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Mycological Profile of Rhinosinusitis in a Tertiary Care Centre

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Abstract

Fungal rhinosinusitis is a common entity encountered with patients admitted in the dept. of ENT. It is caused by a variety of filamentous fungi which can be isolated in culture on saboraud's Dextrose agar. The disease has significant mortality and morbidity which may be reduced to certain extent by early diagnosis and prompt treatment. So many antifungal agents are available nowadays. Hence in – vitro-antifungal susceptibility testing of the fungal isolates is mandatory to assist the clinician in selection of the appropriate antifungal agent. If it is administered at the proper time complications may be avoided in healthy patients and in case of immunosuppressed, it may be life saving. A study was conducted in the Dept. of Microbiology, Govt. Medical College, Thiruvananthapuram in co-ordination with the Department of ENT for a period of one year from August 2015 to July 2016 to find out the prevalence of Rhinosinusitis and the fungal pathogens causing the disease and the antifungal susceptibility testing of the isolates. A total no. of 186 patients with age group 18 to 80 years and both gender irrespective of the duration of symptoms of Rhinosinusitis were included in the study. Fungal culture positivity was 26.88%. Aspergillus species were the predominant isolates (62%). Aspergillus fumigatus was the most common isolate (41.94%) among the aspergillus species. Antifungal susceptibility testing was performed by microbroth dilution method according to CLSI guidelines. The isolates of Aspergillus species showed 100% sensitivity of Itraconazole, 93.5% sensitivity to Amphotericin B and 100% resistance to Fluconazole. Uncontrolled Diabetes Mellitus was the major risk factor (30%). Mortality rate in our study was 4% which is significantly less when compared to many other studies. This may be because of early detection and treatment with appropriate antifungal drug.

Keywords: Fungal Rhinosinusitis, Aspergillus, fumigatus, Aspergillus flavus, Antifungal susceptibility testing

Introduction

Rhinosinusitis is defined as the inflammation of nasal and paranasal sinus mucosa and is associated with mucosal alterations ranging from inflammatory thickening to gross nasal polyp formation. The first documented case of suppurative sinusitis was by Antonio Molinetti in Venice in 1697. The inflammation of the nasal mucosa and sinus mucosa may be due to microorganisms like bacterium and fungi. The role of fungi causing may be proved by demonstration of fungal elements by direct microscopy and its isolation in culture. Most cases occur in patients with serious underlying diseases. Plaignaud in 1791 first described the role of fungi in rhinosinusitis.

Aim of the Study

1. To find out the proportion of Fungal Rhinosinusitis at Govt. Medical College Hospital, Thiruvananthapuram.

2. To isolate and identify different species of fungi causing rhinosinusitis.
3. To evaluate the predisposing factors encountered in patients with rhinosinusitis.
4. To determine the antifungal susceptibility pattern of the predominant species of fungi isolated and identified from clinical specimens.

Materials And Methods

Study Design : Descriptive study

Study Population : Patients with clinical and radiological Features of rhinosinusitis attending ENT department.

Study period : 1 year (from August 2015 to July 2016)

Study setting : Department of Microbiology and Dept. of ENT, Govt. Medical College, Thiruvananthapuram, Kerala

Methodology

Collection of samples

Samples of sinus secretion and debris, sinonasal polyp, scrapings from necrotic tissue material, excised sinus tissue, allergic mucin, fungal ball and mucocele through functional endoscopic sinus surgery were collected under sterile conditions. The nasal cavity was packed with 4% lignocaine and adrenaline before surgery. First step is uncinectomy followed by middle meatal antrostomy where the maxillary sinus ostium is widened. The antrum is then inspected for any polyp, fungal ball, allergic mucin or debris. Samples were collected by endoscopic sinus surgery and sent to the 24 hrs Microbiology Laboratory immediately after collection. If pathology is suspected in ethmoid sinuses, the bulla ethmoidalis is opened and all ethmoid air cells is removed. Sphenoid sinus is opened last.

Sample processing

All samples were collected in a sterile screw capped plastic containers with sterile normal saline to keep the tissue moist. Polyps and interspersed mucus were treated as single sample. All tissue samples were cut into small, 1 mm fragments with sterile scalpel blade in a sterile petri dish. Immediate processing of the samples were carried out in the microbiology laboratory.

Direct microscopic examination was done after wet mount preparation with 10% KOH and lactophenol cotton blue. Fungal hyphae if present may be clearly seen on KOH wet mount preparation.

Culture was done on Sabouraud's Dextrose agar (plain) and SDA with gentamicin. Samples collected from patients with rhinosinusitis are inoculated into the medium using a sterile bent loop and kept at room temperature and also at 37 degree C. The SDA slopes were examined for growth of fungi. If the growth is cottony woolly appearance, tease mount was prepared with lactophenol cotton blue and examined under microscope. Preliminary identification of the fungi may be done based on the morphology. Species identification of the filamentous fungi was done with slide culture. If mucoid, moist colonies were seen, then wet mount preparation with lactophenol blue stain and Gram staining were done. If yeast cells are seen on smear, Germ tube test was done to differentiate candida albicans from non-albicans.

Germ tube test

Colony of the yeast like fungi was picked up with sterile straightware and inoculated into 0.5ml of sterile human serum taken in a sterile test tube, mixed well and incubated at 37 degree C for 2-3 hours. 1 drop of the mixture was taken in a clean glass slide and examined under microscope after 2- 3 hours for the appearance of the tube like structures arising from the yeast cell. If germ tube was present, preliminary identification of candida albicans can be done. If germ tube is absent, it may be taken as non albicans group of candida.

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Antifungal susceptibility testing

Antifungal drugs were obtained in the powder form from Himedia Laboratories and stores at 40C as recommended by the manufacturer. Antifungal stock solutions were prepared at concentrations hundred times more than the highest concentrations tested. Amphotericin B and Itraconazole powders were dissolved in dimethyl sulfoxide (DMSO) and Fluconazole powder was dissolved in sterile distilled water. The medium used was RPMI-1640 (with glutamine, without bicarbonate and phenol red as pH indicator). The buffer used was MOPS (3-N-Morpholino) propane sulfonic acid. Fungal isolates were grown on potato dextrose agar slants until sufficient conidia were formed. (Approximately 3-7 days).

Microbroth dilution method was performed using doubling dilutions and minimum Inhibitory concentration was detected visually. Growth in each MIC well was compared with that of growth control with the aid of a reading mirror. MIC above 8mg/ml has been associated with treatment failure and below 8 ug/ml with clinical cure in the case of Itraconazole.

Results

A total number of 186 patients clinically diagnosed as cases of rhinosinusitis in the Dept. of ENT of Govt. Medical College, Thiruvananthapuram during the study period was included in the study. Samples were collected from these patients under sterile precautions. Of the 186 samples examined under the microscope after 10% KOH mount, fungal hyphae were seen in 40 samples (21.5%). No fungal elements were demonstrated in 246 samples (78.5%). After the preliminary examination with KOH mount, the samples were inoculated into Sabouraud's Dextrose Agar slopes and incubated at 37 degree C and at room temperature. Tubes were examined daily starting from 24 hrs of inculation for 7-10 days for the growth of fungi. If no growth occurs even after 10 days, the culture is declared as negative. If growth occurs after 2 days/4 days/ one week, identification of the fungi were done by colony characteristics, morphology on tease mount with LPCB and special tests. Culture positivity was 26.88% in this study. Identification of the moulds done by slide culture. The predominant fungi isolated was Aspergillus species (62%) culture positivity was more in males (52%) than in females (48%). Most of the samples collected in the study were sinus secretions and debris (36.02%) and the culture positivity in these samples was 24% only. Culture was positive in more no. of patients with age group between 40-49 yrs (34%). The prevalence rate of FRS cases were quite high during monsoon season from July to October (38.7%) and the culture positivity was also high during this period (44%). Diabetes mellitus was the major predisposing factor observed in 30% of FRS cases followed by Hypertension (18.36%). No specific predisposing factor was observed in 26% of cases of fungal rhinosinusitis.

Table 1: Clinical samples versus culture positivity

Sl. No.	Nature of samples	Total No.	Culture positivity
1	Sinus Secretion & debris	67	18 (26.86%)
2	Sino nasal polyp	63	6(9.52%)
3	Necrotic scraping	26	7 (26.92%)
4	Excised sinus tissue	20	10 (50%)
5	Allergic mucin	6	5 (83.35%)
6	Fungal ball	3	3 (100%)
7	Mucocele	1	1 (100%)
	Total	**186**	**50 (26.88%)**

Table 2: Species of Fungal isolates

Sl. No.	Fungal species	No. of isolates
1	Aspergillus fumigatus	13 (26%)
2	Aspergillus flavus	10 (20%)
3	Aspergillus niger	6(12%)
4	Aspergillus terreus	2 (4%)
5	Rhizopus species	2 (4%)
6	Mucor species	2 (4%)
7	Penicillium species	2 (4%)
8	Pseudoallescheria boydii	2 (4%)
9	Fusarium species	1 (2%)
10	Curvularia species	1 (2%)

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11	Trichosporon species	1 (2%)
12	Candida species	4 (8%)

Table 3: Culture positivity based on age and gender

Age	Male	Female	Total
20-29	1	2	3 (6%)
30-39	3	5	8 (16%)
40-49	8	9	17 (34%)
50-59	4	3	7 (14%)
60-69	9	3	12 (24%)
70-79	1	2	3 (6%)
Total	26	24	50 (100%)

Table 4: Seasonal distribution of FRS

Season	Total no. of patients	Culture positive cases
Summer (March – June)	66(35.4%)	16 (32%)
Monsoon (July – October)	72 (38.7%)	22 (44%)
Winter (November – February)	48 (25.87)	12 (24%)
Total	**186 (100%)**	**50(100%)**

Table 5: Predisposing factors associated with FRS

Sl. No.	Predisposing factor	No. of patients
1	Diabetes mellitus	11 (30%)
2	Hypertension	9(24.32%)
3	Allergic rhinitis	7(18.9%)
4	Bronchial Asthma	6 (16.21%)
5	Polypectomy	3 (8.1%)
6	Dental extraction	1 (2.7%)
7	No predisposing factor	13 (26%)

Table 6 : Distribution of clinical symptoms of FRS

Sl. No.	Symptoms	Total no. of patients
1	Nasal obstruction	40 (80%)
2	Nasal discharge	43 (86%)
3	Headache	39 (78%)
4	Recurrent URI	15 (30%)
5	Facial fullness	12 (24%)
6	Hyposmia	5 (10%)
7	Retro-orbital pain	5 (10%)
8	Facial swelling	5 (10%)
9	Lateral rectus palsy	2 (4%)
	Total	**186 (100%)**

Table 7: Sinuses involved in patients with FRS

Sl. No.	Sinus involved	Total no. of patients
1	Maxillary sinus	16 (32%)
2	Ethmoid sinus	6 (12%)
3	Sphenoid sinus	3 (6%)
4	Frontal sinus	2 (4%)
5	Multiple sinuses	22 (44%)
	Total	**50 (100%)**

Table 8: Multiple sinuses involved in FRS

Sinuses involved	No. of cases
Maxillary + ethmoid	9 (40.9%)
Maxillary + Sphenoid	7 (31.81%)
Maxillary = ethmoid + frontal	4918.18%)
Frontal + Ethmoid	2 (9%)
Total	**22 (100%)**

Table 9: Antifungal sensitivity testing of the Aspergillus isolates by microbroth dilution method

Isolates	Amphotericin b		Iraconazole		Fluconazole	
	MIC <2μg/m	>2μg/ml	<8μg/ml	>8μg/ml	Sensitive	Resistant
Aspergillus Fumigatus	13	-	13	-	-	13
A. Flavus	10	-	10	-	-	10
A. Niger	6	-	6	-	-	6
A. Terreus	-	2	2	-	-	2

Discussion

Fungal Rhinosinusitis is confirmed only when the fungal pathogen is isolated in culture. Culture positivity in our study was 26.88%. This is in accordance with a similar study conducted in Amritsar by Shivani et al (2016) in which they have reported of culture positivity 21.29%. Another study in Coimbatore by Sandeep et al reported 30% of culture positivity. Ragini Tilak et al from North India (2012) reported 21.3% of culture positivity in patients with rhinosinusitis.

FRS was recognised slightly more in males (52%) than in females (48%). This is in accordance with a similar study done in Kerala by Shanthi et al (2013) in which FRS was seen in 55% males and 45% females. Male preponderance in FRS was also noted by Banerjee et al and Fazl-al-Wahid et al in this studies.

KOH mount was positive in 21.5% of cases which gives a clue to the diagnosis of Fungal Rhinosinusitis. The percentage of KOH positivity was quite high among culture positive samples. Shanthi et al reported 25% KOH positivity in 25.8% of FRS cases.

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In the present study, 2 species of yeast- like fungi and 7 species of mould were isolated. Yeast like fungi isolated are candida species and Trichosporon species. Species of filamentous fungi isolated were Aspergillus, Rhizopus, Mucor, Penicillium, Fusarium, Pseudoallescheria boydii and Curvularia.

Aspergillus species were the predominant isolates (62%) in this study. This is in accordance with another study by Ananthilakshman et al in Tamil Nadu (70%). Ragini et al (2012) reported 50% and M.P. Kamath et al reported 44.4% prevalence of Aspergillus species.

Among the aspergillus species, Aspergillus fumigatus was the most common species isolated (41.9%) followed by Aspergillus flavus (32.25%), Aspergillus niger (19.34%) and Aspergillus terreus (6.45%). Similar observations made by two studies in Tamil Nadu and Kerala and Vennewald et al Germany) 2012 and 2013.Aspergillus niger was the predominant isolate in a study done in Mangalore (2009-2011) by MP Kamath et al. Aspergillus terreus was isolated in 4.76% of cases in the study Banerjee et al in Uttar Pradesh. This is consistent with our study. Zygomycetes were the second most common fungi (16%) isolated in the present study. Rhizopus species (12%) and mucor (4%). This is similar to the study by Ananthilakshman et al.

Candida species isolated in our study was 8%. Other studies reported higher percentage of candida species. Suresh et al (Tamil Nadu) reported 26.6% and a study from Brazil reported 17.7% of isolates being candida species.

Penicillium species isolated in our study was 4%. Another study from Kerala reported 6.7% and from Srinagar 17.04%. One study from Malaysia reported 14.3% isolates being penicillium species.

Fusarium species isolated in our study was 2% only. This is consistent with other studies from Kerala and Maharashtra (2.7%). Pseudoallescheria boydii isolated was 4% in our study. It is a rare cause of FRS. Dwight D. Bates reported a case of invasive fungal sinusitis caused by this organism in an immunocompromised host. Annam et al (2008) in Karnataka have reported one case with frontal sinusitis caused by this organism. Trichosporon species was isolated from a patient with uncontrolled diabetes mellitus with invasive FRS in this study. Three cases were previously reported from Iran by Hedayati et al. Trichosporon inkin has been reported from Chennai by Janagond et al as a rare cause of allergic FRS. Curvularia species was isolated from one case in this study (2%). Ananthilakshman et al in their study reported one isolate from FRS patients.

In India, the most frequently isolated specie are Aspergillus, mucorales, pencillium, candida and fusarium species. In contrast to thin western studies reported dematiaceous fungi as the most common cause of FRS.

Antifungal sensitivity testing of Aspergillus species done by microbroth dilution method showed 100% sensitivity to Itraconazole and 93.5% to Amphotericin B and 100% resistance to Fluconazole. Drug resistance is a major problem in treating fungal infections. Selection of anifungal drug after doing invitro susceptibility testing is mandatory nowadays.

Symptoms of FRS for more than 3 months of duration constituted 84% of cases in this study. Das et al (2007) Chandigarh reported 42.7% of chronic FRS in their study.

Most of the patients presented with multiple sinus involvement (44%) Unilateral involvement of the sinuses was noted in 54% of cases. The most common sinus involved was the maxillary sinus (32%) followed by ethmoid sinus (12%), sphenoid sinus (6%) and frontal sinus (4%).

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Management of FRS cases

Clinically diagnosed cases of FRS were treated with antifungal therapy and surgical intervention with endoscopic sinus surgery. Aggressive surgical debridement was done in cases of invasive FRS. Surgical excision of nasal polyp and fungal ball were performed immediately after diagnosis.

For antifungal therapy, intravenous amphotericin B was given for patiets with mucormycosis (16%). On discharge, patients were advised to take oral Itraconazole. Patients with invasive FRS with Aspergillus were successfully treated with voriconazole. Allergic FRS was treated with endoscopic sinus surgery and corticosteroid nasal spray (74%)

Treatment outcome

Majority of the patient responded well to the antifungal therapy along with surgical intervention (92%). Two patients admitted with FRS developed complications like orbital cellulitis and lateral rectal palsy (4%). In spite of antifungal therapy 2 patients with invasive FRS expired during treatment. Mortality rate in our study was 4%, which is very low when compared to other studies.

Conclusion

Rapid increase in mycotic infections has been recognised recently. The patients with fungal infections have high morbidity and mortality especially those having acute fungal rhinosinusitis. The disease is often neglected and misdiagnosed in developing countries like India, where FRS is one among the neglected diseases. Early detection of cases and proper collection of samples at the proper time helps the clinician in early diagnosis of the disease so that the mortality rate may be reduced by giving medical treatment earlier with appropriate antifungal drugs and surgical intervention if needed. Facilities are available all clinical microbiology laboratories for doing fungal culture and identification of the organism is made easy by different conventional methods and also by automated methods. In vitro susceptibility testing of the fungal isolates is mandatory for selection of appropriate antifungal agents.

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