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A STUDY OF FUNGAL BALLS IN CHRONIC RHINOSINUSITIS

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ABSTRACT: INTRODUCTION: Fungal ball is a term used for a form of fungal sinusitis. It is always an extramucosal fungus proliferation which fills completely one or more Paranasal sinuses; the most common sinus involved is the Maxillary sinus. Malignancy should always be considered in the differential diagnosis of Fungal ball. **PURPOSE:** To study the etiology of Fungal balls and correlate with Histopathological examination to rule out abnormal findings. **MATERIALS AND METHODS:** Fungal balls removed endoscopically from 20 cases of Chronic Rhinosinusitis from October - 2013 to October - 2014 were studied. KOH wet mount preparations and Fungal cultures in SDA were done as per the standard guidelines. Simultaneously the samples were analysed by Histopathological examination (HPE) also. CT findings of the cases were analysed. **RESULTS:** Among 20 samples, 19 were positive for fungal elements on KOH wet mount showing branched and septate hyphae. Out of these 19, 14 were culture positive - *Aspergillus fumigatus* in 7 cases, *Aspergillus niger* in 5 and *Penicillium* spp. in 2. HPE showed entangled fungal hyphae in 19 samples. One case was negative for fungal elements in KOH wet mount & Culture. Interestingly that case on HPE revealed Squamous cell carcinoma. **CONCLUSION:** A diagnosis of Fungal ball needs to be evaluated by repeated KOH mounts and HPE also in order not to miss Malignancy. A pathological examination & confirmation is mandatory for KOH wet mount & culture negative cases.

KEYWORDS: Fungal ball, Chronic Rhinosinusitis, Squamous cell carcinoma, Keratin pearls.

INTRODUCTION: Sinus infections, also called sinusitis, are defined as inflammation of one or more sinuses. Sinuses are hollow cavities within the bones of the face. The sinuses are lined by mucous membranes that normally produce fluid – this fluid usually drains into the nasal passages and is swallowed throughout the day. However, when the drainage sites for the sinuses are blocked, the sinuses cannot drain, and an infection may develop.

The actual infection of the sinuses is most frequently caused by a viral infection (Such as the common cold), but can also be bacterial in nature (and therefore responds to antibiotics). Some sinus infections are due to fungus in the sinuses.¹

Fungal organisms are ubiquitous and our exposure to these organisms occur on a daily basis. A common location for these organisms to enter the human body is through the sinonasal cavity. Fungal sinusitis is becoming increasingly common due to changes in the distribution of normal flora and to a growing number of diverse immunocompromising factors caused by overuse of drugs like antibiotics and steroids, endocrine diseases like Diabetes mellitus, viral diseases like HIV, Measles, Carcinomas, etc.

PATHOGENESIS: Inhalation of Fungal spores is thought to be the primary means by which fungi gain access to the sinonasal tract. These spores get sequestered in one of the Paranasal sinuses, usually the

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Maxillary sinus (69-86% of cases). Due to deficient mucociliary clearance the spores germinate, proliferate and growth of the fungus occurs evading the host immune system. It develops in immunocompetent individuals without any predisposing factors.²

There is an estimated 20,000 to 1.5 million different fungal species. However, only a few dozen actually cause infectious disease in humans. There are three main forms of fungi – Yeasts, Yeast like and Molds. The yeast is a unicellular organism roughly 3-15µm in diameter. They reproduce asexually by budding. If these buds do not detach a chain of fungal cells results. This chain is known as pseudohyphae. The mould is a multicellular organism measuring 2-10 µm in diameter. These organisms grow by branching into structures termed Hyphae.

CLASSIFICATION OF FUNGAL SINUS DISEASE: Fungal Sinus Disease can be divided into two categories - Noninvasive and Invasive. Noninvasive disease includes Saprophytic Fungal infestation, Sinus Fungal Ball and Allergic Fungal sinusitis. Invasive disease includes Acute Fulminant Invasive Fungal sinusitis, Chronic Invasive Fungal sinusitis, Granulomatous Invasive Fungal sinusitis.³

SAPROPHYTIC FUNGAL INFESTATION: Saprophytic Fungal infestation within the sinonasal cavity occurs when fungus grows on mucus crusts without the involvement of the surrounding mucosa. Patients typically have minimal or no sinonasal symptoms. Diagnosis is done by direct examination and the treatment involves removal of the crusting with sinonasal rinses. In addition, patients should undergo weekly endoscopy with removal of further crusting until the disease process resolves.⁴

SINUS FUNGAL BALL (MYCETOMA): Fungus is an extramucosal fungal proliferation and sequestration of fungal hyphal elements which fills completely one or more Paranasal sinuses, the most common sinus involved being the Maxillary followed by Sphenoid. Involvement of the Ethmoid, Frontal and multiple sinuses is rare.⁵

CLINICAL FEATURES: Fungus ball is usually asymptomatic. The signs and symptoms of the disease are the result of mass effect of the fungus ball and sinus obstruction. However the symptoms are nonspecific. The common symptoms are Post nasal discharge, Anosmia or Hyposmia, Nasal blockage, Facial pain and Headache.

CAUSATIVE FUNGI: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Alternaria*, *Bipolaris*, *Curvularia*, *Penicillium marneffeii*, *Mucor*, *Rhizopus*, *Scedosporium prolificans* etc.

PREDISPOSING FACTORS: Massive fungal exposure, Local tissue hypoxia, Dental treatment, Usage of positive airway pressure apparatus, deviated nasal septum, Osteomeatal complex occlusion.

Shazo, O'Brien, Chapin et al. (1997) laid down criteria for diagnosis of Fungus ball.⁶

1. Partial sinus opacification.
2. Clay like material within the sinus.
3. Conglomeration of hyphae, involving sinus mucosa.
4. Chronic inflammatory inflammation.
5. No predominance of eosinophils, no granuloma, no allergic mucin.
6. No invasion of bones, vessels or mucosa.

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TREATMENT: Complete surgical removal of Fungal ball, Irrigation of involved sinuses, Antifungal therapy only if patient is at risk for invasive disease such as in cases of severely immunocompromised and continued recurrence despite proper medical/surgical management. Topical antifungals should be instituted first followed by the least toxic medications if they are required.

ALLERGIC FUNGAL SINUSITIS (AFS): Allergic fungal sinusitis occurs when a fungus colonizes a sinus cavity and then causes allergic mucosal inflammation through an IgE response to fungal protein. Patients present with nasal obstruction, rhinorrhoea, facial pressure, sneezing, watery/itchy eyes, and periorbital edema. There are five major criteria used to make the diagnosis of AFS. These are the presence of eosinophilic mucin containing noninvasive fungal hyphae, nasal polyposis, allergy to the offending fungus, immunocompetence of the patient, and the classic radiographic findings associated with AFS. Eosinophilic mucin is pathognomonic for AFS, and is described as a thick, tenacious and highly viscous material that is tan to brown or dark green in appearance. Under microscopic examination, the mucin contains branching fungal hyphae, sheets of eosinophils and charcot-leyden crystals. Charcot-leyden crystals are slender and pointed crystals consisting of a pair of hexagonal pyramids joined at their bases. They result from the breakdown of cells by enzymes that are released by eosinophils.⁷

The classic radiographic findings of AFS are best noted by CT imaging. The disease will usually be unilateral in 78% of cases. The involved sinuses are typically expanded, with rare cases of bony destruction.

The treatment of AFS begins with the surgical removal of all mucin while providing permanent drainage and ventilation of the affected sinuses. Following this, systemic steroids are utilized for a period of 2-3 months. As AFS is an allergic response, immunotherapy has also been used as a treatment.

SQUAMOUS CELL CARCINOMA IN SINONASAL CAVITY: It is a malignant epithelial neoplasm originating from the mucosal epithelium of the nasal cavities or Paranasal sinuses.

EPIDEMIOLOGY: Sinonasal squamous cell carcinoma is rare, accounting for <1% of malignant tumours and only about 3% of malignancies of the head and neck. It is extremely rare in children, and men are more commonly affected (about 1.5 times) than women. Patients are generally about 55-65 years of age.

ETIOLOGY: Reported risk factors have included exposure to nickel, chlorophenols, textile dust, smoking and a history or concurrence of sinonasal papilloma. Human papillomavirus (HPV) has been found in some cases, especially those associated with inverted papilloma, but a definite etiologic role has not been clearly established.

LOCALIZATION: Sinonasal squamous cell carcinomas occur most frequently in the Maxillary sinus (about 60-70%), followed by the nasal cavity (about 12-25%), Ethmoid sinus (about 10-15%) and the Sphenoid and Frontal sinuses (about 1%).

CLINICAL FEATURES: Symptoms include nasal fullness, stuffiness, or obstruction; epistaxis; rhinorrhea; pain; paraesthesia; fullness or swelling of the nose or cheek or a palatal bulge; a

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persistent or non-healing nasal sore or ulcer; nasal mass; or, in advanced cases, proptosis, diplopia, or lacrimation. Radiologic studies such as CT scan or MRI may delineate the extent of the lesion, the presence of bony invasion, and extension to neighboring structures such as the orbit, pterygopalatine or infratemporal spaces.

MACROSCOPY: Sinonasal squamous cell carcinomas may be exophytic, fungating, or papillary; friable, haemorrhagic, partially necrotic, or indurated; demarcated or infiltrative.

TUMOUR SPREAD AND STAGING: Maxillary sinus carcinomas may spread to the nasal cavities, palate, other paranasal sinuses, skin or soft tissues of the nose or cheek, orbit, cranial contents, or the pterygopalatine and infratemporal spaces. Lymph node metastases are less common than in squamous cell carcinomas of other sites in the head and neck.

MATERIALS AND METHODS: Fungal balls from 20 suspected cases of Chronic Rhinosinusitis were removed endoscopically by Functional Endoscopic Sinus Surgery (FESS) in the department of ENT, Government General Hospital, Kurnool. The period of study was from October 2013 to October 2014. All the Fungal balls received in the laboratory were subjected to Macroscopic examination, Potassium Hydroxide(KOH) wet mount, Lacto Phenol Cotton Blue(LPCB) mount, Histopathological examination (HPE) after Haematoxylin and Eosin (H&E) staining and Periodic Acid Schiff (PAS) staining, Culture and Radiological examination prior to surgery.⁸

KOH WET MOUNT PREPARATION: The specimen is emulsified in a drop of 10% KOH on a microscopic slide with the help of a loop. Gentle heat is applied over a Bunsen flame for 3-4 times to hasten the clearing. It is then covered with the coverslip and left for 5 -10 minutes. The slide is examined under low (10X) and high power (40X) magnifications.

LPCB MOUNT: A drop of LPCB is taken on a slide and a small amount of growth from culture is teased in it. It is then covered with coverslip and observed under low (10X) and high power (40X) magnifications.

HAEMATOXYLIN AND EOSIN (H&E) STAINING: The section on the slide is deparaffinized in xylene, fixed in alcohol and then dipped in Hematoxylin jar for 3-5 min. It is washed with water and then dipped in 0.05% Ammonia, 70% alcohol and in 1% Eosin (2-3 times) with washing after each step. It is finally dipped in xylene and mounted with coverslip and DPX.

PERIODIC ACID SCHIFF (PAS) STAINING: The slide is deparaffinized in xylene, fixed in alcohol. Periodic acid is poured on the section and kept for 5-10 min. The slide is washed with water and Schiff's solution is put for 5min. After counterstaining with Hematoxylin for 1-2 min the slide is mounted in DPX.

CULTURE: Culture on plain Sabouraud's Dextrose Agar (SDA) and SDA containing Gentamycin (20mg/lit) and Chloramphenicol (50mg/lit).

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RADIOLOGICAL EXAMINATION: Sinus CT scanning had been conducted on all 20 patients.

RESULTS: MACROSCOPIC FEATURES OF FUNGUS BALL: With regards to physical appearance, the fungus ball appeared as a greenish cheese like substance in 11(55%), as a brownish substance in 6(30%), as a darkish substance in 2(10%) and accompanied by calcification in 1 case (5%).⁹

KOH WET MOUNT: Among 20 samples, 19 were positive for fungal elements showing hyaline, branched and septate hyphae.

H&E STAINING: Matted, dense conglomerations of hyphae separated from but adjacent to respiratory mucosa of the sinus were seen in 19 cases. The hyphae were branched at an angle of 45° in 11 cases.

In one case features were suggestive of Keratinizing Squamous cell carcinoma. There was histologic evidence of squamous differentiation, in the form of extracellular keratin (pink cytoplasm, dyskeratotic cells). Tumour cells were opposed to one another in a “mosaic tile” arrangement. Invasion was seen as irregular strands.

PAS STAINING: Hyaline, branched and septate hyphae were observed.

CULTURE: Velvety green growth with reverse white was observed in 7 cases, Woolly black growth with reverse black in 5 cases,, Powdery bluish green growth with reverse white in 2 cases.

LPCB MOUNT: Septate hyphae with unbranched coniodophore terminating in vesicle, covered entirely with chains of round conidia in 4 cases (suggestive of *Aspergillus niger*) and conidia covering only upper half of vesicle in 7 cases (suggestive of *Aspergillus fumigatus*)

Septate hyphae with branched coniodophores, flask shaped sterigmata and chains of round conidia giving Broom Stick appearance in 2 cases (suggestive of *Penicillium* spp.)

RADIOLOGICAL EXAMINATION: In 12 cases radiologic findings suggested sinus Fungus ball. In 7 cases, focal calcification was observed inside the soft tissue lesions of the sinus and in the remaining one case erosion of neighbouring bone tissues was observed. All 20 cases were unilateral and in 17 cases, the Maxillary sinus was involved. In one case Sphenoid sinus was involved.^{10, 11}

AGE AND SEX DISTRIBUTION: Out of 20 samples with Fungus balls 14(70%) were from males and 6(30%) from females. The ages of the patients ranged from 25 - 74. Maximum number of patients were in the age group of 50 – 59 years.

GEOGRAPHIC DISTRIBUTION: Out of 20, 13(65%) belonged to rural area and 7(35%) belonged to urban area.

DISCUSSION: Fungal ball is described as a noninvasive accumulation of a dense conglomeration of fungal hyphae in a Paranasal sinus. It is characterized by a mass of fungal hyphae, inflammatory cells and mucus, with gradual growth in the sinus socket, without involvement of the underlying mucous

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membrane. Fungal balls had been classified erroneously as maduramycosis or aspergilloma in the past. During the last decades there has been an apparent increase in the diagnosis of these pathologies.¹²

It is more common in males from urban area in the age group of 50-59.

While a general H&E stain could be used for histologic diagnosis, staining with PAS or Gomori's Methenamine Silver stain (GMS) was found to be more effective as applying special stain improves the accuracy of diagnosis.

The possible reasons for negative culture could be that fungi are usually degenerated and nonviable and also the mucus is viscous inhibiting the proper contact of the fungus with the medium. Use of mucolytic agent may increase the yield of positive culture to 96%.

KOH wet mount, Culture followed by LPCB mount from growth on SDA, HPE after H&E and PAS staining were the main methods used for diagnosis of Fungus ball. Unusually, features of Squamous Cell Carcinoma were found in one case in HPE.

CONCLUSION: Fungus ball can be found in apparently healthy individuals and appears as Chronic Rhinosinusitis that does not respond to antibiotics. The existence of uncommon manifestation of fungal infection in Paranasal sinuses as reported in the literature helps to alert the physician of the proper diagnosis by thorough and good clinical assessment together with support from laboratory and radiological investigation. Recent developments in CT and MRI technologies have made diagnosing fungal ball easier.¹³

Out of the 19 cases positive for fungal elements, 14 were culture positive. One case clinically suspected as Fungus ball, on HPE revealed Squamous cell carcinoma. A diagnosis of Fungal ball needs to be evaluated by repeated KOH wet mounts, culture and HPE also in order not to miss Malignancy. So Fungal ball, Malignancy should be considered in the differential diagnosis of unilateral sinusitis or recurrent sinusitis not responding to antibiotics.

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Macroscopic appearance of Fungus Balls

KOH Wet Mount – Hyaline, septate and branched hyphae

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LPCB MOUNT

HPE – H & E STAINING

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CULTURE ON SDA

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