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FUNGAL RHINOSINUSITIS:CORRELATIONAL STUDY BETWEEN CULTURE AND HISTOPATHOLOY

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ABSTRACT **Introduction:** 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. **Materials and Methods:** This prospective study was conducted in the Department of Microbiology, Pathology and Otorhinolaryngology, DMC&H Laheriasarai, Darbhanga. 60 study patients were selected among inpatients admitted to department of Otorhinolaryngology. Tissue and blood sample, for each patient, was obtained. Tissue specimens were examined by direct microscopy (20% KOH mount) and culture. Final identifications to species level were made by micro-slide culture method. Blood samples were examined by total IgE ELISA. Histopathological examinations were done by Haematoxylin and Eosin stain, and Periodic Acid Schiff stain. **Results:** Out of 60 cases, 17 were positive for fungal rhinosinusitis. Among 17 positive cases, 13 cases were culture positive consisting of 9 cases of Aspergillus flavus, 2 cases of Aspergillus fumigatus, and 1 case each as Paecilomyces variotii and Cunninghamella bertholletiae. Cases of R. seeberi were further confirmed by Haematoxylin and Eosin and GMS staining. On the basis of histopathological findings, 12 cases (70.59%) were non invasive consisting of 11 cases of AFRS and 1 case of FB and 5 cases (29.41%) were of invasive consisting of 3 cases of CIFS and 1 case each as AFIFS and GIFS. IgE concentration was relatively significantly raised among all cases of AFRS. **Conclusion:** Fungal Rhinosinusitis is a common disorder in and around North Bihar. Early diagnosis and accurate histopathological classification helps in establishing further treatment protocol.

KEYWORDS : Fungal rhinosinusitis, paranasal sinuses, KOH mount, Haematoxylin and Eosin stain.

Introduction

The term "Rhinosinusitis" has become a common replacement for the term "Sinusitis" due to contiguous nature of nasal and paranasal sinus mucosa, as well as their interactions and potentially shared involvement in various inflammatory processes. Rhinosinusitis is widely believed to compromise a spectrum of inflammatory and infectious diseases simultaneously affecting the nose and the paranasal sinuses. The International Classification of Diseases divides rhinosinusitis into acute and chronic forms according to the duration of the symptoms. Acute Rhinosinusitis lasts up to 12 weeks (four weeks in the American Rhinosinusitis Classification) with complete resolution of symptoms, whereas the chronic form persists beyond 12 weeks.[1,2]. Chronic Rhinosinusitis (CRS) accounts for >90% of all cases of rhinosinusitis and the correct diagnosis of each category is important for proper therapy and predicting the course of the disease.[3] Despite awareness, it continues to be under diagnosed. About 1-20% patients of chronic rhinosinusitis are generally considered to be presenting with Allergic Fungal Rhinosinusitis.[3] The final diagnosis requires careful review of direct microscopic examination, histopathology of tissue or the cheesy material obtained from the sinuses, fungal culture results and operative surgical observations.[3] Direct microscopy helps in diagnosis of fungal etiology and culture helps in identification of the etiologic agent. Histopathology is important to distinguish the invasive from the non-invasive type. The distinction is easier and can be diagnosed even clinically when invasion of contagious structures has occurred. But when the lesion is restricted to the sinus, demonstration of histopathological invasion of mucous membrane is the only criterion to rely on.[4] Fungal rhinosinusitis presents in five forms as recently classified by deShazo and colleagues.[3] Each form is distinct pathophysiologically, histologically and clinically. Each require different approach to treatment and carries different prognosis. The five major forms are (1) allergic fungal rhinosinusitis (AFRS) (2) fungal ball (FB) (3) acute fulminant invasive fungal sinusitis (AFIFS) (4) granulamatous invasive fungal sinusitis (GIFS) and (5) chronic invasive fungal sinusitis (CIFS).

Materials and Methods

This prospective study was undertaken in the Mycology section of the Microbiology department in association with Pathology and Otorhinolaryngology departments of DMCH Laheriasarai Darbhanga, Bihar, India. The tertiary care hospital serving people from Bihar, Jharkhand and the neighbouring countries of Nepal and Bangladesh. The study period was from 5th April 2018 to 31st December 2020. A total of 60 study patients were selected from inpatients admitted in indoor wards of Otorhinolaryngology department, DMCH Darbhanga. The study was approved by the Institute ethical committee. For each patient, a tissue specimen was collected from the sinuses by endoscopic sinus surgery and a blood sample was obtained via peripheral venipuncture, and all relevant informations were collected. Tissue specimens were examined by direct microscopy in 20% KOH mount and culture on two Sabouraud Dextrose Agar media incubated at 25^0 C and 37^0 C respectively. Fungal isolates were identified by procedures detailed in standard mycology textbooks by Evans and Larone.[5] Final identifications to species level were made by micro slide culture method. Antifungal Susceptibility Testing was done by Disk Diffusion method as detailed in CLSI document M51-A for Non-dermatophyte Filamentous Fungi. Blood samples were examined by total IgE ELISA Test Kit (de medi tec, Principle: Direct sandwitch ELISA). Histopathological examinations were done by Haematoxylin and Eosin stain, and Periodic Acid Schiff stain.

Results

Out of 60 cases, 17 were positive for fungal rhinosinusitis. Among 17 positive cases, 13 cases were culture positive consisting of 9 cases of *Aspergillus flavus*, 2 cases of *Aspergillus fumigatus*, and 1 case each as *Paecilomyces variotii* and *Cunninghamella bertholletiae*. 4 culture negative cases were positive for sporangia on direct microscopy in 20% KOH mount suggestive of *Rhinosporidium seeberi*. Cases of *R. seeberi* were further confirmed by H & E and GMS staining. *Aspergillus spp.* (64.7%) was most common isolated fungal species with *Aspergillus flavus* (52.94%) being most common followed by *R. seeberi* (23.52%). On the basis of histopathological findings, 12 cases (70.59%) were non invasive consisting of 11 cases of AFRS and 1 case of FB and 5 cases (29.41%) were of invasive consisting of 3 cases of CIFS and 1 case each as AFIFS and GIFS (Table 1 & 3). Histopathologically, out of total 9 isolates of *A.flavus*, 7 isolates

belonged to AFRS, and 1 isolate each belonged to FB and GIFS while all 4 isolates of *R.seeberi* belonged to AFRS (Table 2). The azoles tested in this study (Voriconazole, Itraconazole and Fluconazole) showed an excellent activity against *Aspergillus spp.* as all the isolates were found susceptible to it. One isolate of *Paecilomyces variotii* was resistant to fluconazole, but sensitive to voriconazole and itraconazole. On the other hand azoles failed to have any activity against *Cunninghamella bertholletiae.* Amphotericin B exhibited good activity against the different isolates obtained in the study, only one isolate of *Aspergillus flavus* was found intermediate to it. All the 17 cases of FRS were above 15 years of age and all had IgE conc. above normal range (i.e >100 IU/mL). The IgE concentration was relatively significantly raised among all 11 cases of AFRS. The concentration range for the cases of AFRS was 298.35 to 446.04 IU/mL.

Discussion

The fungal infections in paranasal sinuses are becoming common day by day. In 1893, Mackenzie first reported paranasal sinus mycosis and since then numerous cases have been reported from various parts of world.[3] In previous fifteen years more than 200 cases had been reported in numerous studies at PGIMER Chandigarh **(Chakrabarti *et al.*, 2000)**[4]. One similar study undertaken by **Das *et al.*, 2007**,[6] at Chandigarh found FRS having incidence of 42.7% of total 665 cases of CRS over 5 years study duration. A study conducted by **Joshi *et al.*,**[7] in a tertiary care hospital in Nepal reported FRS in 14% cases of CRS in a total of 100 cases of CRS. Our study has found 17 cases of FRS among 60 suspected cases of CRS over a period of two years and eight months. Various studies have reported different species of *Aspergillus* to be the causative agents of fungal rhinosinusitis.[8, 9] Most cases of *Aspergillus* rhinosinusitis in Sudan and North India have been caused by *Aspergillus flavus*[8,10,11] but in United States, *Aspergillus fumigatus* and *Aspergillus oryzae* have been found in increasing number of cases.[9,12,13,14] **Panda *et al.*,**[15] in their study reported that in all types of paranasal mycoses, *A.flavus* was the commonest isolate (79.7%). *A.fumigatus* was isolated from 11.1% patients. **Joshi *et al.*,**[16] reported *Acremonium* and *Candida* each in 28.6% cases of FRS and *Aspergillus spp*. in 21.5% cases of FRS. In our study *Aspergillus spp.* (64.7%) was the most common isolated fungal species among all the cases of fungal rhinosinusitis with *Aspergillus flavus* (52.94%) being the most common fungal isolate followed by *Rhinosporidiun seeberi* (23.52%) which could not be grown on artificial culture media. *Aspergillus fumigates* was isolated in 11.76% of cases of FRS while *Paecilomyces variotii* and *Cunninghamella bertholletiae* were isolated in 5.88% of cases each of FRS.

Table 1: Histopathological classification of cases of FRS

Histopathological diagnosis		No. of cases	Percentage (n=17)
Non - invasive classes	Allergic Fungal Rhinosinusitis (AFRS)	11	64.70
	Fungal Ball (FB)	1	05.88
Invasive classes	Acute Fulminant Invasive Fungal Rhinosinusitis (AFIFS)	1	05.88
	Granulamatous Invasive Fungal Rhinosinusitis (GIFS)	1	05.88
	Chronic Invasive Fungal Rhinosinusitis (CIFS)	3	17.64
Total		17	

Table 2: Distribution of fungal agents among different histological classes of FRS

Fungal agents	AFRS (n=11)	FB (n=1)	AFIFS (n=1)	GIFS (n=1)	CIFS (n=3)	Total	Percentage (n=17)
A.flavus	7	1	0	1	0	9	52.94
A.fumigatus	0	0	0	0	2	2	11.76
P.variotii	0	0	0	0	1	1	05.88
R.seeberi	4	0	0	0	0	4	23.53
C.bertholletiae	0	0	1	0	0	1	05.88
Total	11	1	1	1	3	17	

Table 3: Histopathological findings in various classes of FRS

Histopathological findings	AFRS (n=11)	FB (n=1)	AFIFS (n=1)	GIFS (n=1)	CIFS (n=3)
Inflammatory cells	11 (100%)	1 (100%)	1 (100%)	1 (100%)	3 (100%)
Allergic mucin	11 (100%)	0	0	0	0
Fungal element	9(81.8%)	1 (100%)	1 (100%)	1 (100%)	3 (100%)
Granuloma	0	0	0	1 (100%)	0
Fibrosis	0	0	0	1 (100%)	0
Tissue invasion	0	0	1 (100%)	1 (100%)	3 (100%)
Eosinophils	8(72.7%)	0	0	0	2 (66.6%)

Goh *et al.*,[17] (2005) reported AFRS in 8 out of 30 cases of CRS (26.7%). **Das *et al.*,**[6](2009) eported incidence of AFRS to be 24% of all cases of CRS. Histopathologically in our study AFRS was diagnosed in 11 out of 60 cases of CRS (18.33%). Out of 11 AFRS, 7 was associated with *A.flavus* while 4 was associated with *R.seeberi*. **Adelson et al.,**[18] had reported that *Aspergillus spp*. is, almost exclusively, the responsible organism in all case series of FB. Histopathologically in our study, FB was diagnosed in only one case associated with *A.flavus* out of 17 cases of FRS. **Das *et al.*,**[6] in their study at Chandigarh had reported 17.3% cases of AFIFS of all the cases of FRS over a period of 5 years. All cases of AFIFS were associated with Zygomycetes. **Michael *et al.*,**[19] in a study done in South India reported 21 cases (10%) of CIFS among 211 cases of FRS diagnosed. *Aspergillus flavus* was the etiological agent in 10 cases and *Aspergillus fumigatus* in 8 cases among all the 21 cases of CIFS detected. A study by **Challa *et al*., 2008** in Hyderabad reported 15.87% cases of CIFS among all the cases of FRS with *Aspergillus spp.* being the most common etiological agent. In our study, we found 3 cases of CIFS out of 17 cases of FRS. Two was associated with *A.fumigatus* and one was associated with *P.variotii*.

Das *et al.*,[6] in their study stated that patients of AFRS had elevated total IgE level. **Goh *et al.*,**[17] found that among the AFRS group, 62.5% had elevated total IgE level. In our study, all cases of FRS had elevated IgE level but it was significantly elevated among cases of AFRS.

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