

A Study of Clinico Mycological Profile of Fungal Rhinosinusitis in a Hospital of Haryana

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Abstract

Background: To correlate clinical and culture results with histopathological findings of fungal rhinosinusitis in a hospital for accurate clinical classification of the disease. **Materials and Methods:** One-hundred suspected patients were included in the study. Data was collected in a brief predetermined format. Samples like nasal lavages, sinus secretions, and tissue specimens were processed and examined by microbiology culture. Slide culture was done to observe the microscopic morphology. Histopathological examination was done by H and E stain and PAS stain for classification. **Results:** Out of 100 cases of rhinosinusitis, 42 cases were culture-positive for fungal rhinosinusitis. On the basis of histopathological findings, 28 cases (66.66%) were found to be of non-invasive fungal rhinosinusitis. *Aspergillus flavus* was the most common fungal isolate. **Conclusion:** Mycological profile of rhinosinusitis in Haryana was thus evaluated. Histopathological and microbiological findings reported 42 cases of fungal rhinosinusitis among 100 suspected cases of rhinosinusitis.

Keywords: MRI, Placenta Accreta, Prenatal Diagnosis, Sensitivity and Specificity.

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Introduction

Colonisation of fungus in nose and paranasal sinuses is a common finding in diseased and healthy individuals. Traditionally, fungal infections of paranasal sinuses have been considered uncommon and were thought to occur only in immunocompromised individuals. However, its incidence in recent years has shown a marked increase in immunocompetent population, especially in South-eastern states of USA and in North India[1]. It significantly impacts quality of life in comparison to chronic debilitating diseases such as diabetes and congestive heart failure. Sinusitis or more accurately rhinosinusitis is a common disorder affecting 20% of the population[2]. Since the past two decades, fungi are increasingly recognized as a significant cause of morbidity and mortality among the patients because of the wider use of broad-spectrum antibiotics, immunosuppressive therapy, cancer chemotherapy, increased incidence of immunodeficiency diseases and increased use of intensive care interventions. Rhinosinusitis occurs in both acute and chronic forms and represents a potential heterogeneity of pathophysiologies and prognosis. Chronic rhinosinusitis accounts for more than 90% of all cases of rhinosinusitis, has a slow protracted course, and has different aetiologies, fungal infections being a major cause[3]. Fungal rhinosinusitis is a common disorder in India. The commonest category appears to be allergic fungal rhinosinusitis. The most common etiological agent being *Aspergillus flavus*.

The exact pathogenesis remains unclear but various etiologies have been implicated such as anatomical variants, microbial infection and/or colonization, fungal stimulation, atopic response, acetyl salicylic acid intolerance and a combination of the above. These factors may be possible initial triggers that up-regulate inflammation

of the lateral wall of the nose resulting in the development of nasal polyposis[4,5]. It is suggested that there may be a unique immune response to fungal antigen in patients with CRS that induces production of cytokines and drives intense heterogeneous eosinophilic inflammation which is absent in healthy controls. The fungal spore germinates in the mucin and continues to provide an antigenic stimulus which ultimately results in polyps and hyperplastic mucosa formation[6-8]. It is suggested that the pathophysiology of disease is probably a mucosal hypersensitivity directed against fungal antigens deposited on sinus mucosa rather than true infections[9,10]. Interestingly IL-17 has recently been implicated in regulating the atopic inflammation in NP by attracting the eosinophils and subsequent tissue reaction[11].

Diagnosis of fungal rhinosinusitis is based on high index of clinical suspicion, because clinical history and physical examination of the patient are rarely conclusive. Clinical presentation can provide a clue to the subcategories of fungal rhinosinusitis; however, the diagnosis depends upon direct microscopic examination, culture and histopathology of the tissue or the cheesy material obtained from the sinuses. Histopathology is important to distinguish the invasive from the non-invasive type and classify the disease. Direct microscopy and culture helps in establishing the aetiology[1]. Hence, a detailed examination along with correlation with culture results is necessary. Early diagnosis and accurate classification of fungal rhinosinusitis may help in deciding the treatment protocol and preventing multiple surgical procedures and lead to effective treatment. Clarification of classification of fungal rhinosinusitis and the criteria for their diagnosis should facilitate clinical trials necessary to establish appropriate treatment.

The aim of this study was to determine accurate classification of fungal rhinosinusitis and establish the aetiology so as to estimate the disease burden in the area and help in determining further treatment protocol.

Materials and Methods

One-hundred suspected patients were included in the study undertaken at Adesh medical college and hospital, Mohri, Haryana, India in the department of Microbiology. Data was included in a

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predesigned format. It included patient's identification number, name, age, sex, patient's history, clinical presentation, radiological findings, microbiological results and histopathological diagnosis. Clinical assessment was done.

Samples collected included nasal lavages, nasal secretions and tissue specimens. The tissue specimens were collected from the sinuses by endoscopic sinus surgery. A portion of surgically excised specimen was received in sterile container containing normal saline to mycology laboratory, and another part of the specimen was received in a sterile container containing 10% formalin in the histopathology laboratory for final histopathological diagnosis.

The tissue specimens received in the mycology laboratory were minced into small pieces (0.5-1 mm in diameter) using sterile scalpel, pestle and mortar. The specimens were examined direct microscopy and culture. Direct 20% KOH mount preparation was made of the specimen and examined. Culture was done on Sabouraud's dextrose agar with Chloramphenicol and incubated at 25 °C and 37 °C, respectively. Further identification of fungal isolates was done. Histopathological examination of the specimen was done by Haematoxylin and Eosin stain and Periodic Acid Schiff stain.

Results

Out of 100 cases of rhinosinusitis, 42 cases were culture-positive for fungal rhinosinusitis. Maximum number of cases were found to be of the age group 31-40 years (44 %), followed by 41-50 years (29 %) and 21-30 years (21%). Male: Female ratio was approximately 1.30:1. Most of the confirmed cases were found to be in the lower socio-economic status (72 %). Twenty-six (61.90%) confirmed cases of fungal rhinosinusitis were found to be from urban areas as compared to 16 (38.10%) cases coming from the rural areas.

Table 1: Distribution of fungal isolates identified among cases of fungal rhinosinusitis

Species isolated	No. of cases	Percentage (n=42)
<i>Aspergillus flavus</i>	24	57.14
<i>Aspergillus fumigatus</i>	6	14.29
<i>Aspergillus niger</i>	3	7.14
<i>Rhizopus spp</i>	2	4.76
<i>Mucor spp</i>	3	7.14
<i>Alternaria spp</i>	2	4.76
<i>Syncephalastrum</i>	1	2.38
<i>Curvularia spp</i>	1	2.38
Total	42	100

Table 2: Histopathological classification of cases of fungal rhinosinusitis

Histopathological diagnosis	No. of cases	Percentage (n=42)
Allergic fungal rhinosinusitis	24	57.14
Fungal ball	4	9.52
Acute fulminant invasive fungal rhinosinusitis	4	9.52
Granulomatous invasive fungal rhinosinusitis	4	9.52
Chronic invasive fungal rhinosinusitis	6	14.29
Total	42	100

Table 3: Distribution of fungal isolates among various histological types of fungal rhinosinusitis

Etiological agents	AFRS	FB	AFIFS	GIFS	CIFS	Total	Percentage (n=42)
<i>Aspergillus flavus</i>	16	2	0	4	2	24	57.14
<i>Aspergillus fumigatus</i>	0	2	0	0	4	6	14.29
<i>Aspergillus niger</i>	2	0	1	0	0	3	7.14
<i>Mucor spp</i>	0	0	3	0	0	3	7.14
<i>Rhizopus spp</i>	2	0	0	0	0	2	4.76
<i>Curvularia spp</i>	1	0	0	0	0	1	2.38
<i>Alternaria spp</i>	2	0	0	0	0	2	4.76
<i>Syncephalastrum</i>	1	0	0	0	0	1	2.38
Total	24	4	4	4	6	42	100

Discussion

Fungal rhinosinusitis, once considered a rare disorder, is now being recognized and reported with increasing frequency worldwide. A study conducted by Das *et al.*, 2007, at Chandigarh reported fungal rhinosinusitis with incidence of 42.7% of all the 665 cases of chronic rhinosinusitis[3]. The current study has, on the basis of clinical, histopathological, microbiological and radiological findings, reported

The most common presentations in cases of fungal rhinosinusitis were nasal obstruction (100%) and nasal discharge (100%) followed by headache (78 %) and facial pain/swelling (61%).

Aspergillus spp. (78.57%) was the most common isolated species among all cases of fungal rhinosinusitis with *Aspergillus flavus* (57.14%) being the most common fungal isolate followed by *Aspergillus fumigatus* (14.29%).

On the basis of histopathological findings, 28 cases (66.66%) were found to be of non-invasive fungal rhinosinusitis. These included 24 cases (57.14%) of allergic fungal rhinosinusitis, and 4 cases (9.52%) of fungal ball. Fourteen cases (33.33%) were of invasive fungal rhinosinusitis, out of which 4 cases (9.52%) were of acute fulminant invasive fungal rhinosinusitis, 4 cases (9.52%) of granulomatous invasive fungal rhinosinusitis and 6 cases (14.29%) of chronic invasive fungal rhinosinusitis. Of all the cases of fungal rhinosinusitis, allergic fungal rhinosinusitis was the most common histopathological diagnosis.

Aspergillus flavus was the most common fungus, isolated in 16 cases (66.66%) of allergic fungal rhinosinusitis, of all the 24 cases of allergic fungal rhinosinusitis. In fungal ball, *Aspergillus flavus* was isolated in 2 cases and *Aspergillus fumigatus* from the other 2 cases. *Mucor spp.* was the fungal species isolated in all 3 cases of acute fulminant invasive fungal rhinosinusitis. *Aspergillus flavus* was isolated in all 4 cases of granulomatous invasive fungal rhinosinusitis. In chronic invasive fungal rhinosinusitis, *Aspergillus fumigatus* was the most common aetiological agent identified, being isolated in 4 cases (66.66%) of the 6 cases of chronic invasive fungal rhinosinusitis.

42 cases of fungal rhinosinusitis among 100 suspected cases of chronic rhinosinusitis.

There is emerging evidence that fungi play an important role in exacerbation and perpetuation of mucosal inflammation in CRS, and only in more recent times has the categorization of FRS been more fully defined.

In some studies from Sudan and North India, fungal rhinosinusitis has been documented commonly in young adult males from rural areas than others[12]. A study conducted by Das *et al.*, in Chandigarh, reported ages of the patients with fungal rhinosinusitis ranged from 2 to 81 years (mean-31 years). There was predominance of fungal rhinosinusitis in male patients with a Male:Female ratio of 1.8:1.³ A study by Michael *et al.*, 2007, conducted at Vellore reported fungal rhinosinusitis in patients with mean age of 45.7 years ranging from 11 to 79 years with male to female ratio 0.8:1[13]. A study by Panda NK *et al.*, reported that there were more patients from rural areas than from urban areas in the same study[14]. In our study, the age of patients in 3rd decade of life followed by 4th decade (28%) with mean age being 37 years and male to female ratio being 1.30:1. Sixty-three percent of patients belonged to urban area and 37% of patients belonged to rural areas with 72% of patients coming from low socio-economic background.

Panda *et al.*, in their study, categorized 178 patients diagnosed as having paranasal sinus mycoses into three disease groups- Allergic (8), non-invasive (92) and invasive (78) on the basis of histopathological and mycological investigations[14]. In a prospective study of 176 cases of fungal rhinosinusitis, Chakrabarti *et al.* classified the patients into allergic (12 patients), non-invasive without bony destruction (81 patients), non-invasive destructive (16), chronic invasive (55) and fulminant (12)[1]. We, in our study, on the basis of clinical, radiological, histopathological and mycological findings, classified 42 patients of fungal rhinosinusitis and following observations were made: 28 cases (66.66%) were of non-invasive fungal rhinosinusitis including 24 cases of allergic fungal rhinosinusitis (57.14%) and 4 cases of fungal ball (9.52%), whereas invasive fungal rhinosinusitis constituted of 14 cases (33.33%) including 4 cases of acute fulminant invasive fungal rhinosinusitis (9.52%), 4 cases of granulomatous invasive fungal rhinosinusitis (9.52%) and 6 cases of chronic invasive fungal rhinosinusitis (14.29%). Allergic fungal rhinosinusitis constituted of 24 cases (57.14%) of all the cases of fungal rhinosinusitis and was the most common histopathological diagnosis[1].

In the initial studies, *Aspergillus fumigatus* was considered the primary aetiological agent of AFRS cases, but later, pigment-producing dematiaceous fungi- *Bipolaris spicifera*, *Exserohilum roibatum*, *Curvularia lunata* and *Alternaria spp.* were found as predominant aetiological agents in Western literature[15]. Data published from various studies from India differ from those reported in the western literature and state *Aspergillus flavus* as the predominant agent in cases of allergic fungal rhinosinusitis in the Indian sub-continent. A study by Saravanan *et al.*, in Chandigarh, reported that among the 32 patients in the allergic fungal rhinosinusitis group, the most common culture isolate was *Aspergillus flavus* (81%), followed by *Aspergillus fumigatus* (9%). *Bipolaris* species was isolated in only 2 patients (6%)[16]. In our study of all the 24 cases of allergic fungal rhinosinusitis, culture was positive in all the cases, whereas smear was negative in 4 cases. The results were correlated with the histopathological findings, and it was found that *Aspergillus spp.* was the most common fungus isolated in 18 cases (75%) of allergic fungal rhinosinusitis with *Aspergillus flavus* being the most common species isolated (66.66% of cases of allergic fungal rhinosinusitis). Phaeoid fungi were isolated in 4 cases (16.66%). In our study, histopathologically allergic fungal rhinosinusitis was diagnosed in 24 cases of all the 42 cases of fungal rhinosinusitis by H and E stain and PAS stain. Inflammatory infiltrates and allergic mucin were found in all 24 cases, whereas non-invasive fungal hyphae and eosinophilic infiltrate was observed in 22 cases[1].

Chakrabarti *et al.*, in a prospective study, reported 12 cases of acute fulminant invasive fungal rhinosinusitis among 176 cases of fungal

rhinosinusitis[1]. Of all the cases of fungal rhinosinusitis diagnosed in our study, 4 cases were diagnosed as acute fulminant invasive fungal rhinosinusitis on the basis of microbiological, histopathological and radiological findings. *Mucor spp.* was isolated in all 3 cases, and histopathologically all 3 cases showed inflammatory infiltrates with fungal hyphae invading into the mucosa and sub-mucosa.

Das *et al.*, in their retrospective study on cases of fungal rhinosinusitis over a period of 5 years in Chandigarh, reported 48 cases of granulomatous invasive fungal rhinosinusitis (16.9%) among 284 cases of fungal rhinosinusitis. In our study, granulomatous invasive fungal rhinosinusitis was reported in 4 cases based on histopathological findings of presence of fungal hyphae invading into the adjacent tissue and granuloma formation in all 4 cases. All 4 cases were caused by *Aspergillus flavus* as in accordance with previous studies[3].

Michael *et al.*, in a study done in South India, reported 21 cases (10%) of chronic invasive fungal rhinosinusitis among 211 cases of fungal rhinosinusitis diagnosed. *Aspergillus flavus* was the aetiological agent in 10 cases and *Aspergillus fumigatus* in 8 cases among all the 21 cases of chronic invasive fungal rhinosinusitis detected. In the current study, 6 cases of CIFS were reported on the basis of histopathological, microbiological, radiological and clinical findings. *Aspergillus fumigatus* was the aetiological agent in 4 cases and *Aspergillus flavus* was isolated in 2 cases[13].

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