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Molecular Characteristics at Species Level and Diseases Associated with Fungus Aspergillus Fumigatus; A Review

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Abstract

Background: Aspergillus fumigatus is type of fungus which is harmful and going to be harmful for human in day to day life. As we are moving to a great scientific era we are not able to find the cure of many of the diseases and aspergillosis is one of them. Aspergillosis is disease which is caused by fungus Aspergillus fumigatus which causes inflammation in lungs and respiratory tract causing respiratory disorder and sometimes it can be so harmful to that extent that it can cause fatality in humans. This fungus glows very well in wide temperature ranges which have humid and warm conditions. A. fumigatus is a thermophilic species, with growth occurring at temperatures as high as 55°C and survival maintained at temperatures up to 70°C.[1] In the (Figure 1) below we can see that in which of the country Aspergillus grows the less to the most:

Keywords: Aspergillus fumigatus; Aspergillosis; Cystic fibrosis.

Introduction

Since the past decades scientist are able to find different types of fungus which are present in the environment which is danger for the human life as well as for the animals also in which one of is Aspergillus fumigatus. As the name suggests it come from the family of Aspergillus which is a type of fungus. It is basically a human pathogen which is responsible for systemic mycoses which attract the lungs or the respiratory tract. And it is the fungus which is responsible for causing immudificiancy in human. Aspergillus fumigatus is type of fungus which grows fast and its colony size may reach up to 4cm within a week when grown on Czapek-Dox agar at 25°C.[2] Basically it is a monomorphous filamentous fungi which comes from Division-Mycota (which means its has no chlorophyll and does not synthesize their own food), Sub Division-Eumycotina, Class-Ascomycetes (which means spore made is asco spore), Order-Aspergillaes, Family-Apergillaceae and Genus-Aspergillus. Aspergillus fumigatus are saprophytic which is found in the decaying matter or the soil such as compost etc. Morphological features of Aspergillus cultures were studied, the major and remarkable microscopic features in species identification were the colony diameter, color (conidia and reverse), exudates and colony texture. Isolation for Aspergillus fumigates is quick and easy but culture is actually slow which creates misconception about is value for its detection. Nowadays with the improved procedural changes and improvised training in the laboratory the diagnostic value of this method is improved. Aspergillus does not have specific mechanism for releasing conidia

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into air only few strong current or wind can make release conidia onto air. But when conidia are in the air their small size makes it buoyant thus keeping them airborne both indoor and outdoor. Many of the survey were done and it was found that at least several hundred aspergillus conidia are inhaled by humans per day.[3] Now if inhalation of conidia is done by immunocompetent humans then it rarely affect and result in release of conidia. A clinical condition observed in individuals who were exposed repeatedly to conidia, or aspergilloma, and an overgrowth of the fungus on the surface of cavities which was existed before in the lungs of patients treated successfully for tuberculosis.[4,5] Over last few decades aspergillus fumigatus has become most prevalent airborne fungal pathogen. Which usually cause fatal invasive infections in immunocompromised hosts in developed countries.[6,7] A drastic increase in invasive aspergillosis (IA) is been observed in the last 12 years. In 1992, aspergillosis was responsible for approximately 30% of fungal infections in patients dying of cancer, and it is estimated that invasive aspergillosis occurs in 10 to 25% of all leukemia patients, in whom the mortality rate is 80 to 90%, even when treated[8, 9, 10]. Nowadays invasive aspergillosis is major cause of death in bone marrow transplant and basic organ transplantation units [11]. Aspergillus fumigatus is the most pathogenic agent among all types of aspergillus fungi which is responsible for 90% of human infection [12]. Different pathogen which causes human infection is Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Aspergillus nidulans basically from same genus. So Aspergillus fumigatus is most common pathogens so this review is exclusively onto it. The topic here will basically cover (i)Macroscopic characteristics (ii)Microscopic characteristics (iii) Clinical and laboratory method for the diagnosis of this disease (iv) Immune response to this fungus (v) Drugs used for the treatment mostly antifungal.

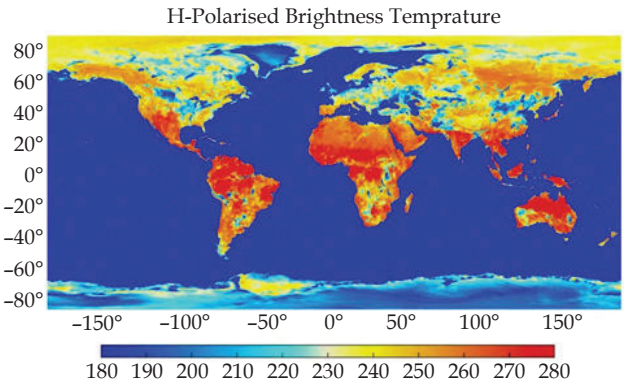

Fig. 1: Variation of temperature in different continent

Microscopic characteristics

As we know Aspergillus fumigatus is types of fungus so it will have macroscopic as well as microscopic characteristics. Now the identification is done basically on morphology of conidia and conidiospore present in Aspergillus. A few variety of Aspergillus fumigatus are pigment less and produce white conidia.[13] The chains of conidia come directly on broadly clavate vesicles in the absence of metulae. As the sample were observed after the culture preparation the following feature was observed which has size of 200-400 micrometer. Aspergillus hyphae are septate with diconomous branching. And the side strips was visible as grayish with same colour at the apex. The surface was smooth walled and the surface also looks like furry or fleshy. Its phialides is in uniseriate pyriform which covers 2/3 in upper hand of vesicles with no metula present. And also phialides are parallel to conidiospore. Its shape is globuse with small column present in it as well as conidia surface is smooth and spinose in which conidio spores are present which short and smooth. Aspergillus normally expands vertically or terminally to form flask shaped vesicles and the size of vesicles varies from 20-30 diameter. So after studying the paper microscopic characteristics are it basically plays important role in recycling of carbon and nitrogen.[14] A. fumigatus is morphologically more variable[15,16] which was originally described by Raper and Fennell.[17] As A .fumigatus is more variable these variation has led to description of several varieties of A. fumigatus, which include acolumnaris, phialiseptus, ellipticus, and sclerotiorum, was based on only slight morphological differences. A. fumigatus, A. brevipes, A. duricaulis, A. unilateralis,A. viridinutans, together makes of species within the perfect genus Neosartorya, it is a genus in which morphologically related same species have been grouped and are classified as Aspergillus sect. fumigate. As A. Fumigatus does not reproduce sexually so the search for a sexual stage of A. fumigatus has been attempted among Neosartorya species, which allow genetics to be pursued and is not relevant for this review and to date so such stage has been discovered.[3]

Biochemical and molecular characterizations

The species was further studied for the better taxonomic definition. Species of Neosartorya from Aspergillus fumigatus was reviewed from the paper.[3] For additional chemical and molecular characterization. As the morphological

characterization was studied for the species detection as compare to that biochemical characterization was also identification of secondary metabolites[18], the identification the ubiquinone system[19], and the examination of isoenzyme patterns.[18,19] Molecular data has been obtained on isolation of total DNA[20], mitochondrial DNA (mDNA)[21], ribosomal DNA (rDNA)[20,21] by using method of RFLP (restriction fragments length polymorphism) visualized with or without hybridization to specific probes and sequencing of characteristic DNA regions.[3] Few of the criteria that was useful in the identification of A. fumigatus a isoemzyme pattern[22,23], ethidium bromide visualized RFLPs[24, 25] and IGS probes[26,27] can be used for index of stain characterization. This criterion was used for comparative analysis of data which was obtained with nuclear or ITS1 and ITS2 ribosomal genes.[28,29] Some of the secondary metabolites which were produced by A. Fumigatus include fumagillin, fumitoxin, fumigaclavines, fumigatins, fumitremorgins, gliotoxin, monotrypacidin, tryptoquivaline, helvolic acid. The secondary metabolites most commonly found in A. fumigatus are families from uncharacterized chemically (FUA and FUB).[30] Some of the species of Neosartorya and A. fumigatus such as A. brevipes, A. duricaulis, A. unilateralis and A. viridinutans produces secondary metabolites. Isoenzymic pattern of some of the Neosartorya species were not sufficient which cannot be used for the identification at species level. So several researcher have attempted to use the analysis of isoenzyme pattern as a taxonomic tool.[31,32,33] The enzymic pattern which was common to all strain of A. fumigates is glutamate dehydrogenase. Some of the enzyme that have been reported were monomorphic such as (lactate dehydrogenase, superoxide dismutase, isocitrate dehydrogenase, aspartate aminotransferase, glucose-6-phosphate dehydrogenase, and phosphogluconate dehydrogenase and some were polymorphic such as (malate dehydrogenase, glucose phosphate isomerase, phosphoglucomutase, hexokinase, esterase, malate dehydrogenase, peptidases, fructose kinase, purine nucleoside phosphorylase, and phosphatases) but still data has not been clear and some of the enzyme electrophoresis pattern is still not investigated and general taxonomy is still unknown.[4] Involvement in the isolation of DNA has shown more concise in the characterization of A. fumigatus. Apart from this the other approach for study of dna was analysis of intron and sequencing the entire portion of genes such as beta-tubulin and hydrophobin genes.[34,35] A method which is successful is hybridization of endonuclease-digested DNA with unique and repeated sequence of different A. fumigatus species. And by amplification by pcr technique we can get the result satisfying. There comes several or many difference to distinguish Neosartorya species and A. fumigatus during sequencing of internally transcribed spacers ITS1 and ITS2 of rDNA.[36, 37]

Some of these method were useful for identification as some of DNA identification method were not useful such as gel electrophoresis which has only presence of five chromosomal band and these ranged from 1.7 to 4.8 Mb and the total size of genome was of at least 15.8Mb which does not showed clearly indication of A. fumigatus species.[38] And if in gel electrophoresis bands correspond to one or more of the chromosomes then it can be aspergilllus spp.which is different from A. fumigatus.[39] Till now chromosomal banding pattern of related species of A. fumigatus has not been done on taxonomic level. And the analysis of mtDNA and rDNA has given limited result according to which the species can't be identified at taxonomic level. Unlike other fungi, researches of the sequence 18S and 28S subunits of rDNA have proved that there is insufficient variability to identify the species taxonomically. An isolated of A. fumigatus with a variable number of 200-Bp repeating units had major fragments which suggested that it was too heterogeneous to use at species level[40]. Therefore, secondary metabolites and sequencing data and DNA isolation with a use of PCR amplicons have been useful in verifying the species taxonomically. And these method showed that A. fumigatus and Neosartorya species are closely related but are genetically and biochemically different. Therefore, research or work must continue on genetic level of A. fumigatus.

Molecular analysis of stain typing

Apart from taxonomic character characterization of these species has important epidemiological and clinical action which was not yet involved. Since for the determination of stain some of the methods are highly contagious, reproducible and seprate from growth condition and phenotypes are based on protein pattern which are detected by antibodies or enzymatic substrate that should be removed from the futher use. For the best result, protein pattern can be used to rank stain at species or sub-species level. some of the molecular test was helpful for the analysis at subspecies level RFLP method for the total digestion of genomic DNA by Xbal, Sal or Xhol shows few level of stain. The banding pattern was complex as large number of faint band was

seen in ETBr-gel. Only few major band was visible detected or was visualized as subspecific cluster. Same as this the IGS region was used to group stain using IGS probes of A. fumigatus at subspecific level and determine the heterogeneity of the species.[41,42] Some of the method were used and out of which only three method were used to describe A. fumigatus stains genotypically, And from these three method two these were with the use of PCR with each of them using different primers and amplification using RAPD method whereas third method was visualized using DNA isolation with RFLP method with repeated DNA sequence. Use of PCR technique using RAPD method is the most commonly method use to determine types of strain of A. fumigatus . The decamer primer R108 with DNA sequence (GTATTGCCCT) is the best strain differentiation till now generated.[43,44,45] However, this method (RAPD) is difficult to execute due to low annealing temperature but can be executed using RT-PCR technique.[46] The RT-PCR method includes microsatellites and this method is used to construct characterization structure of human genome, As described previously thus method is successfully applied recently to A. fumigatus.[47] In contrast to RAPD, this method is highly reproducible with the use of unique and specific primers. And four of the CA repeats have been identified till date which are (CA)9 (GA)25, (CA2)C(CA)23,(CA)8 and (CA)21.[4] Use of restriction enzyme fragments with repeated DNA sequence by hybridization process is used to determine fungal pathogens and also the strain of A. fumigatus. Now for the use of specific probes provides unique and highly blot hybridization pattern for each of the strain of A. fumigatus.[48,49] As it is described above A. fumigatus does not reproduce sexually and there is no methylation of cytosine was detected and was typically associated with mutation of the sequences which is affected by repeated-induced point mutation (RIP). Although, these copies of the sequence does not rely upon the fix time where the A. fumigatus lost its sexual stage.

Regarding the typing of stain most of the researcher has used PCR and RFLP method separately. So, now study is going on to compare their potential to lead better strain discrimination. So, as per now stain typing is most successful method by using micro-satellite chain reaction or analysis of hybridization process which is obtained with repeated DNA sequences.

Clinical symptom and disease associated with Aspergillus Fumigatus

The main site for infection of A. fumigatus in many of the patient is through respiratory tract. But some of the researchers have described the other sites of infection in normal and immunocompromised host such as skin, peritoneum, kidneys, bones, eyes and gastrointestinal tract but these of the disease are uncommon so it is not described. So the major disease caused by A. fumigatus is differentiated according to site of disease within the respiratory tract and second which are influenced by the immunological status of the host. Some of the diseases like allergic bronchopulmonary aspergillosis (ABPA), Aspergilloma and IA syndrome show the mycelium growth of A. fumigatus inside the body which require proper attention. Some of the symptoms and types of aspergillosis are described below in the subsection:

Allergic bronchopulmonary aspergillosis (ABPA)

ABPA is the most severe disease caused by Aspergillus species. It basically attacks the person who is going through atopic asthma or cystic fibrosis. ABPA normally occurs 1%to 2% in asthmatic patients and 7% to 35% in cystic fibrosis patients.[50,51,52] It shows same symptom of asthma with unique cellular response which is caused by response of T-cell products.[53,54] A. fumigates can effect a person by causing asthma to damage of the lungs that can be defined from clinical, serological, radiological and pathological feature.[55,56]

Clinically, ABPA act as bronchial asthma which leads to proximal bronchietasis and lungs fibrosis.[57] This syndrome is difficult to diagnose. As some of the clinically associated diagnostic are not mentioned here this may lead to unimprovised data. But moreover most of the diagnostic does not fill all criteria at the same time.[58] For example central bronchietasis can be detected only at the last stages of the disease.[59] And the prediction may depend upon the age of the patient's studies (who is going through cystic fibrosis as well as asthmatic patients with cystic fibrosis.[60] In some of the cystic fibrosis patients, damage of respiratory mucosa leads to response of conidia of Aspergillus even when all the diagnosis criteria is not fulfilled. And in patients who are not treated may leads to pulmonary fibrosis and respiratory failure. So, there need to be improved and well diagnostic of ABPA syndrome.

Aspergilloma

Aspergilloma is commonly known as 'fungus ball' which occurs in pulmonary cavity which is pre-existed and are caused by tuberculosis,

sarcoidosis and some chronically obstructed par-nasal sinuses.[61] Before, in 1850s the common syndrome was Aspergilloma but yes it still occurs in this era with 10% to 15% of the patients with cavitating lung diseases.[62] A symptom which is common in Aspergilloma is hemoptysis. In hemoptysis the blood vessels get disruptured in the wall of the cavity which is occupied by the fungus or it damages the bronchial aretery supply, which is a few centimeter away from the area of Aspergilloma.[63] Most often internal bleeding is caused but in hemoptysis it is massive and may be even fatal.[64] When we do radiography of chest of a patient we can see Aspergilloma which is usually surrounded by radiolucent cresent.[65] Patients are detected with high antibodies titers (precipitins) who are suffering from Aspergilloma.[66] In case of Aspergilloma patients does not shows any specific symptoms but this can be identified by obtaining chest radiograph for the evaluation of allergic disease. This type of disease is best visualized or can be seen by computed tomography (CT) scan of the chest. So, we can conclude that their presence can be seen during this scan and proper immunosuppressive therapy can be done before it get worsen.[67]

Invasive aspergillosis (IA)

IA has become major cause of death among hematology patients. The average patients with acute leukemia are 5%to 25%. IA is disease which mainly follow soli organ transplant (19% to 26%) and it gradually decreases in transplant of liver, heart, lungs and kidney recipients (1% to 10%).[68] But IA has majorly reported as main fungal infection in cancer patients and it is basically underrated due to low sensitivity of diagnostic tests.[69] IA has also been reported in AIDS suffering patients (1% to 12%).[70] And some of the complication of IA is also found in chronic granulomatous disease (CGD) (25%to 40%).[71] But it is rarely found in immunocompetent host.[72] IA has been described on its four types.[73] (i) Acute or pulmonary aspergillosis which is the most common form of IA (ii) Tracheobronchitis and obstructive bronchial disease which can been seen in the AIDS suffering patients[74] (iii) Acute invasive rhino sinusitis[75] (iv) Disseminated disease which involve brain most frequently (10% to 40% BMT patients) and few of the other solid organs such as heart, kidney, and eyes.[76] Still IA is very difficult to diagnose during its early stage. But there is diagnostic procedure available and its associated problems are being discussed. During early stage of IA one must give histopathological evidence of the mycelia growth within the tissue. So, it can only be demonstrated at autopsy.[77] Therefore, I here can conclude that there is no accurate diagnostic for IA, thus we can say that there might be possible reason to define it. And some of the variation might come from study to study.

Conclusion

There is sudden increase in the aspergillosis which is caused by A. fumigatus in recent years. A. fumigatus is the common airborne pathogen in most of the country causing significant increase in fatal pulmonary Invasive Aspergillosis which was known 20 years ago. A. fumigatus is majorly associated with disease such as Aspergilloma or Aspergillosis. As, this disease affect only few thousand of people worldwide but its mortality is more than 50% as it is nearly approaching to 100% in IA patients. Moreover, there is risk of getting another infection as IA treatment is based on different therapy as well as it is expensive. Now, the studied should be focused on the control of filamentous fungal disease which is caused by the conidia spores and risk of getting fungal infection is high in the patients. After, reviewing few of the paper the disease as well as treatment associated with A. fumigatus still pathogenic behavior for this fungus is not known this is because there is insufficient data in the history for IA as the curing management for this disease is difficult to understand. But progress in diagnosis in recent years has resulted in decrease in the mortality rate but apart from this early diagnosis is the most important criteria. Detection of molecules of fungus in biological fluids is the best if the host does not shows any specific sign or symptoms. But there is a challenge among microbiologist to develop proper kits for the diagnosis which includes use of molecular and immunological tools for the treatment.

And regarding the characteristics of A. fumigatus it does not need any human host to complete it life cycle. It is basically saprophytic fungus which causes disease in immune suppressed host. There is need of advance mechanism to identify the molecular route of phagocytes which are responsible for degrading of A. fumigatus fungi as there are fewer resources known for the cellular and molecular level defense mechanism. In short regarding the treatment of this fungus there must be advance and proper diagnosis which must be affordable for the treatment of Invasive Aspergillosis.

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Conflict of Interest

Author does not shows any conflict of interest

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