Clinico-Mycological Profile of Allergic Fungal Rhinosinusitis (AFRS) In a Tertiary Care Hospital.

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ABSTRACT

Background: Fungus has been associated with the history of human civilization from time immemorial. Allergic fungal rhinosinusitis (AFRS) is the most advanced form of chronic rhinosinusitis. Allergic fungal rhinosinusitis is generally recognized as a disease distinct from other fungal forms of sinusitis. The incidence of AFRS has increased dramatically in recent years. FRS has always remained a diagnostic and therapeutic challenge. Therefore this study was undertaken to correlate mycological and clinical aspects AFRS in our region. Methods: Over one and a half year period 110 cases of FRS were studied. Characterization of cases was done on the basis of clinical presentation, direct microscopy and culture. The samples were subjected to direct microscopy by KOH preparation and were inoculated on Sabouraud's dextrose agar (SDA). Identification of fungal isolates was done as per standard procedures. Results: Out of 110 cases, 57 (52%) cases were of AFRS. Among these males (63.1%) were predominantly involved as compared to females (36.9%). Majority of cases were seen in age group of 21-30 years. AFRS was more in the female from urban set-up. The most common clinical presentation was nasal discharge. 45.6% were positive for fungus by either direct microscopy or fungal culture or both. Aspergillus flavus was the commonest isolate (85%). Conclusion: The incidence of AFRS in our region was 52% and Aspergillus flavus was the commonest fungi.

Keywords: Allergic fungal rhinosinusitis (AFRS), Aspergillus flavus, inflammatory polyp, KOH preparation, Sabouraud's dextrose agar (SDA), Sinonasal polyps.

INTRODUCTION

In the BC era, Egyptians utilized of fungus for making bread and wine. Eversince that time, fungus has been useful to man in different ways. From bread to alcohol, we need help of fungus. But along with it, the harmful effect it causes in our daily life cannot be ignored. Many of the fungal diseases are deadly. In the field of medicine also, fungal diseases were dreaded for a long time. The fungal infections in paranasal sinuses are becoming common day-byday. Fungal rhinosinusitis (FRS) is one of the important health care problems and its incidence and prevalence are increasing from past few decades that significantly impacts the quality of life.[1] Rhinosinusitis is defined as the inflammation of nasal and paranasal sinus mucosa which associated with mucosal alterations ranging from inflammatory thickening to gross nasal polyp formation.[2]

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Rhinosinusitis is a common disease affecting 135 per 1,000 population. It has been estimated to affect

approximately 31 million patients (4% of adult population) in the United States each year. [3]

Fungal infection of the paranasal sinuses is a well-documented disease in the immunocompromised patient, but recently many reports have indicated an increased prevalence in otherwise healthy individuals. Therefore, it results in great socioeconomic effects, including both direct and indirect costs to the society.^[1]

Plaignaud first reported fungal sinusitis in 1791 AD. Since then it has been brought into being the foremost challenges for clinicians, clinical microbiologists and basic scientists. [4] In 1965, Hora recognized two categories of fungal sinusitis: non-invasive behaving clinically like chronic bacterial sinusitis, and the other invasive, in which the infection results in a mass that behaves like malignant neoplasm, eroding bone and spreading into adjacent tissue. [5] In 1980 acute invasive FRS was also attributed to *Aspergillus* spp. [6]

The exact aetiology of FRS is not known. Various agents including bacteria, viruses and fungi have been introduced as aetiological origins of the disease. The most accepted one is allergic or hypersensitivity response to the presence of extramucosal fungi in the sinus cavity. It is more common in atopic individuals that occurs most common in areas with high temperature and high

humidity. The prevalence of the disease and the dominant fungal pathogen appear to vary in different geographic regions and probably are related to individual host conditions.^[8]

FRS is more common in the older age group, possibly due to the risk factors like diabetes and cancer chemotherapy, which are common in that age group. Some studies from Sudan and north India, allergic fungal rhinosinusitis (AFRS) has been documented commonly in young adult males from rural areas than others. On basis of these reports it was postulated that young adult males who commonly go to the field in a hot, dry climate sustain frequent mucosal injuries of paranasal sinuses and acquire the agent from the field. [9]

The incidence of paranasal sinus mycoses (fungal sinusitis) is significantly higher in Sudan, Saudi Arabia and south western states of USA and north India, which have hot and dry climate. North India has been identified as an endemic zone of paranasal sinus mycoses.^[10]

AFRS is not only reported in immunocompromised patients but also in immunocompetent individuals. Fungal infection of the sinuses can occur when fungal organisms are inhaled and deposited in the nasal passages and paranasal sinuses, causing inflammation. The impact of FRS is not withstanding, the disease is often neglected and misdiagnosed especially in developing countries like India, where FRS is one among the neglected diseases. The most important aetiological agents of fungal sinusitis are Aspergillus, Alternaria, Bipolaris and Curvularia species. [11]

Allergic fungal rhinosinusitis (AFRS) is characterized by two or more symptoms like blockage/congestion, discharge, anterior/posterior nasal drip, facial pain/pressure, reduction or loss of smell. There can be either endoscopic signs like polyps, mucopurulent discharge from middle meatus, oedema/mucosal obstruction primarily in middle meatus, or CT (computed tomography) changes like mucosal changes within ostiomeatal complex and/or sinuses.^[12]

Parameters Used for Classification

According to Pinheiro *et al*, classification of rhinosinusitis should be done along five axes:

- i. Clinical presentation (duration: acute, subacute, and chronic)
- ii. Anatomical site of involvement (ethmoid, maxillary, frontal, and sphenoid)
- iii. Responsible microorganism (viral, bacterial, and fungal)
- iv. Presence of extra sinus involvement (complicated and uncomplicated)
- v. Modifying or aggravating factors (e.g., atopy, immunosuppression, osteomeatal obstruction, etc.)

Classification of fungal rhinosinusitis (FRS)

FRS is categorized into two groups: Invasive and Non-invasive fungal rhinosinusitis. Invasive disease includes: 1) acute invasive (fulminant) FRS; 2)

granulomatous invasive FRS and; 3) chronic invasive FRS. The non-invasive disease includes: 1) saprophytic fungal infestation; 2) fungal ball and; 3) fungus related eosinophilic FRS that includes AFRS.^[13]

Noninvasive FRS

Eosinophil related FRS: Allergic fungal rhinosinusitis (AFRS): After the early observations of Safirstein, Millar, and Katzenstein, Bent and Kuhn proposed five diagnostic criteria AFRS: type I hypersensitivity, nasal polyposis, characteristic findings on CT scan, presence of fungi on direct microscopy or culture, and allergic mucin containing fungal elements without tissue invasion.^[14]

FRS presents with different types of clinical features. According to a study done in north India it was found that rhinorrhoea with nasal polyposis (45.8%) and proptosis (46.4%) were the most common presentations, followed by headache (11.3%), cheek swelling (9.5%), diminished vision (8.9%), blindness (5.3%) and seizures, vomiting and altered sensorium (5.3%).^[10] One another study revealed that AFRS is a disease of younger age, mainly occurring in 2nd & 3rd decade of life, with male to female ratio 1:1.3. Allergic rhinitis (91%) and nasal polyposis (91%) were important associated factors. Nasal obstruction (96%), nasal discharge (91%), post-nasal discharge (87%) and unilateral multi sinus extension were important clinical features.^[15]

In a study conducted in north India on 178 patients diagnosed with paranasal sinus mycoses, A.flavus was the commonest isolate (79.7%) followed by A. fumigatus (11.1%). Rhizopus arrhizus was detected in patients with the invasive type only. Alternaria spp. and Candida albicans were rare isolates. The maxillary (45.8%) and ethmoid sinuses (39.3%) were the most commonly involved paranasal sinuses. The frontal and sphenoid sinuses were involved in 16.7% and 11.3% patients. [16]

In another study, 28 consecutive cases of allergic nasal polyposis were studied. Out of these 11 patients had allergic fungal sinusitis. The ethmoid sinuses were most commonly involved, followed by the maxillary, sphenoid and frontal sinuses. Fungal culture revealed A.flavus in 9 patients, A.fumigatus and A.niger in one patient each.^[17]

In another study the incidence of AFRS in 210 consecutive patients with CFRS with or without polyposis were evaluated of whom, 101 were treated surgically. Fungal cultures of nasal secretions were positive in 202 (96%) patients. Allergic mucin was found in 97 (96%) of 101 consecutive surgical cases of CRS. Allergic fungal sinusitis was diagnosed in 94 (93%) of 101 consecutive surgical cases with CFRS.^[3]

Laboratory Diagnosis

a. Direct examination:

The fungal elements are directly seen in the KOH (potassium hydroxide) mount preparation. For the

clinical specimens, a KOH mount (10% potassium hydroxide) is performed to identify yeast, pseudohyphae, and hyphae; identification of the fungal forms is facilitated with the KOH which clears the epithelial cells and other debris and identifies the fungal elements in the clinical specimens.

The histopathological examination characteristically contains allergic mucin containing sheets of eosinophils, necrotic eosinophils and cellular debris within an amorphous stroma. High-power examination reveals the presumptive diagnosis of AFRS is made by closely examining the specimen for mucosal or bony invasion. Tissue invasion precludes the diagnosis of AFRS and favors the diagnosis of chronic invasive fungal sinusitis.

b. Fungal Culture:

The clinical specimen is inoculated on Sabouraud's dextrose agar with antibiotics and without cyclohexamide at 25°C and 37°C. The cultures should be examined daily during first week and twice a week for further four weeks before being considered as sterile.

The contamination of culture media during incubation with different fungi is not uncommon, therefore, histopathological examination of tissue specimen from infected host is highly recommended as back up for culture isolation and to correlate its clinical significance.

c. Immunology:

The immunological tests have been used as important tools in diagnosis of fungal sinusitis. The use of serology may be an answer to diagnosis and several serodiagnostic tests have been used as an alternative or to substitute culture isolation.

These include indirect immunofluorescence, immunoelectrophoresis and enzyme-linked immunosorbent assays. The skin test reactivity to various antigen extracts is used for patients with suspected sinusitis. Intradermal skin tests are performed using 0.5 ml of fungal antigen in the patients and controls. Type I hypersensitivity showing erythema and wheal is noted within 1 hour and Type III-Arthus reaction develops in 4 to 10 hours. [18]

d. Radiodiagnosis

The radiological findings in fungal sinusitis include haziness, calcification and bone destruction of the involved sinuses on X-rays. CT scan is more sensitive than conventional X-ray in detecting the classical focal areas of hyper-attenuation and calcification seen in soft-tissue masses of fungal sinusitis.^[19]

MATERIALS AND METHODS

This prospective study was conducted over a period of one and a half year in the Department of Microbiology, Dayanand Medical College & Hospital, Ludhiana. Clinically suspected cases of

fungal rhinosinusitis of any age, either gender, positive by fungal smear and/ or culture were included.

Data collection

The following data was collected: -

- Patient's details including name, age, sex, admission number, history (present, past, personal, family, treatment)
- Presenting features like nasal polyposis, proptosis, headache, cheek swelling, diminished vision, blindness, seizures, vomiting, and altered sensorium.
- Investigation details

Sample collection and transportation

Specimens like nasal mucosa & crusts/ nasal scrapings/ excised nasal polyp/ biopsy were collected and transported in saline in a sterile, screw capped container. The specimens received in the Department of Microbiology were processed as per standard procedures.

Potassium Hydroxide Mounts [20]

a) Slide KOH method:

Nasal mucosa & crusts nasal scrapings were placed on clean glass slide. Samples like excised nasal polyp/ biopsy were first homogenized and then processed further. A drop of 10% KOH was poured on specimen and coverslip was placed over it. The slide was heated gently over flame and examined under microscope after few minutes. If specimen was not properly dissolved, it was kept for some more time in a wet petridish and examined. Overheating was avoided so that crystals of KOH were not formed.

b) Tube KOH method:

Tube KOH method was used for nasal mucosa, excised nasal polyp/ biopsy which dissolve with difficulty.

Presumptive identification was made based on direct microscopic examination of material from clinical samples.

Observations of KOH mount preparation: - Septate or aseptate fungal hyphae, with or without branching and budding yeast like cells were seen.

Culture Media: - Media used were:

- **1.** Sabouraud Dextrose Agar (SDA) With Antibiotics And Without Cycloheximide [20]
- Sabouraud Dextrose Agar (SDA) With Antibiotics And Cycloheximide [20]

<u>Inoculation</u>: - After processing of the clinical specimens inoculation was done on SDA media. Inocula were gently implanted into agar at well-spaced intervals on each tube having a sterile cotton stopper.

Incubation: - Inoculated tubes containing SDA with antibiotics and cycloheximide were kept at two temperatures, 25°C and 37°C. All tubes were examined for growth and incubated for up to 3 weeks before recording no growth. [21]

The gross appearance of the colony served as the first important step in the recognition and

identification of a fungus. The following characteristics were noted:-

- Rate of growth of the colony
- Texture, colour and shape of the upper thallus
- The production of pigment on the underside.

Lactophenol Cotton Blue (LCB) Mount was made from growth on culture media to study morphological features of fungal isolates.

<u>Lactophenol cotton blue mount (Tease mount)</u> <u>preparatio^[22]</u>

Growth on culture tubes was teased out on a glass slide in a drop of LCB stain using two teasing needles. Coverslip was placed over it and examined under microscope. Edges of coverslip were sealed with nail polish to keep it for pretty longer time.

Transparency Tape method of preparing LCB mount [22]

An elongated drop of LCB was placed on a clean microscope slide. A piece of clear vinyl adhesive tape 6-7 cm long was held with forceps and introduced into the culture tube. The centre of the adhesive side of the tape was allowed to touch the colony lightly under its own weight. The tape was transferred to the slide, placing the area with adhered mycelium on to the lactophenol cotton blue.

Statistical Analysis

Data collected on various variables was analyzed statistically. Mean and standard deviation (SD) was computed.

RESULTS

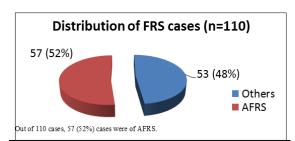


Figure 1

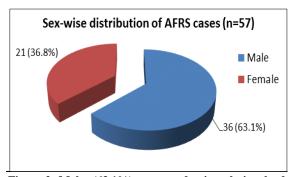


Figure 2: Males (63.1%) were predominantly involved as compared to females (36.9%).

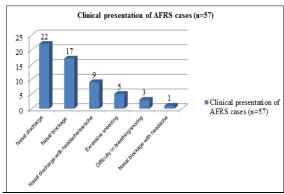


Figure 3: The most common clinical presentation was nasal discharge

Table 1: Age-wise distribution of AFRS cases (n=57).

Age group (in years)	No. of patients (n=57)
0-10	1 (0.9%)
11-20	14 (24.5%)
21-30	15 (26.3%)
31-40	10 (26.3%)
41-50	10 (17.5%)
51-60	4 (7.01%)
61-70	1 (0.9%)
71-80	2 (3.5%)

Majority of cases were seen in age group of 21-30 years.

Table 2: Rural/urban distribution of AFRS cases (n=57).

Social set-up	Male	Female
Rural (35)	31	4
Urban (22)	5	17
Total	36 (63.1%)	21 (36.8%)

In our study 63.1% cases were males among these 31 males were from rural background and 5 were from urban background while 36.8% were females, 4 females were from rural background and 17 females were from urban background.

Table 3

Clinical type Radiological findings	AFRS (n=57)
Opacity in sinuses	11
Sinonasal polyps	30
Mucosal hypertrophy	6
Widening of nasal ostium	1
No abnormality suggestive of	9
sinusitis	

Table 4

KOH results	Growth on SDA		
AFRS (n=57)	Growth on SDA	No growth	
	(n=20)	(n=37)	
Positive (n=21)	15	6	
Negative (n=36)	5	37	
p value < 0.001; Highly significant			

Correlation of direct examination with culture in AFRS cases (n=57).

Aspergillus flavus (85%) was the commonest fungus isolated.

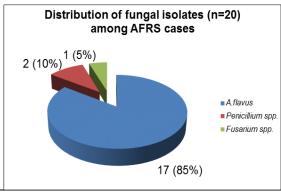


Figure 4: Distribution of fungal Isolates among AFRS Cases.

DISCUSSION

During the recent decades, paranasal sinus mycosis has been recognized more frequently in different parts of the world due to the increased awareness of physicians. A significantly higher incidence is reported in restricted areas that have warm and dry climate [9,23,24] Its incidence in recent years has shown marked increase specially in north Sudan, in southwestern states of USA and in north India.

Rhinosinusitis is a common disorder affecting approximately 20% of the population at some time of their lives. Over one and a half year period, 110 patients that presented with clinical suspicion of fungal sinusitis were studied. The overall prevalence of AFRS among the patients with clinical suspicion was 52% [Figure 1]. In a study done in USA prevalence of fungal rhinosinusitis was 93%.^[3] The reasons for this difference are a matter of speculation but several factors may be involved.

There was predominance of AFRS in male patients [Figure 2]. This result is similar to the study done by Manning SC et al that noted a male predominance with 1.6 male per female. [25] However, study done by Micheal et al. [26] showed female predominance. The results obtained in our study can be attributed to the fact that the males are more commonly exposed to irritating pollutants of traffic, dust, and factories.

In the present study age of patients ranged from 9 years to 80 years. The most affected age group was 21-30 years with 26.3% cases while the least affected age group was of 0-10 years with 0.9% cases [Table 1]. Our finding is nearer to the observation of Michael et al in which the age group 11-79 years was found to be more commonly affected. However, in other studies the affected median age was 30 years. This is possibly due to risk factors like diabetes, chemotherapy which are common in older age group.

Males (63.1%) were predominantly involved as compared to females (36.9%) but in urban set-up females were more [Table 2]. This finding is similar to the study done in Nepal where urban cases were reported more as compared to rural. Reason could be the fact that the population residing in the urban area

is more commonly exposed to the irritant pollutants of traffic, dust, factories residuals in compare to the population in the rural region; these irritants causes rhinitis and lead to the fungal sinusitis.

Most common presenting complaint was nasal discharge (38.5%) followed by nasal blockage (29.8%), nasal discharge with headache/earache (15.7%). Less commonly found features were (8.7%), excessive sneezing difficulty breathing/snoring (5.2%) [Figure 3]. In a similar study done in Nepal nasal discharge was the chief presenting symptom in 78.5%, followed by headache in 50% while, 42.9% complained of nasal blockage, either bilaterally or unilaterally.^[28] In one of the study done at PGI Chandigarh rhinorrhoea with nasal polyposis (45.8%) and proptosis (46.4%) were the most common presentations, followed by headache (11.3%),cheek swelling diminished vision (8.9%), blindness (5.3%) and seizures, vomiting and altered sensorium (5.3%).[16] Although the AFRS cases among the females residing in urban areas were more as compared to females from rural set up. Yet the difference in the incidence was statistically not significant (p>0.05) [Table 2]. This could be due to the fact that the population residing in the urban area is more commonly exposed to the irritant pollutants of traffic, dust, factories residuals in comparison to the population in the rural region; these irritants cause rhinitis and lead to sinusitis. Other reason for the predominance of AFRS among females in urban setup could be due to the frequent use of various types make-up products and fragrance products like body sprays and deodorants. All these products can trigger allergies, headache and various types of chronic allergic sinus conditions.

Among the males with urban background invasive (acute, chronic and granulomatous) form of disease was most common as compared to rural set-up. It can be explained by higher incidence of obesity and other lifestyle associated diseases like diabetes mellitus due to sedentary working conditions prevail more in cities. Acute invasive FRS was also seen more in older age group. This was similar to a study done in north India. [29] In our study acute invasive FRS is more common in the older age group which is possibly due to the risk factors like diabetes and cancer chemotherapy, that are common in this age group. [30]

Overall KOH positivity was 36.8% and fungal culture positivity was 26.3%. Cases with positive direct KOH smear examination yielded a negative culture which may be due to the inadequate specimen as well as the faulty technique of SDA slant inoculation or antifungal therapy of the patient. The common fungal isolate in this group was Aspergillus flavus. This could be due the fact that dust and frequent sand storms during the contain large numbers of Aspergillus conidia that can easily settle on the injured mucosa of the sinuses.^[10] This

finding was similar to the study done by Chhabra et al.^[17] Another prospective study of 176 cases of FRS done in north India showed that A. flavus was the causative agent in 80% of the patients.^[10]

Despite recognition of fungal rhinosinusitis as a serious disease entity for more than two centuries, our knowledge about the epidemiology and medical mycology of the disease remains incomplete and subject to newer findings and research. AFRS can range from the benign localized fungal colonization to the extremely aggressive acute invasive FRS

CONCLUSION

In conclusion, this study highlights the importance of paranasal sinus mycosis in North India. As fungal diseases are not notifiable infections like viral, bacterial or parasitic disease hence these are not given much attention and usually diagnosis is established very late. Therefore early diagnosis and recognition of fungal sinusitis is very important, not only because it is curable in the early stages, but also to prevent progression of the disease in to the more serious and destructive invasive forms. These days since the awareness among people is increasing and people are becoming more concerned about the health related issues, there is better recognition of this disease entity. The mycological assessment are essential to confirm the diagnosis. Therefore our suggestion to clinicians is that all the rhinosinusitis patients should be screened for fungal aetiology.

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