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Prevalence of fungal infections in Chronic Rhinosinusitis patients in a tertiary care hospital, South India

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ABSTRACT

Fungal sinusitis (FS) is an important health care problem both in immunocompetent and immunocompromised individuals; but which is less understood. Clinically FS patient presents with Chronic Rhino Sinusitis (CRS) refractory to medical and surgical treatment and they should be properly evaluated with history, CT scan, histopathology, fungal culture and allergic markers. Total of 300 patients with documented CRS based on clinical and radiological findings in the ENT clinic were enrolled as study group. Sinus washings, biopsy samples from sinusoidal mucosa and polyposis were collected in 2 sterile containers, for microbiology and histopathology and blood for various allergic parameters (total IgE concentration, absolute eosinophil count and precipitation test using fungal antigens). Total prevalence of fungal sinusitis was 13% in CRS patients. Commonly affected age group was between 21-40 years (69.3%) and male preponderance was noted in our study (1.6:1). *Aspergillus* species (87.1%) were the predominant isolate in our study. Allergic fungal sinusitis was commonest clinical presentation (61.5%). This study revealed that the prevalence of fungal sinusitis in CRS patients was significantly high. To estimate the real magnitude of the problem, multicentric study as well as compulsory fungal screening for the all the CRS patients should be undertaken.

Introduction

Rhinosinusitis (RS) is the term representing the symptomatic inflammation of the Paranasal sinuses and the nasal cavity mucosa. Fungal sinusitis (FS) is an important health care problem both in immunocompetent and immunocompromised individuals but which is less understood. (1) Fungal spores are ubiquitous everywhere in the environment, upon inhalation they can colonize and cause non invasive and invasive forms depends upon the host immunity and the degree of inoculum. (2)

Aspergillus species are the most common agent in FS in Indian subcontinent whereas dematiaceous fungi are most common in western countries. Four types of fungal sinusitis are described and classified as noninvasive and invasive form based on the histopathological findings. The invasive types are acute necrotizing, chronic invasive and granulomatous fungal rhino-sinusitis; which are life threatening systemic illness especially in immunocompromised group patients. The noninvasive FS is allergic fungal sinusitis (AFS) and presence of sinus fungal ball in immunocompetent individuals. Allergic fungal sinusitis is defined largely by the presence of allergic fungal mucin, which is a thick, tenacious; clay like eosinophillic secretion contains eosinophillic polynuclear cells, Charcot-Leyden crystals and fungal elements under microscope. (3,4) In this HIV and diabetic era, any type of FS may progress to aggressive and dreadful disease, which illustrates the importance of early recognition of increasingly encountered disease. The incidence, prevalence and the causative fungal agents are varying from region to region could be due to the lack of awareness among physician and availability of diagnostic techniques.

Aim of the study

Considering the burden, seldom research on fungal sinusitis and the existing favorable hot, dry climate for fungi in our area, we have undertaken this study to estimate the prevalence of fungal infections in chronic rhino-sinusitis patients and a detailed analysis of various risk factors and to find the various allergic parameters.

Materials and methodology

Materials

This cross sectional study was conducted in our tertiary care hospital. Total of 300 patients with documented chronic rhino-sinusitis based on clinical and radiological findings in the ENT clinic were enrolled as study group. Demographic, clinical history and co morbidities were recorded from all the patients and informed oral consent obtained.

Methods

Sinus washings, biopsy samples from sinusoidal mucosa and polyposis were collected in 2 sterile containers, for microbiology and histopathology.

Microbiology

Biopsy samples processed according to the standard protocol. Sample was mixed with 10% KOH and examined under microscope for the presence of fungal elements and inoculated onto Sabouraud's Dextrose Agar (SDA) with and without chloramphenicol for the growth of fungi and incubated

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at 25°C and 35 ° C for the minimum of 3 weeks. Growth was confirmed macroscopically on noting the rate, colour, and texture, pigmentation of fungal colony both on obverse and reverse and microscopically by Lacto Phenol Cotton Blue (LPCB) mount. To explore the fungal morphology in detail, slide culture was performed. [6]

Histopathology

Hematoxylin and Eosin staining and Gomaris methanamine silver staining was done for allergic mucin and fungal elements to know the stage of the disease. [6]

Immunological evaluation

5ml of blood sample were taken aseptically from all these patients in a sterile vacutainer for serological examinations (Absolute eosinophil count, Total IgE concentration and precipitation test using fungal antigens).

Absolute eosinophil count

According to the standard method, the absolute eosinophil count was estimated (normal range-40-440 cells /mm^3)

Total IgE concentration:

Serum samples were tested for the total IgE concentration using sandwich ELISA BASED COMMERCIAL KIT from EUROIMMUNE. It is a solid phase immunoassay, intended for *in vitro* use only. This kit utilizes polyclonal antibodies against human IgE. All serum samples were tested according to the manufacturer's literature guidelines provided along with the kits. Optical Density (OD) was read at 450 nm and a reference wavelength between 620nm and 650nm in an ELISA reader. A value of 100 IU/ml is considered to be the upper IgE concentration limit of the reference range for non-atopic subjects. [5]

Precipitation test using fungal antigens:

Serum samples from the entire patient in study group were tested for the precipitin by Ouchterlony's gel diffusion techniques using the fungal antigen of *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* (obtained from Madras University, Chennai) and observed for the precipitation bands. [7]

Intradermal skin test:

Intradermal skin test was performed using 0.1 ml on fungal antigen, at the medial aspect of forearm and observed for type I hypersensitivity reaction and the results observed and interpreted. [6]

Results

Distribution of fungal sinusitis among chronic sinusitis patients:

In our study, out of 300 chronic rhino-sinusitis cases 13% had fungal sinusitis (Table-1)

Table-1: Distribution of fungal sinusitis

Total no of cases	Positive for fungal elements	Percentage	Negative for fungal elements (non-fungal sinusitis)	Percentage
300	39	13%	61	87%

Age and sex distribution:

More no of cases has been observed between 21-40 years (69.3%), followed by 31-40 years (30.8%). 61.5% males were affected compared to 38.5% females. (Table-2)

Presence of allergic mucin in fungal sinusitis

Allergic mucin was observed in 24 out of 39 (61.5 %) fungal sinusitis patients. None of the non fungal chronic rhino-sinusitis had allergic mucin. (Table-3)

Table 2: Distribution of age and sex in fungal sinusitis

Age in years (n=39)	Male (%)	Female (%)	Percentage (%)
11-20	3	-	03 (7.7)
21-30	9	6	15 (38.5)
31-40	6	6	12 (30.8)
41-50	3	3	06 (15.3)
51-60	3	-	03 (7.7)
61 <	-	-	
Total	24 (61.5)	15(38.5)	39 (100%)

Table 3: shows the distribution of allergic mucin in fungal sinusitis

N=39	Total no & percentage
Allergic mucin positive	24 (61.5 %)
Allergic mucin negative	15 (38.5 %)

Clinical spectrum and fungal species in fungal sinusitis:

Aspergillus species (87.1%) were the predominant isolate in our study. *Aspergillus fumigatus (69.2%), Aspergillus flavus (17.9%), Alternaria spp (5.1%), Bipolaris spp (2.6%), Cladosporium spp (2.6%) and Fusarium spp (*2.6%) were the species isolated in our study. Allergic fungal sinusitis was commonest clinical presentation (61.5%), followed by fungal ball (15.38%) and acute invasive sinusitis (15.38%). (Table-4)

Table 4: Shows Clinical spectrum and fungal species in fungal sinusitis:

Fungal species	Allergic fungal sinusitis	Fungal ball	Acute invasive sinusitis	Chronic invasive sinusitis	No & percentage
Aspergillus fumigatus	17	4	4	2	27 (69.2%)
Aspergillus flavus	4	1	1	1	7 (17.9%)
Alternaria spp	1	1	-		2 (5.1%)
Bipolaris spp	1	-	-	-	1 (2.6%)
Cladosporium spp	1	-	-	-	1 (2.6%)
Fusarium spp	-	-	1		1 (2.6%)
Total	24 (61.5%)	6 (15.4%)	6(15.4%)	3 (7.7%)	39 (100%)

Distribution of occupation and fungal sinusitis:

In our study, farmers were commonly affected (30.8 %). Garment workers, Chemical company workers, and Painters were affected 23.0 %, 15.4 % and 15.4 % respectively. (Table-5)

Table -5: Distribution of fungal sinusitis according to various occupations

Occupation	No of cases	Percentage
Farmers	12	30.8 %
Garment workers	9	23.0 %
Chemical company workers	6	15.4 %
Painters	6	15.4 %
Carpenter	3	7.7 %
Office worker	3	7.7 %

Distribution of allergic history and fungal sinusitis:

Patient with of atopy, asthma and nasal polyposis were analyzed in relation to fungal (66.7%, 100% and 71.4% respectively) and nonfungal sinusitis (33.3%, 0% and 28.6% respectively).(Table-6)

Table -6: Co-relation of history and FS

	Total no of cases	Fungal sinusitis	Nonfungal sinusitis
Atopy	18	21 (66.7%)	6(33.3%)
Asthma	15	15 (100%)	-
Nasal Polyposis	21	15 (71.4%)	2(28.6%)

Distribution of sinuses in fungal sinusitis

Maxillary sinuses (69.2%) were commonly affected, followed by ethmoid (15.4%). Both maxillary and ethmoid sinuses were affected in 15.4%. Unilateral involvement is more common (77%). (Table-7)

Table-7 Involvement of sinuses in fungal sinusitis

Sinuses	Unilateral	Bilateral	No of cases	Percentage
Maxillary	21	6	27	69.2
Ethmoid	6	0	6	15.4
Maxillary and ethmoid	3	3	6	15.4
Frontal	0	0	0	-
sphenoid	0	0	0	-
Total	30 (77%)	9(23%)	39	100%

Distribution of allergic parameters in fungal sinusitis

In our study various allergic parameters analyzed with fungal (n=13) and non fungal sinusitis (n=87) were: Intradermal skin test for fungal antigen (54% vs 3,5%), Absolute eosinophil count(54% vs17.2%), Total IgE (62 % vs 9.2%). Precipitin test (69% vs 1.1%)

Table-8-allergic parameters and fungal sinusitis

Allergic tests	Total no of positive cases	Fungal sinusitis (n=39)					Non fungal sinusitis (n=87)
		Allergic fungal sinusitis (n=24)	Fungal ball (n=6)	Acute invasive sinusitis (n=6)	Chronic invasive sinusitis (n=3)	Total no of fungal sinusitis (n=39)	
Intradermal skin test	30	21 (87.5%)	-			21(54%)	8 (9.2%)
Absolute eosinophil count	66	15 (62.5%)	3(50%)	3(50%)	-	21(54%)	45 (17.2%)
Total IgE	51	15 (62.5%)	6(100%)	3(50%)	-	24(62%)	27 (10.3%)
Precipitin test	30	21 (87.5%)	3(50%)	-	1(100%)	27(69%)	3 (1.1%)

Discussion

Fungal sinusitis is an important health problem which may lead to serious complications. Allergic fungal sinusitis was 1st reported by Katzenstein and her colleagues in 1983. [8] The incidence and prevalence is increasing in the last 3 decades, could be due to the awareness among physicians. Higher incidence was reported in Southwestern states of the USA, Sudan, northern India and Saudi Arabia. North India has been identified as an endemic zone of paranasal sinus mycoses. **[9]**

According to the various reports, the prevalence varies from 5% [10] to 96%. [11] In our study, the prevalence fungal sinusitis was 13%. Various prevalence rates were reported are 6.7 %[12], 7.4 %[1], 28 %. [13] The wide variation in the prevalence could be due to the awareness, availability of diagnostic techniques and climate in that region.

Maximum no of cases has been observed between 21-40 years (69.3%) which is similar to the various studies. [10, 14, 15, 16] With many studies, peak prevalence has been noted in young, male, occurring between 2nd and 4th decade. 61.5% male were affected compared to 38.5% female. Male preponderance was noted in our study (1.6:1). It is concordant with the various studies. [9, 14, 17] In contrary to this, higher preponderance in female has been observed in many studies. [4, 18, 19]

We observed 61.5% allergic fungal sinusitis, 15.38% each of fungal ball and acute invasive form and 7.7% chronic invasive fungal sinusitis. It correlates with many studies. [18,20] Patient with acute invasive form had uncontrolled type 2 diabetes.

Maxillary sinuses (69.2%) were commonly affected, which is followed by ethmoid (15.4%). 15.4% were affected by both maxillary and ethmoid. Unilateral involvement is more common (77%). It is consistent with many studies. [9, 14, 22]

Aspergillus species (87.1%) were the predominant isolates [*Aspergillus fumigatus* (69.2%), *Aspergillus flavus* (17.9%)] in our study. The other species were*: Alternaria spp* (5.1%), *Bipolaris spp* (2.6%), *Cladosporium spp* (2.6%) *and Fusarium spp (*2.6%). *Aspergillus spp* was the most common isolate observed in various studies in India, whereas dematiaceous fungi are the commonest causative agent in western countries. According to the various studies, *Aspergillus spp*. was the commonest agent. [18.20, 22.23] Fungi isolated from various studies were: *Penicillium spp, Cladosporium spp*,[24]. *Candida spp*, [25] *Fusarium, Rhizopus, Bipolaris* [4] *Alternaria* **[13]** and more than 40 fungi were isolated in a study conducted by Mayo Clinic. [11]

Allergic fungal sinusitis was commonest clinical presentation (61.5%) which is followed by fungal ball (15.38%) and acute invasive sinusitis (15.38%). It is consistent with many studies. [18, 9, 23]

Farmers were commonly affected (30.8 %) which is followed by Garment workers (23.0 %,) Chemical company workers (15.4 %), and Painters (15.4 %). Young male farmers were the commonest group affected in the study of Chakrabarti et al, 1992. [9] Working environment, ubiquitous saprophytic spores, exposure and very importantly host immune status could favor the seeding and pathogenesis of these fungi. Climate appears to be an important factor as higher incidence of FS has been reported from areas, which have a warm and dry climate. Dusty, arid conditions predispose to rhinitis and recurrent sinusitis facilitates the growth of fungi. [21]

Among the fungal sinusitis patients, 80% had atopy, 100% had asthma and 71% had nasal polyposis in our study. Similar observations were made by many studies. **[4, 14, 22, 23)** It has been suggested that the fungal elements trapped in the mucus in sinus release antigenic material that stimulates IgE, IgG, and IgA production, and with initial insult cause accumulation of secretions and predisposing to fungal hypersensitivity. Hyper reactivity to fungal organisms could be one of the mechanisms which trigger and up regulate inflammation in the mucosa of nose and sinuses,

Immunological factor evaluation in our study showed significant difference in the various allergic parameters among patients with and without fungal sinusitis [Intradermal skin test for fungal antigen (54% vs 3,5%), Absolute eosinophil count (54% vs17.2%), Total IgE (62 % vs 9.2%). Precipitin test (69% vs 1.1%)]. Many of the study group, the total IgE was more than 3000 IU/ml. None of the patient showed type IV hypersensitivity in our study. According to the report of Chakrabarti A et al, besides mechanical and environmental factors speculated for the progression of the disease, presence of allergy was also found to be significant factor in all the varieties of illness and he reported a significant rise in IgE , positive intradermal skin test for fungal antigen and precipitin test. Various reports also suggested the same. [11, 14]

Limitation of the study

Seldom research works are available about prevalence of fungal sinusitis in southern part of India; as well as in our area. So for comparison and for reference enough reports were not available. Due to financial constraints, we limited our study group; hence to correlate the data obtained by our study to community, a large scale study has to be conducted.

Conclusion:

This present study highlights the prevalence and other associated risk factors of fungal sinusitis in our area. The diagnosis of fungal sinusitis requires high index of suspicion. Clinically FS should be suspected if the patient presents with chronic rhino-sinusitis refractory to medical and surgical treatment and they should be properly evaluated with history, CT scan, allergic markers, histopathology and fungal culture. Maximum no of cases was seen between 2^{nd} and 4^{th} decade. Young immunocompetent male were commonly affected in this study. Allergic fungal sinusitis was the predominant presentation compared to the invasive form. *Aspergillus spp* was the most common isolate in our study. Early diagnosis is very important to arrest the further progression and dreadful complication especially in this immunocompromised and HIV era.

Summary of research:

This prevalence study was helpful; as it provided information about prevalence and variable risk factors of fungal sinusitis. Effective preventive measures should be undertaken to reduce the disease burden and to prevent mortality and morbidity.

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