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Mycological Profile of Chronic Rhinosinusitis

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A B S T R A C T

Aim of the to assess the purpose of fungal rhinosinusitis at MNR Medical College & Hospital, Sangareddy, and to correlate histopathological findings with culture results for accurate clinical classification of the disease. 217 suspected patients were included in the study. The relevant clinical details of the patients including the co-morbidities in the medical records were examined. Samples like nasal swabs collected during nasal endoscopy under sterile conditions, Sinus washings, Allergic mucin, tissue biopsy from polyps & tissue biopsy taken from sinus mucosa during nasal surgery, were processed and examined by microbiology culture using recommended techniques. Slide culture was done to observe the microscopic morphology. Histopathological examination was done by H and E stain and PAS stain for classification. Out of 217 cases of rhinosinusitis, 37 samples showed fungal isolates in culture. *Aspergillus flavus* was the most common isolate (8.29%). *Aspergillus fumigatus* was the second most common isolate (5.99%). *Candida albicans* was isolated in one sample and candida species, other than albicans was isolated in one more sample. On the basis of the histopathological findings, the fungal isolates were grouped. Among the 37 patients with fungal rhinosinusitis 26 patients belonged to non-invasive type. They were mostly of allergic etiology. The organisms were mostly *Aspergillus fumigatus* and *Aspergillus flavus*. Isolates that cause fungal ball were not isolated. 11 isolates belonged to invasive group. Most of them were *Aspergillus flavus* causing chronic granulomatous fungal sinusitis Mycological profile of rhinosinusitis at MNR Medical College, Sangareddy was thus evaluated. Histopathological and microbiological findings reported 37cases of fungal rhinosinusitis among 217 suspected

Introduction

Chronic rhinosinusitis (CRS), defined as infection of the sinuses lasting for more than 3 months, is one of the most prevalent chronic illnesses in India affecting persons of all age groups. It is generally a mild disease. However, it is important to realize that it afflicts a significant percentage of the population, and causes considerable long term morbidity. Many patients with chronic rhinosinus disease are subjected to multiple courses of antibiotics and surgeries, with little or no improvement in their condition. Despite the tremendous advances in medicine over the last few decades, there have been relatively few advances in the diagnosis and treatment of chronic sinus disease. Long-term results of

medical and surgical therapies have resulted in cure rates that vary between 29 and 80% (Hamaguchi *et al.,* 1986; Melen *et al.,* 1986; Murray and Jackson, 1983). We feel that this lack of progress is largely due to the paucity of knowledge on the microbiology and histopathology of chronic sinus disease available to us, and this was the impetus for our study.

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 etiologies, bacterial and fungal infections being a major cause.

A deeper understanding is thus critical, for Otolaryngorhinologists to move from an empiric decision making process, to a more evidence-based or culture-directed therapy paradigm. We undertook this study to prospectively examine the fungal etiology of chronic rhinosinusitis and for accurate clinical classification of the disease.

Aims and Objectives

The aim of the study was:

To study the spectrum of Fungal etiological agents among patients clinically diagnosed as Chronic rhinosinusitis and attending the ENT Outpatient Department MNR Medical College and Hospital Sangareddy, by:

Subjecting specimens collected from these patients to microscopy and to fungal culture.

Identifying the organism isolated.

To study the predisposing factors associated with the range of etiological agents isolated in culture.

Inclusion criteria

All patients with chronic rhinosinusitis diagnosed on the basis of clinical and radiological findings.

Allergic rhinitis patients with chronic sinusitis.

Patients above 10 years of age, both males and females were included.

Exclusion criteria

The patients with acute sinusitis, malignancy of paranasal sinuses were excluded from study.

Materials and Methods

This study was conducted at MNR Medical College and Hospital, Sangareddy, Medak Dist., for a period of one year from March 2012 to June 2013. Specimen processing was done in the Department of Microbiology, MNR Medical College and Hospital, Sangareddy. A total of 217 specimens were collected from patients suffering from Chronic rhinosinusitis attending the ENT Outpatient department of MNR Hospital Sangareddy from 1-11-2011 to 1-8-2013. The relevant clinical details of the patients including the co-morbidities in the medical records were examined. These patients underwent a rigid nasal endoscopy with swabs and biopsies from the middle meatus to assess fungal etiology. CT scans of the paranasal sinus were performed to look for bone erosion and heterogeneous soft tissue opacity if any. Majority of these patients were not responding to conservative line of management with antibiotics.

Specimens included

Nasal swabs collected during nasal endoscopy under sterile conditions, Sinus washings,

Allergic mucin, collected during nasal resection, Tissue biopsy from polyps and Tissue biopsy taken from sinus mucosa during nasal surgery.

Lab methods for isolation and identifiction of fungi

Macroscopic examination

Samples were inspected for colour, odour and whether they were purulent, blood stained (during surgery), muco purulent, mucoid or clear.

Microscopic examination

A KOH preparation of the test material was made on a slide using 10% KOH. The slide was examined for the presence or absence of hyphae & budding yeast cells. A second smear was made on a slide, stained with Gram's stain and examined for Gram positive budding yeast cells if any.

Culture media used

The fungal samples were inoculated with a loop onto the following media:

2 tubes of Sabouraud's Dextrose with chloramphenicol.

Corn meal agar for Candida species.

Preparation of Sabouraud's dextrose agar: The Himedia Sabouraud's dextrose agar was dissolved in distilled water by gentle heating. pH was adjusted to 5.4. It was sterilized in the autoclave at 121^{o}C for 15 min at 15lbs pressure and 20ml was poured into sterile petridishes.

Preparation of corn meal agar: The Himedia corn meal agar was dissolved in distilled water by gentle heating. It was sterilized in the autoclave at 121^{o}C for 15 min at 15lbs pressure and 20ml was poured into sterile petridishes.

1 tube of SDA with chloramphenicol was incubated at 37^{o}C and the other tube was incubated at room temperature. Both tubes were examined on alternate days for four weeks and colony characters were noted. A portion of the colony was picked up with a straight wire, mounted on a slide with Lactophenol cotton blue solution. It was teased with a pair of teasing needles and the fungus was identified by its morphology. A slide culture was also done for confirmation.

Slide culture preparation

A sterile microscopic slide was placed on bent glass rod at the bottom of petri dish. A piece of one square centimeter block of Cornmeal agar was put on the slide. Fungal strain was inoculated at four sides of agar block. The inoculated block was covered with sterile coverslip and incubated at 25^{o}C in BOD incubator. A little sterile distilled water was added on filter paper to avoid drying of agar. When growth appeared, a drop of LCB was placed on slide and coverslip from block and examined microscopically to identify the fungus (Sanam Jindal, 2013).

A Gram's stain was also done where indicated, and examined for Gram positive budding yeast cells, indicative of Candida species.

Specimens that were positive for Candida were further subjected to Germ tube test. 0.5 ml of serum was taken in a test tube and was inoculated with small portion of colony from Sabouraud's dextrose agar growth and incubated at 37^{o}C in a water bath for 2 hours. One loopful of serum yeast culture was taken on to a microscopic slide and examined for the formation of pseudohyphae.

The colonies were also inoculated on cornmeal agar and incubated at 25°C for 72 hours. Abundant branched pseudohyphae and true hyphae with blastoconidia were noted. Terminal chlamydoconidia were formed with extended incubation.

Fungal isolates were diagnosed and grouped depending on the histopathological reports.

Statistical analysis

Statistical analysis of data was recorded and analysed using SPSS version 11.5 and the study was evaluated using CHI square test.

Results and Discussion

Among the 217 pts, who presented with CRS, there were 0 pts., in the age group 0-10yrs., 27 (12.44%) between 11-20yrs., 56(25.80%) between 21-30 yrs., 54(18.43%) between 31-40yrs, 37 (17.05%) between 41- 50 years, 20 (9.25%) between 51- 60 years and 23 (10.59%) pts, above 60 years. The maximum number of pts., included in our study was in the 21- 30 years age group followed by 31-40yrs age group. The age incidence in the present study is shown in Table.1.

Table.2 shows age incidence and culture positivity. Maximum fungal culture positivity (20%) was seen in the age group 51-60 years.

Among 46 pts., who had nasal allergy, fungal growth was seen in 6 pts., and Among 18 pts., with Diabetes mellitus fungal growth was seen in 5 pts. Among 41 pts with dental caries, 6 had fungal growth 37 smokers 8 positive for fungus and among 6 swimmers were –ve for fungal growth as shown in table 3.

The most common symptom was nasal discharge in 76.03% with 20% fungal culture positivity. In pts with nasal block 21.77 were positive for fungus. Among pts with head ache 18.42% had fungal growth. In patients with foul smelling breath 20.45% had fungal growth as shown in Table 4.

Among 116 pts., with deviated nasal septum, out of 217, positive fungal cultures in 29 cases. Among 5 pts., with polyp, 2 had fungal growth. And among 138 pts with hypertrophied turbinate, 25 were positive for fungus as shown in Table 5.

Out of 37 samples that showed fungal isolates in culture, *Aspergillus flavus* was the most common isolate, seen in 18 (8.29%) out of 217 specimens. *Aspergillus fumigatus* was seen in 13 (5.99%) out of 217 specimens, *Candida albicans* in 1 (0.46%), Non *Candida albicans* in 1(0.46%) specimens, *Mucor* species in 2(0.92%), and *Penicillium* species was seen in 2(0.92%) out of 217 specimens as shown in Table.6.

Among the 37 patients with fungal rhinosinusitis 26 patients belonged to non-invasive type. The organisms were *Aspergillus fumigatus* 12, *Aspergillus flavus* 10, *Candida albicans* 1 and Non *Candida albicans* 1. Isolates that cause fungal ball were nil.11 isolates belonged to invasive group. They were *Aspergillus flavus* 8 isolates, *Aspergillus fumigatus* 1 and *Mucor* species 2 shown in table 7.

Among 217 specimens, nasal swabs collected during endoscopy were 102 with 9 showing fungal growths. Among 87 sinus washings samples, 5 were positive for fungus. Among the 14 tissue biopsies collected from sinus mucosa, 13 had fungal growth. Among the 5 polyps 5 had fungal growth. This is shown in Table.8. Number of fungal positive cultures in, Type II Diabetes mellitus cases is shown in table 9. *Aspergillus fumigatus* was isolated in 1 (5.55%), *Mucor* spp. In 2 (11.11%), *Candida albicans* in 1 case (5.55%) and non-candida in 1 (5.55%) case.

Table.1 Age Incidence

Age in years	No. of pts. tested	% of pts. tested
0-10	-	-
11-20	27	12.44
21-30	56	25.80
31-40	54	18.43
41-50	37	17.05
51-60	20	9.25
Above 60 (61-83)	23	10.59

Mean age: 36.5yrs.

Table.2 Age incidence and culture positivity

	No. of cases tested	%	Fungal culture +ve	%
0-10	-	-	-	-
11-20	27	12.44	5	18.51
21-30	56	25.80	11	19.64
31-40	54	18.43	8	14.81
41-50	37	17.05	5	13.51
51-60	20	9.25	4	20
Above 60	23	10.59	4	17.39

Chi-Square Value=16.681 DF= 5 P-Value=0.005
There is Significant association among the age group.

Table.3 Predisposing factors- culture positivity

PREDISPOSING FACTOR	TOTAL	FUNGAL ISOLATE	%
Nasal allergy	46	6	13.04%
Dental caries	41	6	14.63
Smoking	37	8	20%
DM	18	5	27.77%
Swimming	6	0	0%

Table.4 Symptoms-culture positivity

Clinical presentation (symptom)	Total No.	Fungal culture +ve	%
Nasal discharge	165	33	20
Nasal block	124	27	21.77
Head ache	114	21	18.42
Foul smell	44	9	20.45

Table.5 Clinical finding – culture positivity

NASAL OBSTRUCTION	TOTAL	FUNGAL CULTURE +VE	%
POLYP	5	2	40%
DNS	116	29	25%
TURBINATE HYPERTROPHY	138	25	18.11%

Chi-Square Value=88.62 DF=2 P-Value=0.000
There is Significant Accosiation of Clinical finding

Table.6 Fungal isolates from 217 cases of CRS

FUNGAL ISOLATE	NUMBER	%
Aspergillus flavus	18	8.29%
Aspergillus fumigatus	13	5.99%
Mucor spp	2	0.92%
Penicillium spp	2	0.92%
Candida albicans	1	0.46%
Non Candida albicans	1	0.46%

Table.7 Types of fungal isolates depending on histopathology

HISTOPATHOLOGY	FUNGAL GROWTH	TYPE OF FUNGAL SINUSITIS	NO. OFCULTURE +VE PATIENTS
NON INVASIVE			
1)allergic mucin +ve	*Aspergillus fumigatus*	ALLERGIC FUNGAL SINUSITIS	12
2)degenerated eosinophils +ve	*Aspergillus flavus*		10
3)segmental branching +ve	*Candida albicans*		1
4)hyphae +ve	Non *Candida albicans*		1
	Penicillium spp		2
5)fungal balls	Nil	FUNGAL BALL	----------
INVASIVE			
Acute fulminant	*Mucor* species	ACUTE FULMINANT FUNGAL SINUSITIS	2
Granuloma +	*A. flavus*	CHRONIC INVASIVE GRANULOMATOUS SINUSITIS	8
No granuloma	*A. fumigatus*	CHRONIC INVASIVE NON GRANULOMATOUS FUNGAL SINUSITIS	1

Table.8 Specimens with culture positivity

SPECIMEN	TOTAL	FUNGAL ISOLATES	
Nasal swab	102	9	8.82%
Sinus washings	87	5	5.74%
Tissue biopsy from sinus mucosa	14	13	92.85%
Allergic mucin	6	5	83.33%
Polyp	5	5	100%
Throat swab	3	0	0.00%

Chi-Square Value=168.02 DF=5 P-Value=0.000
There is significance between Specimens and culturePositivity

Table.9 Type II DM with Fungal Culture Positivity

Aspergillus fumigatus	1	5.55%
Mucor spp	2	11.11%
Candida albicans	1	5.55%
Non *Candida albicans*	1	5.55%

Rhinosinusitis is a common medical problem encountered in patients attending the Department of Otorhinolaryngology. Rhinosinusitis can be acute or chronic, classified according to the duration of symptoms. Acute rhinosinusitis lasts upto 12 weeks with complete resolution of symptoms, whereas the chronic form persists beyond 12 weeks. Patients with acute sinusitis present with fever, head ache, common cold and other complaints relating to oral or nasal infections. In the chronic form, fever may be low grade. Patients may present with nasal stuffiness, nasal discharge (of any character from thin to thick and from clear to purulent), postnasal drip, facial fullness, discomfort, headache, chronic unproductive cough, hyposmia, sore throat, fetid breath, malaise and easy fatigability.

The etiology, pathogenesis and management of CRS have been one of the most controversial topics in Otolaryngology. The literature available regarding this is sparse and difficult to interpret. In the present study an attempt was made to study the predisposing factors, to examine the fungal etiology of CRS. 217 patients who were clinically diagnosed as Chronic rhinosinusitis, that attended the Department of ENT, MNR Medical college and Hospital Sangareddy, were studied over a period of 1 year and eight months from 1-11-2011 to 1 -8-2013.

Endoscopic specimens from these patients were subjected to microscopy, fungal culture. Those that showed fungal elements on microscopy were also subjected to histopathological examination. The specimens examined were nasal swabs collected during nasal endoscopy, sinus washings, allergic mucin collected during nasal resection, tissue biopsy from polyps and tissue biopsy taken from sinus mucosa during nasal surgery.

In our study the common age group of CRS was from 20-70 years. High fungal culture positivity was seen in the age group 51-60 years (20%) followed by 21-30years age group (19.64%). It is similar to the study done by (Prateek *et al.,* 2013).

In the present study male population affected were 55.76% among the 217 patients studied and their culture positivity was 19% for fungal infections. The female populations

affected were 44.23% with fungal culture positivity of 14.58%. There is slight male dominance in the incidence of CRS or in the positivity of the cultures in our study, although less significant than the male dominance reported in studies by (Prateek *et al.,* 2013) and (Shilpa K. Gokale *et al.,* 2010).

In the present study the rural population is high i.e. 158 out of 217 with 18.35% of them being fungal culture positive. The urban population is 59 with 13.55% fungal positivity showing predominance of fungal infections in the rural population which is similar to the other studies from Sudan and North India (Chakrabarti *et al.,* 1992).

Among all the predisposing factors studied, like nasal allergy, dental caries, diabetes mellitus, smoking and swimming, the most common predisposing factor was found to be nasal allergy (21.19%) followed by dental caries (18.89%) in our study. The values are similar when compared with the studies done by Shapira (1985), Turner *et al.,* for nasal allergy and studies of Melen and colleagues for dental caries.

The most common pathological finding in CRS patients is nasal obstruction in our study. Among 217 patients studied, 138 patients had turbinate hypertrophy, 116 patients had deviated nasal septum and 5 patients had nasal polyps. In all three conditions fungal culture positivity was 18-25%. Itzahk Brook *et al.,* (1982), Berry 1930 reported nasal obstruction as a cause of CRS in 54.54% of cases. It correlates with our study where nasal obstruction is present in more than 50% of cases. This is the commonest pathological predisposing factor described for CRS.

The common presenting symptom in our study group was nasal discharge with bacterial culture positivity of 73.33% followed by foul smelling breath with bacterial culture positivity (72.72%). This is comparable with most of the other studies. Nasal block, on the other hand, was the commonest symptom in fungal sinusitis.

Among all the 217 patients in our study, aerobic fungal growth was positive in 17.05%. In a study by (Panduranga Kamat *et al.,* 2013) the fungal growth was 7.4%. When compared with these studies, our study showed higher prevalence for fungal growths. This shows the high prevalence of fungal infections in our study area.

Out of 37 samples that showed fungal isolates in culture, in our study, *Aspergillus flavus* was the most common isolate (8.29%). *Aspergillus fumigatus* was the second most common isolate (5.99%) similar to the studies of (Chakrabarti *et al.,* 1992) and (Panda *et al.,* 1998). *Candida albicans* was isolated in one sample and candida species, other than albicans was isolated in one more sample. Even though candidial infection is rare in CRS we presume the infection in our study may be due to the associated Diabetes mellitus in these patients. On the basis of the histopathological findings, the fungal isolates were grouped. Among the 37 patients with fungal rhinosinusitis 26 patients belonged to non-invasive type. They were mostly of allergic etiology. The organisms were mostly *Aspergillus fumigatus* and *Aspergillus flavus*. Isolates that cause fungal ball were not isolated.11 isolates belonged to invasive group. Most of them were *Aspergillus flavus* causing chronic granulomatous fungal sinusitis. Our results correlated well with the reports of Prateek *et al.,* (2013).

Among the various specimens examined, nasal swabs taken during endoscopy were highest in number. Fungal isolates were more in tissue specimens like nasal polyps (100%), followed by tissue biopsy from sinus mucosa (92.85%) and allergic mucin (83.33%).

In our study among 217 patients with CRS 18 patients was Diabetics. Fungal isolates were 27.77% among them. When compared with other etiological factors, diabetes mellitus as a predisposing factor is less common. The infection rate is more in patients with diabetes mellitus.

From our study we conclude that the most common predisposing factors for CRS are nasal allergy, dental caries and nasal obstruction. 37 patients had fungal infections associated with CRS which is also a significant association. The common fungal infections are due to *Aspergillus flavus* and *Aspergillus fumigatus*. Hence, it is suggested that culture directed therapy is the gold standard for the management of CRS.

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