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A study on the etiology of fungal sinusitis and the associated predisposing factors in patients with chronic sinusitis: A prospective study

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Abstract
Background: Fungi are uncommon causes of sinusitis. Many fungi have been associated with fungal sinusitis including Aspergillus species, Zygomycetes species, several of the dematiaceous fungi including Curvularia, Bipolaris and Exserohilum. The etiological agents of fungal sinusitis reported from India vary from there of the western countries, where in dematiaceous fungi are common. Aspergillus spp are more commonly isolated from the Indian subcontinent.
Aim: The aim of the study is to determine the aetiology of fungal sinusitis and the associated predisposing factors in patients with chronic sinusitis, attending Department of Otolaryngology, Gandhi Hospital, Hyderabad, a tertiary referral centre.
Materials and Methods: Prospective study approved by the institution ethical committee, conducted from September 2011 to August 2014. 50 patients age group of 10-63 years (with mean age of 39.03 years) attending the Department of Otolaryngology, Gandhi Hospital were studied.
Clinically suspected cases of Chronic Rhino Sinusitis (CRS), lasting longer than 3 months was included in the study. Children below 10 years were excluded from the study. Patients were evaluated on the basis of the clinical, radiological, mycological findings. Specimens include allergic mucin, exudates from the nasal mucosa, tissue biopsy from nasal polyps and sinus mucosa obtained intra operatively were collected and processed. All samples were received in sterile containers. Specimens were subjected to microscopy (10%KOH, Histopathology) and culture.
Results & Discussion: Out of the 50 cases studied, Fungal culture was positive in 22 (44%), found to be common in young adults (20-42 yrs). Aspergillus species (14) were the common isolates followed by *Rhizopus species* (3), *Alternaria species* (1), *Curvularia species* (1), *Penicillium species* (1), *Mucor species* (1) and *Aurobasidium pullorum* (1) each. Nasal obstruction (76.6%) and Nasal discharge (56.6%) are the common complaints in these patients. In India, Aspergillus spp., are commonly isolated and vary from there of the western countries, where in dematiaceous fungi are common. In India a large proportion of the population live in rural or semi-rural areas and their exposure to certain fungi will differ from urban population in developed countries.
Conclusion: In India no population based data is available and more studies are needed to address the problem.

Keywords: Sinusitis, fungal sinusitis, aspergillus, chronic fungal sinusitis

Introduction
Fungal Rhino Sinusitis (FRS) has been referred in the medical literature for over two centuries. However, for the past 25 years it has received the due attention, with increased clinical suspicion and improvement in laboratory techniques for detection of fungi. It's classification based on immunological relation between fungi and their host and level of mucosal invasion is important in the selection of an effective treatment and for the definition of the prognosis. The knowledge of the type of fungal flora, its prevalence, clinical presentation, clinical radiological findings and supplementary tests in patients with Chronic Rhinosinusitis (CRS) will enable better understanding of the disease and raising awareness of

the physicians for appropriate diagnosis, treatment and formulating prognosis of the disease. The common causes of FRS are *Aspergillus* spp, followed by *Zygomycetes* spp, *Penicillium* spp, and *dematiaceous fungi.* There have been reports of rare fungal isolates from India and other countries [1, 4-7].

The objectives of the present study was to assess the various types of fungal sinusitis and commonest modes of presentation, predisposing factors, causative agents, to study the anti- fungal susceptibility pattern of fungal isolates and analyze the data by appropriate statistical methods. Patient's follow-up was performed for 1 year.

Materials and Methods

This is a prospective observational study conducted in the Department of Otolaryngology, Gandhi Medical College & Hospital, Hyderabad, for a period of 3 years from September 2011 to August 2014 with a follow up period of 1year. The study was approved by the Institutional Ethics Committee.

Study population included 50 patients in the age group of 10 to 60 years with history of chronic sinusitis lasting longer than 3 years with males and female ratio of 1:1. Patients presenting with chronic headache, purulent nasal discharge, nasal obstruction, facial swelling, and symptoms of visual impairment, halitosis, and epistaxis were studied.

Statistical analysis

Statistical tool Statistical analysis was performed using statistical package for social sciences (SPSS Version 17). Numerical data was entered as such and categorical data was appropriately code.

Radiographic and Computed tomography imaging of nose and paranasal sinuses was done in all the cases of fungal rhinosinusitis to assess the patency of the osteomeatal complex, involvement of sinuses, and erosion of bony margins or expansion of the sinus cavity.

Otorhinolaryngological examination by Functional endoscopic sinus surgery (FESS): All the patients underwent diagnostic nasal endoscopy to rule out any anatomic variations of the osteomeatal complex, the presence of polyps, and nasal discharge. Specimens for fungal culture and pathologic examination were obtained by diagnostic nasal endoscopy and during surgery.

Microbiological and Mycological analysis of the samples done by microscopy (KOH mount, Histopathological examination) culture and Antifungal susceptibility of fungal isolates done culture of the material done in medium Sabouraud Dextrose Agar (SDA) with or without chloramphenicol and cycloheximide. Incubated at 25 ℃-35 ℃, and cultures were observed up to 20 days before release as negative for fungi. The identification of fungi made from microscopic morphology by LPCB Mount. Antifungal susceptibility done for the filamentous fungi, the MICs was determined by using the reference procedure of the Antifungal Susceptibility Testing for spore-forming molds. Testing was performed in flat-bottom micro dilution plates with RPMI 1640 medium supplemented with 2% glucose and an inoculum size of 2×10^5 to 5×10^5 CFU/ml. MIC end points were visually determined at 24 and 48h. For the polyenes and the azoles, MIC end points were determined spectrophotometrically at 24h. For AMB, the MIC end points were defined as the lowest drug concentration that resulted in a reduction in growth of 90% or more compared with that of a drug-free growth control well. For the azoles the MIC end point was defined as a 50% reduction in the optical density. The MFCs, which were considered the lowest drug concentrations that resulted in 99% killing, were determined as described previously (6).

Patients' Follow up

The follow-up of patients was performed by the authors using anamnesis, complete ENT examination and nasal endoscopic exam. The follow-up was performed for a minimum of 6 months.

Results

A total of 50 patients (25 males and 25 females) in range 16-63 yrs with mean age 39.03 yrs M: F Ratio 1:1 were studied (Table- 1 & 2).

In our study in 50 cases of CRS 22(44%) people were positive for fungal culture (Table -3).

Presenting symptom was nasal obstruction 48(96%), nasal discharge 32(64%), polyposis 40(80%), headache 18(36%), facial pain 8(16%), facial swelling 4(8%) (Table-4).

Diagnostic Nasal endoscopic findings were: nasal secretion in 28 patients (56%), divided into 14(28%) yellowish colour, 08 (16%) greenish, 4 were brown and 2 was black; medium meatus obstruction in 38 (76), polyposis in 22 (44%), bilateral in 8 (16%) and unilateral in 06 (12%), lower conchae hypertrophy in 1 (2%) and adenoid hypertrophy in 1 (2%).

Radiological evaluation showed unilateral involvement in 42 maxillary sinus is most affected followed by ethmoid, frontal, and sphenoid sinuses. Opacification seen in all cases (100%) Middle meatus obstruction, Bone erosion, Sinus expansion (Table-5).

In our study depending on clinical, radiological, histopathological mycological findings, fungal rhino sinusitis was found Non Invasive in 16 (72.72%) and Invasive in 6 (27.27%) of cases. Amongst non-invasive variety, Allergic Fungal Rhino Sinusitis 15(68.1%), Fungal ball 1(4.5%) were identified. In Invasive variety, Chronic Invasive Fungal Sinusitis 5(22.5%) and Chronic Granulomatous 1 (4.5%) identified. (Table -6).

Tables

Table 1: Demographic data & age distribution of the study population

Age	No	Percentage %
10-20	05	10%
21-30	18	36%
31-40	17	34%
41-50	07	14%
51-60	03	06%

Table 2: Sex distribution of the Patients

Sex	Number	Percentage %
Male	25	50%
Female	25	50%

Table 3: Fungi Isolated

Type of fungi	Number (n=22)	Percentage
Aspergillus	14	63.63%
Rhizopus	03	13.63%
Mucor	01	4.54%
Alternaria	01	4.54%
Curvularia	01	4.54%
Penicillium	01	4.54%
Aurobasidium	01	4.54%

Table 4: Clinical presentation of studied patients

Signs and Symptoms	Number	Percentage %
Nasal Obstruction	48	96
Polyp	40	80
Nasal Discharge	32	64
Post nasal dripping	30	60
Cough	25	50
Allergic rhinitis	20	40
Sore throat	18	36
Facial pain	08	16

All patients presented some type of opacification of paranasal sinuses CT scan.

Table 5: CT scan findings

Findings	Number (n=50)	Percentage %
Opacification	50	100
Middle metus obstruction	28	56
Bone erosion	15	30
Sinus expansion	08	16

Table 6: Types of Fungal rhino sinusitis

Types of Fungal rhino sinusitis	Number (n=22)	Percentage %
Fungal ball	01	4.5%
Allergic fungal rhino sinusitis	15	68.1%
Chronic invasive fungal sinusitis	05	22.72%
Granulomatous invasive fungal sinusitis	01	4.54%

Discussion

Fungi are ubiquitous; they exist in the soil, water and organic debris. Approximately 300 species of fungi were documented of which few were attributed to human disease. Fungal Rhino Sinusitis is a common disease with significant morbidity. Assessment and management of these conditions may require multispecialty involvement including the otorhinolaryngologist, microbiologist, and pathologist, providing appropriate diagnosis and treatment options to prevent permanent sequelae and mortality. The aim of this present evaluation was to examine the etiologic of chronic fungal rhino sinusitis and the associated predisposing factors in patients from south India.

In our study, out of 50 cases studied 22 (44%) were Positive by culture. The results of our study correlating with the other studies in India [8, 9] (Chakrabarthi, Panda, Das. *et al.* (45.7%) Usha K *et al.* (44%) And abroad Janes U Ponikau *et al.* (42.9%).

In our study Aspergillus species 14 (63.63%) were the commonest agents of fungal sinusitis, a finding that is in agreement with other studies from India and abroad. Our results were correlate to previous observation made by Donald [10] and Rupa *et al.* [11] Vennewald *et al.* [12] in patients with the same pathology.

Various authors propose that FRS implicate a spectrum disease including allergic, colonizing, invasive forms. In our study AFS 68.18% is most common form observed. Diagnostic nasal endoscopy revealed multiple polyps in majority of cases. CT findings correlate with the surgical and Histopathological reports similar to findings of Shah *et al*, [14].

Fungal Ball-A. *niger* was the isolate in one case in our study. It is smudging of fungus with mucous without an immunologic response (Morpeth JF *et al*).

Zygomycetes spp are the predominant isolate in Chronic invasive fungal sinusitis (22.5%) is associated with immuno compromised states like diabetes mellitus, HIV infection which was supported by the statement Luong A, Blitzer A, Washburn RG *et al;* Satish *et al*. [15]. In the present study, 10% patients have diabetes mellitus. Poverty line and hence may be malnourished. Although they are not "immunocompromised" in the classical sense their poor nutritional status may render them more susceptible to invasive disease. The second reason may be that diabetes mellitus is known to be extremely common in India [17] and some of the patients with invasive sinusitis may have had undiagnosed diabetes mellitus, predisposing them to this form of disease.

Aspergillus flavus was the most common etiological agent in allergic disease, as has been previously reported from north India, with *A. fumigatus* the next most common species [8, 9] In North America, dematiaceous fungi, such as *Bipolaris* spp and *Curvularia* spp were found to predominate in allergic sinusitis [16]. In India *A flavus* is predominant causative agent and different from western countries where dematiaceous fungi are important agents of AFS. This may be due to different geographical distribution of fungi in different areas in which the patient lives. Ambient mold spores are widely distributed in nature and demonstrate distinct regional trends bases on latitude, climatic differences, humidity and other factors like vegetation. The prevalence of individual species reflecting the prevalence of spores in the regional outdoor air. In India, a large proportion of the population live in rural or semi-rural areas and so their exposure to certain fungi will differ from arguably a more urban population in developed countries. Another contributory factor may be related to the type of housing in the two countries. In India, houses are often open to the environment and have an open-plan style and this may lead to prolonged exposure to fungi that occur in the outside environment. Houses in developed countries are more likely to be closed to the outside environment and hence the fungal population within the home may be different to that found outside and so exposure will be to those fungi colonising indoors, rather than outdoor fungi. There is paucity of data linking allergic rhinitis to indoor mold exposure in India.

Allergic fungal sinusitis is incompletely understood, often misdiagnosed, relatively new disease entity characterized by unique clinical, radiological, histopathological presentation. Understanding of the disease is very important for early diagnosis and efficient management of the disease.

Conclusion: In Fungal Rhino Sinusitis is common in India and should be considered in all patients with Chronic Sinusitis. In our study positivity of FRS is 44% in CRS cases. More in young age group 23-42yrs. Women (50.09%) are more affected than Men (33.33%), Maxillary sinus (80%) was the commonest sinus involved. *Aspergillus species* were the major isolates, *63.63%*. Common category is AFRS 68.18% *Aspergillus flavus 36.36% is* most common etiological fungi. Prompt clinical suspicion, early diagnosis and treatment will go a long way in the management of patients with FRS.

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