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A Rare Case of Aspergillosis of the Oral Cavity.

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Running title: Aspergillosis

Clinical significance: Aspergillosis is caused by a common mold, Aspergillus that is present in the environment

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ABSTRACT

Aspergillus species are universally ubiquitous saprophytes found in a large number of ecological sites. Nearly about 200 species of Aspergilli have been identified, of which less than 20 of them are known to cause human disease. The fungus Aspergillus causes a group of conditions called Aspergillosis. Its' spores (conidia) are found scattered in the air, and people breathe in the spores every day without being affected by it. However, when there is a reduced immunity, the organism becomes pathogenic.

Aspergillosis causes multiple problems in an individual. They include the allergies, infections and necrosis in various parts of the body, and rarely the oral cavity.The route of entry for the organism is through the respiratory tract; from where they spread through the blood, to the eyes, brain, heart or sometimes even the kidneys causing destruction and necrosis of the tissues, they localize. This can be life- threatening and serious if not attended to, promptly. Early diagnosis is mandatory for early treatment.

Aspergillosis can be treated with long-term antifungal drugs and in some cases; surgery may be required to remove a lesion. Though not a commonly occurring fungal infection, it can increase the morbidity and mortality to nearly about 90% in an already ill patient.

Keywords: Aspergillosis; aspergillus; fungal infection; fungus; immunocompromised individuals.

INTRODUCTION

Aspergillus is an opportunistic organism second only to candida. [1] Aspergillus species are saprophytic fungi that play a major role in envoirnmental carbon and nitrogen recycling.[2]It comprises of about 200 subspecies[3] that are commonly found in nature; of which less than 20 of them are known to cause human disease.[2]They are normally present indoors, and some of them even have medicinal value. [3]Due to their enzymatic profile they have been used in food and pharmaceutical industries. [2]Only some species of the organism, like the A fumigatus and A flavus are pathogenic; causing disease in both humans and animals.[3]

Aspergillis are filamentous saprophytes in the form of conidia (asexual spores) that live in the soil and decaying vegetations.[1]Once the organism gains entry into the respiratory tract, it spreads by its hyphael form, and releases toxins. The B-glucans on its cell wall initiates an inflammatory reaction. Various enzymes released by the organism such as phospholipases, proteases, [3]peptidase; [4]and toxins such as haemolysin, aflatoxin, gliotoxin, phthalic acid [3]etc. that causes invasion and necrosis. [4]

The spores of the fungus are inhaled; and when they lodge within the lungs, they cause infection. They disseminate to distant sites such as the oral cavity, skin, etc. through the blood. The organism usually spreads into the walls of the blood vessels by releasing their toxins; thereby causing secondary thrombosis, bleeding, and necrosis of the tissues with a yellow or grey slough; resulting in a complete and rapid systemic involvement.[2]The B-glucans on its cell wall initiates the inflammatory reaction. [4]

In the oral cavity the gingiva and [4]palate, followed by the tongue are predominantly affected sites. [3] The palatal involvement may result in a perforation while descending from the maxillary sinus into the oral cavity.[4]

Pathogenesis of the organism is enhanced by environmental changes such as construction activities, incomplete removal of dust, rotten leaves; and dead and decaying plants, etc.

The Aspergillosis may present as 3 variants:

- Saprophytic; superficial or non-invasive
- Allergic; as a hypersensitive reaction
- Invasive; infection into viable tissues.[1]

CASE REPORT

A 36-year-old male, who was a farmer and who maintained poultries, visited the peripheral cancer center with a complaint of pain and foul smell in the left upper teeth region since 1 month.A month earlier, he had undergone an extraction of 16 under local anesthesia. The foul smell with a purulent discharge emanated from the extraction site of 16. The patient consulted a dental surgeon who diagnosed the condition as a dry socket: a complication of the extraction and treated him with antibiotics, sedatives, and dressing. Routine blood examination was advised; as the lesion failed to heal, and the patient was diagnosed with diabetes. The foul odor was obvious when the patient was asked to open his mouth; and on intraoral examination, an ulcerated and necrotic area was visible at the site of the extraction socket. (**Figures 1 and2**)There was no bleeding, but pus discharge was obvious on gentle palpation. The maxillary sinus examination was inconclusive.

FIGURES 1 and 2

Orthopantomogram (OPG) indicated a diffuse radiolucency about the 16-socket and in the inferior border of the right maxillary antrum,(**Figure3**) findings that made a cytosmear examination of the purulent discharge, mandatory.

FIGURE 3

Periodic Acid Schiff staining of the cytological smear showed acute angled septae of the hyphae that was bright magenta in color: along with a collection of inflammatory cells, dispersed within a necrotic background.

A provisional diagnosis of Aspergillosis was given. Hematoxylin and Eosin stained biopsy specimen showed a non–specific maxillary sinus lining and areas of hemorrhage and necrosis in the tissue sample from the palate. PAS stained section showed a few magenta-colored hyphae that bent at an acute angle of 45°, predominantly near the blood vessels: features that were suggestive of Aspergillosis.(**Figures 4 and 5**)(**Figure 6**)

FIGURES 4 and 5: H and E and PAS (right) (5x view) stained section of the tissue

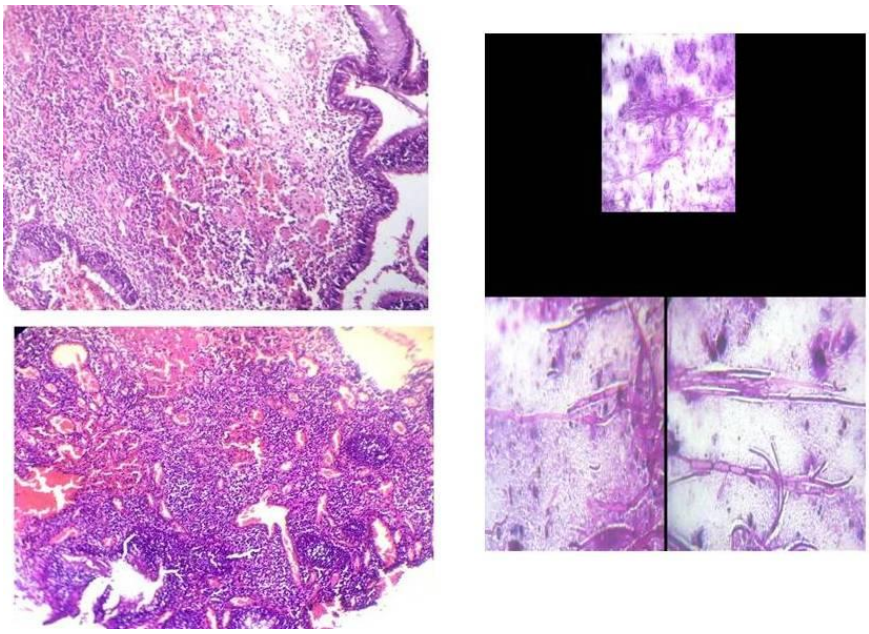
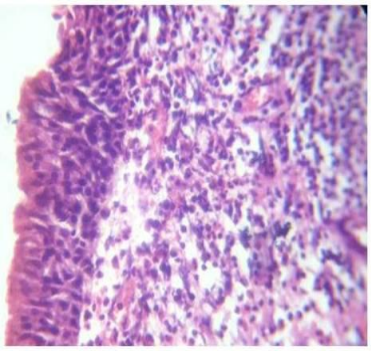


FIGURE 6: H and E stained section of tissue showing hyphae (10x)



The patient was referred to a higher medical center where the patient's diabetes was brought under control, and a Hemi maxillectomywas done. The condition healed after 20 days of antifungal treatment and surgery,and the healing has been uneventful.

DISCUSSION

Katzenstein described the first case of aspergillosis of the paranasal sinus in 1983. [4] The head and neck are rarely affected by Aspergillosis, and the involvement of the nasal cavity, paranasal sinuses, skin of the face and the oral mucosa are still rarer. [1] In the oral cavity, the periodontal tissues, especially the marginal gingiva may show necrosis [6]which may spread into the underlying alveolar bone, and further into the cranium; [5]extreme salivation, swelling of facial tissues and a constant fever are other manifestations of head and neck Aspergillosis. [6]

The lesion on the gingiva is yellow or grey[1] with necrotic and ulcerated base [1] and when present in:

- ✓ an immunocompromised patient,
- ✓ in patients with fever that persists in spite of the broad-spectrum antibiotics,
- ✓ in maxillary sinusitis cases,
- ✓ in febrile neutropenic patients,
- ✓ asthmatic individuals,
- ✓ individuals with cystic fibrosis;
- ✓ diabetes,
- ✓ individuals with a weakened immune system,
- ✓ individuals with HIV infection: recent reports have observed an increase in incidence of aspergillosis in HIV patients, [8]indicate an infection by the invasive Aspergillosis.[3]

The maxillary sinus, hard palate, soft palate, and tongue (A flavus[9]) are the other intraoral sites, affected.[6]

A single palatal ulcer, with yellow or black necrotic slough on its base, must be differentiated from necrotizing sialometaplasia, oral tuberculosis, malignant ulcers, syphilis, and deep fungal infections.[4]

The most common form of invasive aspergillosis is an aspergilloma; which presents as a 'fungal ball'. The A flavus is said to be predominant in this lesion. The 'ball' consists of a twisted collection of mucus, fungus fibers, tissue debris, blood clotting factors (fibrin) and inflammatory cells. [10]

Among the 200 subspecies of aspergillis, themost pathogenic among them is the A fumigates, followed by the A flavus and sometimes even the A terreus, [5]A verscicolor [9]and A Niger. [2]The fumigates is smaller than the flavus, and is therefore able to reach the alveoli of the lungs.

The organism exists in the spore (conidia) and hyphael forms. The organism is found to show dichotonomous (Y shaped), septate (cross-walled) branching hyphae, with non-parallel and irregular cell walls. It produces numerous spores but it does not show dimorphism. [11]The cell wall of the organism shows chitins, glucans, and galactomannans. The GAG or "galactosaminogalactan", found exclusively in the A fumigates may probably enhance the fungus' ability to outsmart the host immune system especially the neutrophils. [12]

The melanin and sialic acid are found on the surface of the conidia, and a hydrophobic layer is present on both the conidia and hyphae.[13]The melanin in its outer layer protects the conidia against damage from UV radiation. Additionally, it is also said to protect against phagocytosis both in vitro and in vivo. Melanin is said to minimize the complement opsonization by 'camouflaging' attachment sites, which can,therefore, reduce the C3 (complement 3) ability to bind conidia. It is said to aid in the fungal survival rather than play a role in its pathogenesis. [9.]

Iron is an essential nutrient for Aspergillus species, and is one of the main components of its enzymes; that participate in a variety of cellular activities. [14]Iron-specific chelators called siderophores are engaged by the fungus to regulate iron load, which is important from the point of view of fungal virulence. [13]

The diagnosis of Aspergillosis is usually made by histological examination and culturing the organism.

Aspergillosis is found to affect males and females in equal numbers.[10]

PATHOGENESIS

Role of neutrophils: Cyclophosphamide, a DNA alkylating agent, used for transplant patients, or those with hematological diseases; binds to the DNA and interferes with cellular duplication, thereby reducing the number of circulating white blood cells including polymorphonuclear leucocytes.

> In both clinical and experimental studies of neutropenia, **invasive aspergillosis** is the consequence of the thrombosis and hemorrhage that occurs due to the rapid and widespread hyphal growth. There is; therefore, alack of inflammatory infiltrates in spite of the production of tumor necrosis factor alpha (TNF-α) that further results in low levels of inflammatory neutrophils. In the absence of neutrophil recovery, the organism spreads through blood and disseminates to other organs.

Role of macrophages: Corticosteroids that is prescribed for prophylaxis or for immunosuppressive therapy, suppresses the functional ability of macrophages, to kill A. fumigatus conidia and hyphae.

Therefore, the neutrophils are recruited to the site resulting in tissue injury and necrosis. This exaggerated inflammatory response is mostly the cause of death.

Role of A fumigatus: A. fumigatus is pathogenic mainly due to its thermotolerant nature, as compared to the other disease-causing species; grows well at 37°C, and can withstand temperatures above 50°C; similar to the environment in decaying vegetation, which is their frequently inhabited site**.** Thegermination rates and pathogenic prevalence when compared, show that the germination rates of A**.** fumigatus, A. flavus,and A. niger were similar at temperatures up to 30°C but differed at 37°C and 42°C suggesting a lowered virulence in the latter two strains.

1. **Role of antioxidants:** Antioxidants is said to play a significant role in the response of the body to A. fumigatus, but its specific role in pathogenicity must be further investigated.
2. **Role of toxins:** Gliotoxin, the toxin liberated by the A fumigates, in vitro has been shown to have a large number of immunosuppressive activities; a few among them being; the inhibition of macrophage phagocytosis, mast cell activation, mitogen-activated T-cell proliferation, cytotoxic T-cell response and induction of apoptosis in inflammatory and cancer cells. [2]
   Aflatoxin, another toxin liberated by Aspergillus species, on the other hand contaminates crops and makes it carcinogenic; it also suppresses phagocytosis, superoxide production by macrophages, and inhibits intracellular killing.[9]
3. **Role of the hyphae per se**: Invasion by the hyphae occurs from the abluminal to the luminal side of endothelial cells, inducing the endothelial cell activation and causing little cell damage. This results in the fragmentation of the hyphael fragments into the bloodstream and an invasion of the endothelium at other sites; resulting in a hematogenously disseminated disease. During the invasion of organs, the hyphal invasion begins on the luminal side and proceeds to the abluminal side of endothelia resulting in significant damage. A protein called fumigillin secreted by aspergillis organism is said to cause this damage.
4. **Role of albumin**: Albumin in-vitro, facilitates the growth of A fumigates but suppresses the growth of the A flavus and A niger.[2]

HISTOPATHOLOGY

The histological appearance of Aspergillosis in tissue samples of H and E stained section is characterized by hyphae that are 3–8 μm in diameter, showing a repetitive dichotomous branching at an angle of about 45°, and show septaes. Histological findings are usually confirmed with a Periodic acid-Schiff (PAS) staining.[3]

LABORATORY DIAGNOSIS

- The organism can be identified by culturing the sample obtained from the purulent, in a Sabouraud's dextrose agar broth. [3]
- The detection of galactomannan, a cell wall polysaccharide of Aspergillus spp., contributes as a diagnostic marker for the disease in the absence of culture.[14]Recently introduced assays, like the B-glucan assays, galactomannan assay, and polymerase chain reaction has been helpful in the detection of invasive aspergillosis. [4]

  The positivity for the Galactomannan antigen is detected nearly 5 to 8 days on an average, before the development of the clinical signs. Positivity in two consecutive serum samples is an indication for invasive aspergillosis.[15]
- The use of PCR is promising, but it is yet to be standardized.[14]
- A potential marker for invasive aspergillosis is β-d-glucan, which however is not very specific.[16]

DIFFERENTIAL DIAGNOSIS

An aspergillosis ulcer may mimica:

- Bacterial infection (acute necrotizing ulcerative gingivitis)
- Viral infection (herpes simplex) [3]
- Deep fungal infection: Filamentous fungal infections, such as Fusariosis and Scedosporiosis [16]
- Tuberculosis
- Actinomycosis
- Malignant infiltration[3]
- Pseudomonas [1]
- Mucormycosis, like aspergillosis, is an entity, which commonly presents in diabetic individuals. Microscopy can help distinguish between the two lesions and PAS and methenamine silver stains together with culture can confirm the diagnosis. [4]

TREATMENT

1. Antifungal treatment such as a:
    - High-dose Amphotericin B----- Liposomal Amphotericin B
    - Triazole, Itraconazole.

- Initial therapy with Voriconazole may improve survival with fewer side effects. [1]In cases of systemic disease this drug is found to be less toxic.[14]
- Echinocandins[4]
- Where there is a confirmed diagnosis in cases of intolerance or refractoriness, the lipid formulations of amphotericin B, such as posaconazole or itraconazole are advisable, except where is a history of the previous usage of voriconazole, caspofungin or micafungin.[14]
- Caspofungin in combination with Amphotericin B or an Azole has been recommended in refractory cases where initially administered antifungals have not worked.[3]
- However, though not a treatment of choice, a combination therapy is not routinely recommended. It is recommended in cases of intolerance or refractoriness

The duration of the treatment ranges from 6-12 weeks; and in immunocompromised patients, the treatment needs to be carried on until the lesion is reabsorbed and the immunological state has improved.[16]

2. Surgical excision of the involved tissue provides a better outcome than antifungal therapy alone. Nevertheless, in case of immunocompromised patients, a close consultation with the anesthetist and hematology specialist is mandatory before surgery.[3]

CONCLUSION

Aspergillosis is a grave condition that results in a high morbidity and mortality between 40-90% if a prompt diagnosisis not made and the treatment of choice includes surgery combined with systemic antifungal therapy. In spite of the newer antifungal drugs available and improved measures for the management of the immunocompromised state, the outcome for invasive Aspergillosis of the oral tissues remains inadequate.

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