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STUDY OF FUNGAL ETIOLOGY IN CHRONIC SINUSITIS

Microbiology

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ABSTRACT

Frequency of mycotic infections of nose and paranasal sinuses has been increasing over the past three decades. Present study is undertaken to isolate and identify the fungi causing chronic sinusitis and also to determine their antifungal susceptibility patterns. Prospective study was done on 100 cases of chronic sinusitis. Samples were processed, identified and AFST done according to CLSI guidelines. Out of 100 samples, 32 were positive for fungal culture. Aspergillus species is the most commonly isolated fungi. Other fungi isolated were Rhizopus spp, Penicillium spp, Scopulariopsis spp, Fonsecaea spp. Allergic fungal rhinosinusitis was the most common presentation. MIC's for itraconazole and amphotericin B were within susceptible ranges as per CLSI.

KEYWORDS

fungal infections, chronic sinusitis, AFST

INTRODUCTION:

Sinusitis or more accurately rhinosinusitis is a common disorder affecting 20% of the population.[1] It occurs in both acute and chronic forms. Chronic rhinosinusitis(more than 12 weeks duration) 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.[2] The frequency of fungal infections of the nose and paranasal sinuses have been increasing over the past three decades in India.[3,4]. Fungal sinusitis is broadly classified into invasive and non invasive sinusitis based on clinical and histopathological findings.[5] and can occur in immunocompromised as well as immunocompetent individuals. The disease process is chronic and indolent in immunocompetent individual whereas it will be rapidly progressive and fulminant in immunocompromised individuals.[6]

AIMS AND OBJECTIVES

1. To isolate and identify the common species of fungi causing chronic sinusitis.
2. To determine antifungal susceptibility pattern among isolates.

MATERIALS AND METHODS

This is a prospective study done in 100 cases clinically diagnosed as chronic rhinosinusitis, after getting the approval from Ethical Committee of the College.

Patients clinically diagnosed as chronic rhinosinusitis were included and those with acute rhinosinusitis were excluded.

Sample collection and processing[7,8,9]

All the relevant history like age, sex, presenting symptoms, diagnosis at admission, duration of illness, history of chronic illnesses like diabetes were taken into consideration. Biopsy from the paranasal sinus tissue, mucinous material and necrotic material obtained during functional endoscopic sinus surgery were collected in two sterile containers, one containing sterile 0.85% NaCl which was transported immediately to the Microbiology lab for mycological processing. The other container containing formalin was sent to Pathology lab for histopathological examination and reports were obtained. For mycological processing, the tissue specimen was cut into small pieces in a sterile petridish using sterile scalpel and blade. Then a portion of specimen was put into 0.5ml of 10% KOH for direct microscopic examination. Then the remaining specimen was inoculated into two sterile SDA tubes containing 0.05% chloramphenicol. After inoculation, the tubes were incubated, one at 25ºC and the other at 37ºC respectively. Cultures were examined for growth, daily in the first week and twice a week for the subsequent period. Cultures were held for an entire period of 4 weeks before being labelling it as negative for fungal growth. If growth was obtained in any test tube, they were identified by colony morphology, macroscopic, microscopic examination by LPCB mount and slide culture technique.

Antifungal susceptibility testing(AFST) was done by broth microdilution method according to CLSI M38A2 reference method. Synthetic medium RPMI 1640 with glutamine, without bicarbonate (Hi Media laboratories) is used. Amphotericin B(AMB) powder from HiMedia and Itraconazole(ITR) powder from Sigma-aldrich were obtained. Antifungal stock solutions were prepared using DMSO and then serial dilutions were made. Inoculum is prepared separately. Each well of a microtitre plate was inoculated with- 100 µl of inoculum +100 µl of diluted drug of each concentration. This brings the drug concentration and inoculum density to the final desired concentrations. Positive and negative controls are kept and the microtitre plate is incubated at 35˚C for 48 h. For Rhizopus species, incubation for 24hrs is sufficient. MIC(Minimum Inhibitory Concentration) is defined as the lowest concentration of an antifungal agent that substantially inhibits growth of the microorganism as detected visually.

RESULTS AND DISCUSSION:

Out of 100 cases of chronic sinusitis, 32 were diagnosed to have fungal rhinosinusitis based on culture findings. The remaining 68 samples did not show any growth on culture.

Out of 100 cases of Chronic rhinosinusitis(CRS) which underwent functional endoscopic sinus surgery, paranasal sinus tissue bits along with mucinous material was collected in 81% cases. Only necrotic debris was seen in 19% cases.

Out of 100 cases of chronic sinusitis**.** number of females (59) affected were more than number of males (41) with a male to female ratio of 0.7:1 which is close to a study done by Michael et al(0.8:1).[10] But compared to other studies done by S Prateek et al(1.33:1),[11] Usha Krishnan et al(1.25:1),[12] Das et al(1.8:1)[13] and Shivani et al(1.4:1),[14] there was a female predominance in our study.

Among the cases which were fungal culture positive (32), majority were females (59.4%) compared to males (40.6%). This may be due to more number of females presented in the study or could be due to delayed seeking of medical advice incase of female population.

Out of the 32 cases diagnosed as fungal rhinosinusitis, majority i.e, 17 (53.1%) are from age group 31 to 40years followed by 7(21.9%) are from 21 to 30years which correlated with Prateek et al[11] which showed maximum number of cases were from 31 to 40 years age group(42.86%), followed by 41-50years(28.57%) and 21-30 years(19.05%).

In a study done by Usha Krishnan et al,[12] age of patients varied from 14 to 62 years with majority of patients (37%) belonging to an age group

of 21-40 years. A study conducted by Das et al,[13] reported age of patients ranged from 2 to 81 years with a mean age of 31 years.

From the above studies, middle age group was the most commonly affected which may be due to more frequent exposure to external environment and hence more exposure to fungal spores.

Table I: Age wise distribution of patients with fungal rhinosinusitis (n=32)

Age group in years	Number of culture positives	Percentage of culture positives
0 to 10	00	0%
11 to 20	02	6.25%
21 to 30	07	21.9%
31 to 40	17	53.1%
41 to 50	03	9.4%
51 to 60	01	3.1%
61 to 70	02	6.25%

The percentage of fungal culture positivity in the present study is 32% which nearly correlates with S Giri et al(25%).[15] But it is slightly high compared to S Prateek et al(21%)[11] , Kini JR et al(14.5%)[16] and low compared to K Usha Krishnan et al(44%)[12]

All the patients with CRS presented with nasal obstruction. Most of the patients presented with nasal discharge(86%), post nasal drip(78%). Headache was the presenting symptom in 60% cases, sneezing in 44% cases, smell disturbance was seen in 21% cases. This is nearly similar to a study done by Prateek et al,[11] who reported the most common presentations as nasal obstruction (100%) and nasal discharge (100%) followed by headache (71.43%) and facial pain/swelling (57.14%).

In the 32 cases diagnosed as FRS, majority (65.63%) were maxillary sinusitis, 28.12% were pansinusitis cases, and 6.25% were ethmoid sinusitis.

Out of the 32 isolates, majority were Aspergillus species (84.375%), other fungi were 15.625%. This nearly correlated with studies done by other workers like UshaKrishnan et al,[12] Prateek et al,[11] Michael et al,[10] who reported 74%, 76.19%, 78.67% isolation of Aspergillus species respectively, while the other fungi isolated in their studies were 26%, 23.81%, 21.33% respectively.

Among the Aspergillus species, Aspergillus flavus (59.4%) was the most common isolate, which correlates with Giri et al[15] (60%), Prateek et al[11] (57.14%) and UshaKrishnan et al[12] (52%).

Aspergillus fumigatus isolation was 15.6%, which correlates with Prateek et al[11] (14.29%). UshaKrishnan et al[12] and Giri et al[15] reported 8.7% and 6.67% isolation of A.fumigatus. Aspergillus niger isolation was 9.3% in our study while Usha Krishnan et al[12] reported 13%.

Other fungi isolated were Rhizopus species (6.25%), Penicillium species (3.1%), Scopulariopsis species (3.1%), Fonsecaea species (3.1%). After Aspergillus spp, Rhizopus spp was most commonly isolated in many studies, but there is a wide variation in the other fungi isolated. This could be due to variation in geographical distribution of fungi.

Based on histopathological examination, most common type of fungal sinusitis in the study was allergic fungal rhinosinusitis (n=24, 75%). Chronic invasive fungal sinusitis was seen in 6 cases (18.75%) and fungus ball in 2 cases (6.25%) i.e., 81.25% cases were non invasive sinusitis and 18.75% were invasive sinusitis. This nearly correlates with studies done by Prateek et al[11] (66.67% non invasive FRS, 33.33% invasive FRS), UshaKrishnan et al[12] (82.6% non invasive FRS, 21.7% invasive FRS), Giri et al[15](66.67% non invasive and 33.33% invasive FRS).

Allergic fungal rhinosinusitis(75%) was the most the most common type of sinusitis in the present study. This correlates with studies by Usha Krishnan et al [12](66.6%), Prateek et al[11] (57.14%), Kavitha et al [17] (68%) all of which showed that majority of cases were of AFRS type.

Chronic invasive rhinosinusitis(18.75%) was the next common type of sinusitis in the present study which correlated with UshaKrishnan et al [12] (12.5%), Prateek et al[11] (14.29%) and Kavitha et al [17] (19%). Fungus ball was seen in 6.25% cases in the present study, 12.5% in study by Usha Krishnan et al,[12] 9.52% by Prateek et al,[11] and 5% by Kavitha et al.[17]

Table II: Distribution of isolates in different types of fungal rhinosinusitis

Isolates	Allergic fungal rhinosinusitis	Fungus ball	Chronic invasive fungal rhinosinusitis
Aspergillus flavus	15	01	03
Aspergillus fumigatus	04	01	-
Aspergillus niger	03	-	-
Rhizopus species	01	-	01
Penicillium species	-	-	01
Scopulariopsis species	-	-	01
Fonsecaea species	01	-	-
Total	24	02	06

Among the 32 culture positives, KOH was positive in 28 cases. On histopathological examination by H&E, 23 showed presence of fungal hyphae along with tissue reaction, 9 showed presence of eosinophils and other inflammatory cells, whereas on GMS, fungal hyphae were seen.

Table III - Antifungal susceptibilities for *Aspergillus* species in various studies

Various studies	MIC ranges (µg/ml)					
	A.fumigatus		*A.flavus*		*A.niger*	
O C Abraham et al	Amphotericin B	Itraconazole	Amphotericin B	Itraconazole	Amphotericin B	Itraconazole
	0.5-8	0.25-4	0.5-8	0.25-4	0.5-8	0.25-4
R Kachuei et al	0.125-4	0.031-16	0.125-4	0.031-16	0.125-4	0.031-16
F Sabatelli et al	-	-	1	0.5	-	-
Present study	0.06-0.5	0.06-0.5	0.03-0.25	0.03-0.25	0.03-0.5	0.03-0.5

Minimum Inhibitory Concentration(MIC) of Amphotericin B was <0.5µg/ml and Itraconazole <0.5µg/ml for all the Aspergillus species were within the susceptible ranges as per CLSI. This correlated with Sabatelli et al[18] who reported susceptible MIC's for Amphotericin B(1µg/ml) and Itraconazole (0.5µg/ml) against A.flavus. Abraham et al [19] reported high MIC's for Amphotericin B(8µg/ml) against all the Aspergillus species.

R Kachuei et al [20] reported high MIC's for Itraconazole (16 µg/ml) against all the Aspergillus species.

Table IV- Antifungal susceptibilities of other fungi in various studies

Various studies	MIC ranges (µg/ml)							
	Rhizopus spp		*Pencillium spp*		*Scopulariopsis spp*		*Fonsecaea spp*	
Suarez et al	AMB	ITR	AMB	ITR	AMB	ITR	AMB	ITR
	-	-	1-2	0.125-1	-	--	-	-
Anuradha chowdhary et al	0.03-1	0.125-16	-	-	-	-	-	--
F Sabatelli et al	1	4	-	-	-	-	-	-
Najafzadeh et al	-	-	-	-	-	-	0.5-2	0.03-0.25
Denis et al	-	-	-	-	0.25-32	1-32	-	-
Present study	0.06-0.5	0.25-0.5	0.25	0.5	0.5	1	0.25	0.25

In the present study, MIC of Penicillium spp for Amphotericin B(0.25 µg/ml) and Itraconazole(0.5 µg/ml) was within the susceptible ranges according to CLSI. This correlated with Suarez et al[21] who reported susceptible range of MIC for both Amphotericin B and Itraconazole.

MIC for Amphotericin B (0.06-0.5 µg/ml) and Itraconazole (0.25-0.5 µg/ml) against Rhizopus spp were within susceptible range according to CLSI. This correlated with Anuradha chowdhary et al[22] and Sabatelli et al[18] for Amphotericin B.Anuradha choudhary et al reported high MIC's for Itraconazole(16 µg/ml) against Rhizopus spp. MIc's of both

the drugs(0.25 µg/ml) were within the susceptible ranges for Fonsecaea spp which correlated with Najafzadeh et al.[23]

In the present study, MIC for Scopulariopsis spp was 0.5 µg/ml for Amphotericin B and 1 µg/ml for Itraconazole. This did not correlate with Denis et al[24] who reported high MIC's for both the drugs (32 µg/ml).

CONCLUSION

Fungal rhinosinusitis has been increasing over the past three decades. Early diagnosis can be obtained if all the cases of chronic sinusitis are screened for fungal infections. Progression to invasive form can be prevented by correct and prompt treatment.

Aspergillus species were the most common species isolated in cases of fungal rhinosinusitis, of which Aspergillus flavus was the most common isolate.

Antifungal susceptibility testing of the isolates against the drugs Itraconazole and AmphotericinB showed susceptible MIC's. No resistant strains were isolated in our study with reference to the standard testing method.

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