay. Test showed negative to colonization but positive to infection. The result also shows growing hyphae during the invasion of fungus.[44] Serum Galactomannan shows no sensitivity in non-neutrophenic patients. The LFD is the recommended method for the diagnosis of invasive aspergillosis. The benefit of GM screening in COVID-19 patients shows data that attracts attention on recently published trial. The data shows GM in serum is on the rise.[45] The new norms of the European Organisation for Research and Treatment of Cancer/Mycoses Study Group showed diagnostic criteria for ICU patients due to missing hosts. The criteria broadly include chest imaging of lungs and microbiological evidence of Aspergillus presence. Presently influenza and aspergillosis trial shows GM in serum and bronchoalveolar lavage fluid in mycological criterium to overcome imperfect culture limitation and sensitivity of Aspergillus. Below is the chart for colonization.[46]

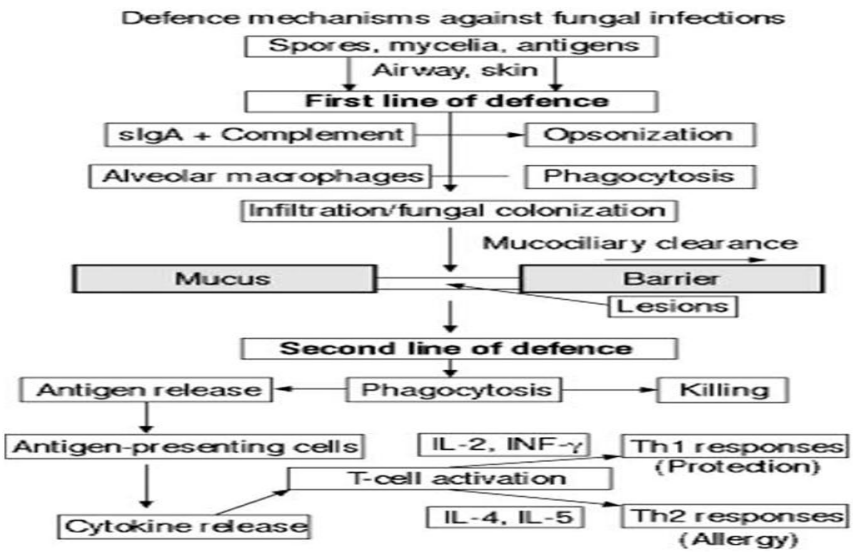

Figure 3: The chart for colonization.

Etymiology and Diagnosis

It has been discovered that mucormycosis has common agents such as Rhizpisspp, Mucorspp. Genera of Mucorales, varies from country to country. Mucorspp, Lichthemiaspp and Rhizopussppat 34%, 19% and 19% are common in Europe. In India Rhizopusspp is the most common causing disease Apophysomyceselegans, A. variabilis and Rhizopushomothalliusare emerging. Another species namely Apophysomycesis reported in Mexico. By inhalation of sporangiospores, mucormycosis is caused.[47] These are air borne or direct inoculation of organisms into skin or gastrointestinal tract mucosa. Recent studies suggest they are seasonal infections occurring from August to November, but patients in Australia showed mucormycosis occurring anytime with uncommon species such as non Rhizopus and some infected with Apophysomyces or Sakenaspp localised in skin and tissues.[48]

Mucormycosis has been a centre of attention all around the globe. But there seem difference in species and effect on human body differing from developed country and developing nations. In developed nation this disease in less common and seen only in patients with haematological malignancies(HM). The developing countries paint a different picture, it is common is patients with uncontrolled diabetes mellitus or trauma. In India, mucormycosis is seen in 14 out of 100000 patients. In Europe and US it is seen in 0.01 per 100000 population . Below is the image of formation of mucormycosis inside lungs.[49]

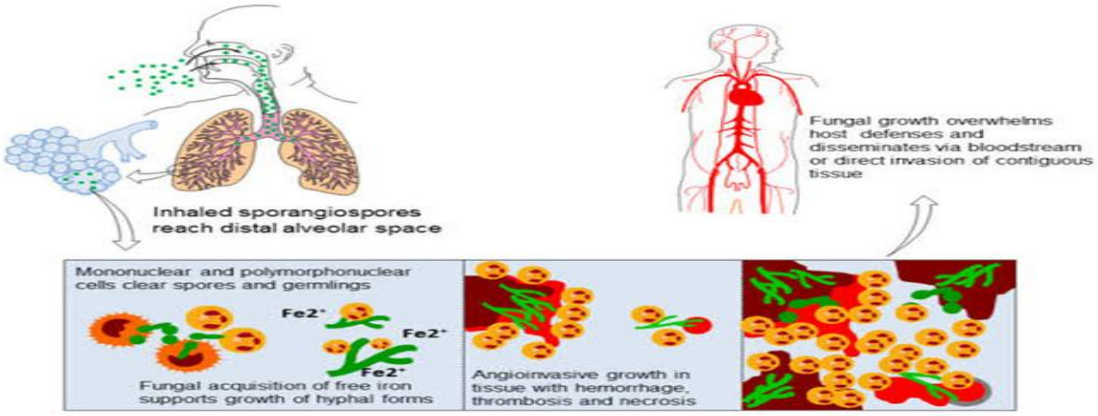

Figure 4: The image of formation of mucormycosis inside.

The percentage associated with mucormycosis in rhino-orbito- cerebral pulmonary are 27%, 20% and 18%. In Europe it is 27%, 32%, 26%. Considering patients with HM is less compared to patients in India with DM. In Mexico, 72% of people were associated with diabetes underlying malignancies, sinus and pulmonary. Below is the figure for diagnosis of

mucormycosis.[50] Infections from Mucorales are usually rapid, they were initially reported in farmers from China. Some reports show they are opportunistic fungus like Mucorirregularis, that has completely different epidemiology. Their infections are highly chronic but without any risk factors effecting only skin and tissue cells.[51]

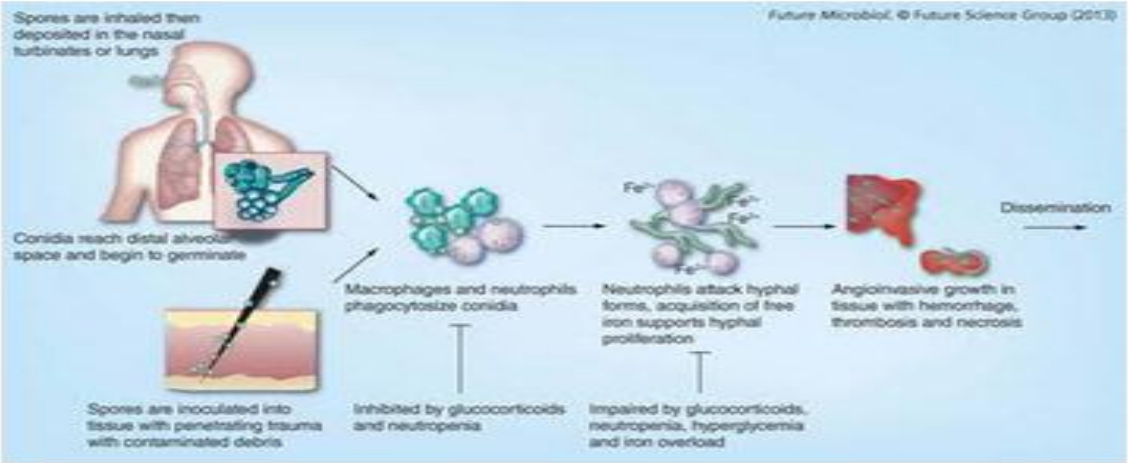

Figure 5: The figure for diagnosis of mucormycosis.

Fungal isolates such as Rhizopusspo and Lichtheimiasppalong with Mucorsppwere found in children in some cases. Keeping the factors of HM and malignancies such as solid organ transplant, surgery, DM and underlying many medical conditions the fungus targets the above and attacks lungs, skin, soft tissues, sino orbital and rhino cerebral region. Mortality rate for such studies were more than 60%. In Children it was 15% with certain infection.[51]

Clinical Diagnosis and Possibilities

The diagnosis has prerequisite and with great deal of suspicion and recognition host, we can incur to conclusion through testing methodologies such as histology, imaging modalities, advanced molecular methods and microbiology. As a revision, rhinocerbral pulmonary is the most common clinical presentation for Mucorales with representation in soft tissues, pulmonary vessels, disseminated diseases and any other organ that shows infection. Tissue necrosis is the benchmark for mucormycosis. Fungi such as Aspergillus or Fusarium show some sign. Below chart shows pathophysiology of mucormycosis.[52]

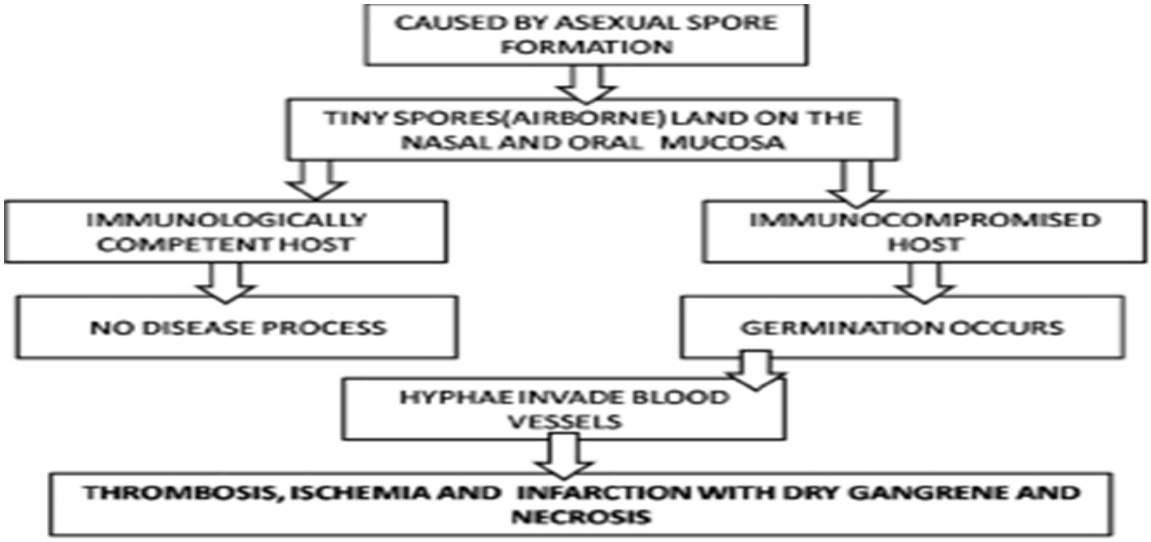

Figure 6: Chart shows pathophysiology of mucormycosis.

In many countries where tuberclosis is relevant, two infections coexist. However with diabetic patients the index is higher for invasive pulmonary mucormycosis. The list that should be considered as symptoms are cranial nerve palsy, diplopia, sinus pain, orbital apex syndrome, ulcer, preorbital swelling. In radiology, most common symptom is pleural effusion. Computerised topography(CT)scan that indicates mucormycosis is the reverse halo sign (RHS), when sequential thoracic CT scans are performed in more than 100 patients RHS were observed in 92 patients during initial stage of disease. Hence we conclude RHS on CT scan is a strong indicator of presence of pulmonary mucormycosis. In another study of patients with lung mucormycosis, presence of RHS is common on all of them. Symptoms were centrilobular nodules, bronchial wall thickening, petribronchial consolidation with Aspergillus in it. Another method for aggressive lab technique to identify mucormycosis is positron emission tomography-computed tomography (PET/CT) with fluorodeoxyglucose(FDG). Endobrochial ultrasound guided injection is the best and useful diagnostic tool for mucormycosis. Below graph shows presence on mucormycosis and growth.[53]

Laboratory Work and Culture technique

Laboratory work and culture on petridishis the effective clinical tool for mucormycosis. Below is the microscopic view of mucormycosis.[52]

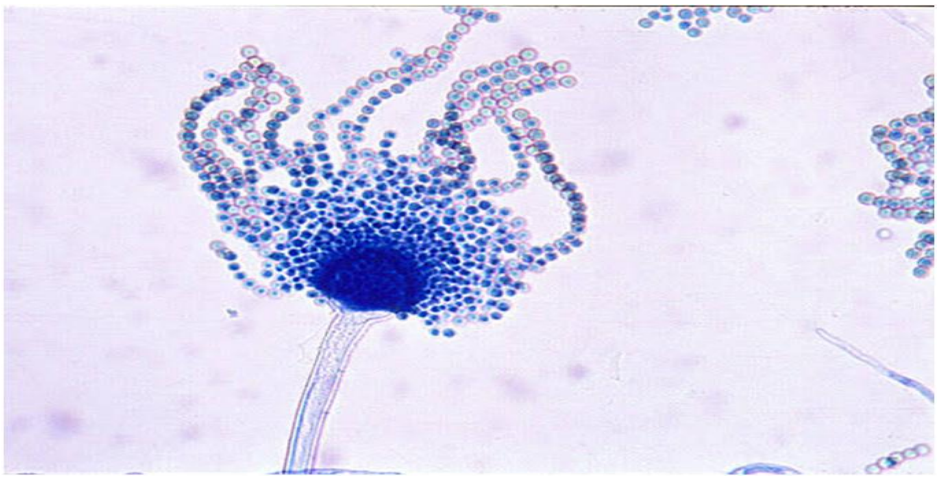

Figure 8: Microscopic view of mucormycosis.

Hypea and Mucorales have a width of 6-25 nano meters and are nonseptate and have a ribbon shaped appearance. The angle and branching are at 90 degrees and fungal element is seen at hematoxylin and eosin regions. To elevate morphology more, periodic acid Schiff or Grocott were used for silver staining to highlight hypea. Tissue hispathology shows inflammation regions, in some cases these are absent in immune suppressed patients. In cases of nerve cells, perineural invasion is present when done by tissue histopathology, but that is not the best method always, tissue differentiation is most effective to differentiate between hypea of Aspergillus and hypea of Mucorales. It distinguishes all fungi and helps in pathogen of specimen in laboratory culture containment.[53]

It is observed that Mucorales grow upto 3-7 days on fungal media, namely potato dextrose agar and Saboraud agar incubated at 25 degrees. In some cases, it aids in yield of culture, because hypeas are friable in nature and gets damaged easily. The main target of this culture in situ hybridization is 5s and 18s ribosomal RNA sequence, hence a specific mouse monoclonal anti- Rhizomucorantibidy is employed to target analysis and to react strongly on murcorales and Entomophthorales.[54]

Identification of species

Identifying species is more important for better understanding of epidemiology of mucormycosis. Mucorales fungi differentiates from Aspergillus fungi on culture and provides high level of accuracy in fungal identification. Test kits used are ID32C combined with positive melezitose assimilation detects L.remosa. Another one is thematrix assisted laser desorption/isolation time of flight (MALDI-TOF) along with mass spectrometry.[55] Serology used are ELIZA assays immunoblots and immunodiffusion tests that are invasive towards

Mucorales and mucormycosis. Specific T cells were detected from above, they are used as surrogate diagnostic markers for further research. Molecular assays such as PCR restriction fragment length polymorphism analysis (RFLP), DNA sequencing of defined genes and melt curve analysis were part of assays that help in analysis of Mucorales. They targeted internal transcribes 18s rRNA genes.[56]

Specificity in cure

Multimodel approach is necessary to cure mucormycosis. Early dosage of anti-fungal agents, rapid correction of metabolic abnormalities are mandatory features. For diabetic patients, sodium bicarbonate(with insulin) to reverse ketoacidosis regardless of whether acidosis is mild or severe, has the ability to reverse Mucorales to invade hosts.

Drugs such as corticosteroids should help in early diagnosis such as to stop tissue invasion. Mucormycosis has characteristics to invase angio vessel that leads to thrombosis and tissue necrosis. The suggestion by European conference for such harsh invasion is lipid formulation of amphotericin B. The suggested dose is 5mg/kg/day upto 10mg/kg/day for injection in central nervous system. The results conducted on patients with mucormycosis showed a response rate of 78% in week 1 to 87% on week 12.[57]

Posaconazole and isavuconazole are used as maintenance therapy dosages recommended by ECMM at dose of 200 mg q6h of oral. This has some effect on fungus and recovery rate is high. Another option is salvage treatment, combining effects of liquid amphotercin B and caspofungin or posaconazole, impact showed survival rates much higher on patients with rhino orbital cerebral mucormycosis. The use of hyperbaric oxygen to enrich cytokine environment to lower fungal cell area works at high percentage. This oxygen helps in simulating granulocyte- macrophage colony giving way for interferongamma response to fight the Mucorales. Final treatment can be done with the usage of drug VT-1161, an inhibitor with selective activity against fungus. They are ergosterol synthesis inhibitor and prove an additional asset to fight mucormycosis.[58,59]

CONCLUSION

Mucormycosis is a disease that is rare but poses an important burden on immune compromised patients. Newly developed medications have several pathogenesis but cure to mucormycosis is still a challenge. Several methods have delayed the mortality but still posses a challenge in curing mucorales. The clinical presentation is non specific, and early diagnosis

target the hisptopathology efficiency and it is time consuming. Direct examination of culture, molecular diagnostic techniques, PCR and situ hybridisation offer an alternate to initiation the treatment. The management of mucormycosis depends on underlying factors such as injection of antifungal agents, surgical intervention and timely dosage of antifungal therapy. Immunologic and metabolic profiling is the way to approach this black fungus i.e mucormycosis.

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