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A Cross-sectional Study on Chronic Fungal Rhinosinusitis in a Tertiary Care Hospital in Central Delhi, India

Prafulla Songara*[1], Gaurav Saxena[1] and Ramesh Agrawal[2]

[1]Department of Microbiology, Government Medical College, Ratlam, Madhya Pradesh, India.
[2]Department of Microbiology, Government Medical College, Khandwa, Madhya Pradesh, India.

Abstract

Chronic Rhinosinusitis is a common disorder, and its prevalence vary from 1-20% globally. The incidence of fungal sinusitis has increased to such extent in recent years that fungal infection is a common diagnosis in patients with Chronic Rhinosinusitis. The objectives of this current research were objectives of estimating the prevalence of Fungal aetiology in chronic sinusitis patients and their drug sensitivity pattern with common antifungal drugs. A total of 61 Cases present with Chronic Rhinosinusitis (CRS), visited in a tertiary care hospital based in Central Delhi, were included in our study. Excision of sinus tissue, including polyps and masses, were collected in the operation theatre during Functional Endoscopic Sinus Surgery (FESS) procedure in a sterile manner. All the tissues brought in sterile normal saline were processed for bacteriological and mycological examination. Tissues, obtained in 10% formalin were processed for histopathological and cytological analysis. A total of 14 (22.9%) cases of Chronic Rhinosinusitis were affected by fungal etiologies. By E test, the MIC range for isolates of *Rhizopus arrhizus* after 24 hr of incubation was 1-2 µg/mL, and the mean was 1.5 µg/Ml. Similarly, the MIC range for isolates of *Aspergillus flavus* after 48 hr of incubation was 0.5-16 µg/mL, and the mean was 4.09µg/mL. By the M38-A broth dilution method, the MIC range for the isolates of *Rhizopus arrhizus* after 24 hr of incubation was 0.5-2 µg/mL, and the mean was 1.25 µg/ml. Similarly, the MIC range for isolates of *Aspergillus flavus* after 48 hr of incubation was 0.5-4 µg/mL, and the mean was 1.95 µg/mL.

Keywords: Rhinosinusitis, New Delhi, Fungus, *Aspergillus flavus, Rhizopus arrhizus*, India

***Correspondence:** drprafulla07@gmail.com

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INTRODUCTION

Rhinosinusitis is a spectrum of inflammatory and infectious diseases that involve one or more of the paired paranasal sinuses and nasal mucosa. Because of the contiguous relationship of the sinuses and nasal mucosa, rhinitis and sinusitis coexist and are concurrent in most individuals[1].

Chronic Rhinosinusitis (CRS) is a multifactorial illness. Air pollution, viruses, bacteria, fungi, genetic factors, immune deficiency, and anatomical abnormalities within the sinus system may play a contributory role in the same patient. It is often difficult to define a precise cause of illness in an individual patient[2]. Gender, geographical location, and race or ethnicity influence the frequency of disease[3].

Chronic Rhinosinusitis is a common disorder, and its prevalence is 1-20% in different parts of the world[4]. CRS poses an immense economic burden to the patient via the treatment cost and the indirect costs due to restricted activity days[5]. Many patients with chronic sinus disease take 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[6,7].

Previously fungal Rhinosinusitis was considered a uncommon disease, but it has gained importance in recent past due to increase in the incidence. The incidence of fungal sinusitis has increased to such extent in recent years that fungal infection is a common diagnosis in patients with Chronic Rhinosinusitis. Whether fungi can exist in the sinus mucous without causing disease is unclear. However, over the last two decades, there is an increased frequency of recognized and reported cases of fungal Rhinosinusitis worldwide[8].

Due to increased cases of invasive fungal infection in immunocompromised patients and emergence of resistance to antifungal agents it became essential to perform drug susceptibility testing in mold infections[8,9].

In our literature review, we have not found any study on the pattern of antifungal drug sensitivity among chronic fungal sinusitis cases in New Delhi. Therefore, we did this current research with the objectives of estimating the prevalence of Fungal aetiology in chronic sinusitis patients and their drug sensitivity pattern with common antifungal drugs.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Departments of Microbiology, Pathology, and Otorhinolaryngology of Lady Hardinge Medical College (LHMC) and associated hospitals, New Delhi. Data collection was done in the year 2010. A total of 61 eligible cases, as per our inclusion and exclusion criteria, visited our hospital, were included in our study.

Inclusion criteria

1. Cases present with Chronic Rhinosinusitis (CRS) in Hospital OPD (inflammation of paranasal sinus for more than 12 weeks with or without radiological evidence).
2. Age of participant should be more than 14 years.

Every participant was provided with a patient information sheet and informed written consent was taken from each enrolled patient. Three such patients who has not given consent to participate in the study were excluded.

Sample collection and transport

Excised sinus tissue, including polyps and masses, were collected in the operation theatre during Functional Endoscopic Sinus Surgery (FESS) procedure in a sterile manner. The specimen was divided into two separate parts. One part was kept in a sterile container having sterile normal saline which is used for bacteriological and mycological examination. The other part was placed in 10% formalin, used for histopathological examination. These samples were transported and processed in Microbiology and Pathology laboratories immediately.

Sample processing

All the tissues brought in sterile normal saline were processed in the Microbiology department, LHMC for bacteriological and mycological examination. Tissues, obtained in 10% formalin were processed for histopathological and cytological examination in the department of Pathology LHMC, New Delhi.

Antifungal drug susceptibility testing

Antifungal drug susceptibility testing for Amphotericin B was performed for all the isolates using the CLSI M38-A broth dilution method and

E-test method following the instructions of the manufacturer[10].

RESULTS

A total of 64 eligible patients were contacted for the present study. Few (3) Patients refused to participate in the study, so the final sample size was 61. In the current study, 34 (55.7%) males and 27 (44.2%) females were included. The most common (47.5%) age group involved was 31-50 years, followed by less than 30 years (31.1%) and 51-70 years (21.3%).

On culture of samples from 61 patients of CRS, a total of 21 isolates were identified and out of which, 13 were fungal, and 8 were bacterial.

Out of 61 samples, seven samples were positive for KOH, culture, and histopathology, six samples were positive for KOH and culture, and one was positive for KOH and histopathology. Therefore, a total of 14 (22.9%) cases of Chronic Rhinosinusitis were affected by fungal etiologies. Out of these 14 cases fungal agent were isolated from 13 patients. Out of these 13 isolates 11 (84.61%) were *Aspergillus flavus* and 2 (15.38%) isolates were *Rhizopus arrhizus* [Table 1]

In this study, among all chronic fungal sinusitis patients, most (7 cases, 50.0%) of the participants presented in summer season followed by winter season (5 cases, 35.7%) and the rainy season (July to October, 2 cases, 14.2%).

By E test, the MIC range for isolates of *Rhizopus arrhizus* after 24 hr of incubation was 1-2 µg/mL, and the mean was 1.5 µg/Ml. Similarly, the MIC range for isolates of *Aspergillus flavus* after 48 hr of incubation was 0.5-16 µg/mL, and the mean was 4.09µg/mL. [Table 2]

By the M38-A broth dilution method, the MIC range for the isolates of *Rhizopus arrhizus* after 24 hr of incubation was 0.5-2 µg/mL, and the mean was 1.25 µg/ml. Similarly, the MIC range for isolates of *Aspergillus flavus* after 48 hr of incubation was 0.5-4 µg/mL, and the mean was 1.95 µg/mL. [Table 3]

On comparison, MICs results of E-test and CLSI standard broth dilution testing (M38-A) showed MICs of all isolates were within ±1 log2 dilution for *R. arrhizus* and agreement was found to be 100% for both ±1 log2 and ±2 log2 dilution. Similarly, MICs of most of the isolates of *Aspergillus flavus* showed 45.45% and 90.90% agreement at ±1 log2 and ±2 log2 dilution, respectively.

DISCUSSION

In a present study, a total of 61 samples from patients of chronic Rhinosinusitis were included. Out of them, 59 samples were polyps/ tissue/ mass collected from the patients who underwent functional endoscopic sinus surgery, and the remaining two samples were sinus secretions from patients managed conservatively. In our study, we observed that 34 (55.73%) CRS patients were males, and 27 (44.27%) were females. So, the prevalence of chronic Rhinosinusitis in male patients was 1.25 times higher than females. Aghakhani et al. and Razmpa et al. also observed similar findings among the CRS group, 58% male and 42% females, and 68% male and 32% female, respectively.

In our study, we observed that 34 (55.73%) CRS patients were males, and 27 (44.27%) were females. So, the prevalence of chronic Rhinosinusitis in male patients was 1.25 times higher than females. Aghakhani et al.[11] and Razmpa et al.[12] also observed similar findings

Table 1. Distribution of Chronic Fungal Rhino-sinusitis cases according to KOH, culture, and Histopathology finding

10% KOH	Fungus on Culture	Fungal Histopathology (H/P)	Number	Cultured isolates
Yes	Yes	Yes	7	13 11 (84.61%) *Aspergillus flavus* and 2 (15.38%) *Rhizopus arrhizus*
Yes	Yes	No	6	
Yes	No	Yes	1	0
No	No	No	47	0
Total			61	13

Table 2. *In vitro* susceptibilities of fungal isolates to Amphotericin-B as determined by E-test

Parameter	Isolates	
	R. arrhizus	*A. flavus*
Incubation time	24 hr.	48 hr.
MIC range (µg/ml)	1-2	0.5-16
Mean (µg/ml)	1.5	4.09

Table 3. *In vitro* susceptibilities of fungal isolates to Amphotericin B as determined by CLSI M38-A broth dilution method

Parameter	Isolates	
	R. arrhizus	*A. flavus*
Incubation time	24 hr.	48 hr.
MIC range (µg/ml)	0.5-2	0.5-4
Mean (µg/ml)	1.25	1.95

among the CRS group, 58% male and 42% females, and 68% male and 32% female, respectively.

In the present study majority (26.2%) of CRS, patients were seen in 31-40 years of age group, followed by 24.6% in 21-30 years of age group. Aghakhani et al.[11] found the majority of CRS patients (24%) in 30-39 years, followed by 20% in 40-49 years of age group.

FRS was diagnosed in age groups ranging from 17 to 63 years. Most cases were from 31-40 years of age group, and the mean age was 34.3 years. Study done by Razmpa et al.[12] also found highest number of cases in 30-39 years of age group, with mean age of 37 years. Venugopal et al.[13] found the age range was from 16 to 68 years (average: 35 years). Chakrabarti et al.[14] in their study found that younger age groups are more prone to fungal Rhinosinusitis. Jahromi et al.[15] reported age ranging from 17 to 58 years (mean of 33.4 years). As per our study no specific age group can be considered as risk factor for the development of FRS.

The FRS observed in the present study was 22.9 %[14], based on KOH, culture, and histopathology among the patients with the clinical presentation of chronic Rhinosinusitis. Worldwide prevalence rate varies from 6.7% to 75.5%. In comparison to other studies from various parts of India we observed less prevalence rate. Chakrabarti et al.[14] from Chandigarh and Venugopal et al.[13] from Tamil Nadu reported 42% and 45% of prevalence rate, respectively. Singh et al.[16] identified fungal elements in 62.1% of surgical specimens from patients with Chronic Rhinosinusitis in Gujarat.

From outside India, in Brazil Dall'Igna et al.[17] estimated 6.7% prevalence of fungal Rhinosinusitis. In Nepal, paranasal mycosis was found to be 14% in chronic maxillary sinusitis[18]. In Malaysia, the prevalence of allergic fungal Rhinosinusitis among patients with chronic refractory sinusitis was 26.7%[19]. In an Iranian study, paranasal mycoses were proved in 46% of cases with suspected fungal sinusitis[15]. Another study from Iran found a 42% prevalence rate on direct microscopy with nasal polyposis[12]. Braun et al.[20], in Europe, found 75.5% surgical specimens from patients with Chronic Rhinosinusitis with or without polyposis were positive for fungal elements.

Except for a few reports, our results fall in between most of the studies. By using improved and novel samples processing technique for the detection of fungi significantly higher prevalence rate has been reported in some studies. However, similar methods were adopted in one study still gave a low prevalence rate[19]. Different climate conditions in different geographical areas may be the reason for the varying in prevalence of pathogenesis of FRS.

In our study, out of 14, only 8 (57%) samples showed fungal elements in histopathology. Similarly, Aghakhani et al.[11] found fungal culture-positive in 49 (49%) of cases of paranasal sinuses and on histological examination fungal elements ware seen in 41 (41%) of cases.

Aspergillus flavus which is isolated in 84.61% cases, was the most frequent causative fungi and this finding is concordant to other studies conducted in various part of world. Rao et al.[21] isolated from 87.5% of *Aspergillus flavus*. Chakrabarti et al.[14] identified the same species from 80% of cases. Similarly, Panda et al.[22] isolated *Aspergillus flavus* from 79.7%.

From outside India, Razmpa et al.[12], and Jahromi et al.[15] from Iran isolated *Aspergillus flavus* as a commonest spp. Rahman et al. [23] from Saudi Arabia also separated similar spp.

In our study, the prevalence of fungal

Rhinosinusitis in male patients was 1.3 times higher than females. Iwen et al.[24] also found male: female ratio in fungal rhinosinusitis 1.125:1. Chakrabarti et al.[14] also reported male predominance with male: female ratio being 2.8:1.

To know the antifungal sensitivity/ resistant pattern of the fungal isolates from chronic Rhinosinusitis, we tested all isolates for antifungal susceptibility by E-teat and CLSI standard broth dilution testing (M38-A). We preferred amphotericin B drug because for the life-threatening fungal infections the conventional form of this drug is still the treatment of choice. According to CLSI guideline, for most of the species MIC of amphotericin B are clustered between 0.5 and 2.0 µg/mL, MIC above 2µg/mL have been associated with treatment failures and MIC below 2µg/Ml with the clinical cure. As per our result, most of the MICs felled in between the MIC range of the CLSI guideline. Only one isolate MIC found to be higher than 2µg/mL. For E- test, no MIC was mentioned by the manufacturer (AB-BIODISK) for amphotericin B. So we could not interpret sensitivity/ resistance pattern. E-test MIC was used only for the comparison of E-test MIC and CLSI standard broth dilution testing (M38-A) MIC. In our study, isolates showed good agreement (90%) within ±2 log2 dilution. Szekely et al.[25] demonstrated 50% and 60% agreement for ±1 log1 and ±2 log2 dilution respectively for amphotericin B. Ingroff et al.[26] showed 12% and 31% agreement for ±1 log1 and ±2 log2 dilution respectively for amphotericin B. This agreement could vary, depending upon the strain.

In general, the discrepancies between E test and broth dilution results were due to higher E test MICs. In our study, the E test Showed comparable results with CLSI M38-A. For antifungal susceptibility testing of mold pathogens E-test has potential value and it is more natural to perform, and less cumbersome compare to M38-A. The wider amphotericin B MICs obtained by the E-test for *Aspergillus flavus* and *Rhizopus arrhizus* also suggest that this method could also be useful for MIC detecting.

CONCLUSION

The prevalence rate of fungal Rhinosinusitis was found to be 22.9% among the clinically suspected patients of chronic Rhinosinusitis, and our region may be considered a moderate to higher prevalence area after comparing it with data from the other regions.

Aspergillus flavus was most commonly isolated from patients with fungal Rhinosinusitis establishing it as the commoner etiological agent in our region.

On antifungal susceptibility, 11 out of 12 isolates were found to be susceptible to amphotericin B by CLSI standard broth dilution testing M38-A. So, amphotericin B should be used by the clinician in confirmed cases of FRS. The E-test result was also found to be comparable with M38-A. On comparison of E-test MICs and M38-A MICs, all isolates had shown good agreement at ±2 log2 dilution. However, we found that M38-A is a time-consuming and cumbersome procedure compare to the E test.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

ETHICS STATEMENT

Prior approval has been taken from the institutional ethical committee.

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