



CLINICOMYCOLOGICAL PROFILE OF FUNGAL RHINOSINUSITIS IN AND AROUND DARBHANGA (BIHAR) – A STUDY IN A TERTIARY CARE CENTRE AT NORTH INDIA

Microbiology

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ABSTRACT

Fungal rhinosinusitis is a group of disorders characterized by inflammation of mucosa of nose and paranasal sinuses. It has remained a diagnostic and therapeutic challenge since its prominence about two and a half decades ago. Its incidence in recent years has shown a marked increase in immunocompetent population especially in India. To identify the fungal isolates from patients of rhinosinusitis and correlate histopathological findings with culture results for accurate classification of the disease. This prospective study was conducted in the Department of Microbiology, Pathology and Otorhinolaryngology, DMC&H Laheriasarai, Darbhanga, Bihar over a period of 2 years. 50 study patients were selected among inpatients admitted to department of Otorhinolaryngology, DMCH Darbhanga. For each patient, a tissue specimen and relevant informations were collected. Examined by direct microscopy and culture. Final identifications to species level were made by micro slide culture method. Antifungal Susceptibility Testing was done by Disk Diffusion method. Out of 50 cases, 15 were positive for fungal rhinosinusitis. 4 culture negative cases were positive for sporangia on direct microscopy in 20% KOH mount suggestive of *Rhinosporidium seeberi*. *Aspergillus* spp. (60%) was most common isolated fungal species. 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

INTRODUCTION

Fungal rhinosinusitis has remained a diagnostic and therapeutic challenge since its prominence about two and a half decades ago¹. 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.^{2,3} 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 disease¹. Despite awareness, it continues to be under diagnosed. 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 observations¹. 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 contiguous structures has occurred. Fungal rhinosinusitis presents in five forms as recently classified by deShazo and colleagues¹. Each form is distinct pathophysiologically, histologically and clinically. Each requires 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) granulomatous 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 neighboring countries of Nepal and Bangladesh. The study period was from 5th April 2018 to 4th May 2020. A total of 50 study patients were selected from inpatients admitted in indoor wards of Otorhinolaryngology department, DMC Darbhanga. For each patient, a tissue specimen was collected from the

sinuses by endoscopic sinus surgery 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^o C and 37^o C respectively. Fungal isolates were identified by procedures detailed in standard mycology textbooks by Evans and Larone¹¹. 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. Histopathological examinations were done by Haematoxylin and Eosin stain, and Periodic Acid Schiff stain.

RESULTS

Out of 50 cases, 15 were positive for fungal rhinosinusitis. Among 15 positive cases, 11 cases were culture positive consisting of 8 cases of *Aspergillus flavus*, and 1 case each as *Aspergillus fumigatus*, *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. (60%) was most common isolated fungal species with *Aspergillus flavus* (53.33%) being most common followed by *R. seeberi* (26.66%) [Table 1]. On the basis of histopathological findings, 11 cases (73.33%) were non invasive consisting of 10 cases of AFRS and 1 case of FB and 4 cases (26.66%) were of invasive consisting of 2 cases of CIFS [Table 2]. Histopathologically, out of total 8 isolates of *A. flavus*, 6 isolates belonged to AFRS, and 1 isolate each belonged to FB and GIFS while all 4 isolates of *R. seeberi* belonged to AFRS (Table 3). 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.

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 world¹. 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.*, 2007⁵ 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.*,⁶ 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 15 cases of FRS among 50 suspected cases of CRS over a period of two years. Various studies have reported different species of *Aspergillus* to be the causative agents of fungal rhinosinusitis.^{7,8} Most cases of *Aspergillus* rhinosinusitis in Sudan and North India have been caused by *Aspergillus flavus*⁷ but in United States, *Aspergillus fumigatus* and *Aspergillus oryzae* have been found in increasing number of cases.⁸ Panda *et al.*,⁹ 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. Das *et al.*,⁵ (2009) reported incidence of AFRS to be 24% of all cases of CRS. Histopathologically in our study AFRS was diagnosed in 10 out of 50 cases of CRS (20%). Out of 10 AFRS, 6 was associated with *A.flavus* while 4 was associated with *R.seeberi*. Michael *et al.*,¹⁰ in a study at CMC Vellore found that out of 211 cases of FRS, 51 cases (24%) were of AFIFS with *Rhizopus spp.* being the most common etiological agent. In our study, we found only one case of AFIFS associated with *Cunninghamella bertholletiae* out of 15 cases of FRS.

Table 1: Fungal agents isolated among cases of FRS

Fungal isolates	No. of cases	Percentage (n=15)
<i>Aspergillus flavus</i>	8	53.33
<i>Aspergillus fumigatus</i>	1	6.66
<i>Paecilomyces variotii</i>	1	6.66
<i>Rhinosporidium seeberi</i>	4	26.66
<i>Cunninghamella bertholletiae</i>	1	6.66
Total	15	

Table 2: Histopathological classification of cases of FRS

Histopathological diagnosis	No. of cases	Percentage (n=15)
Non - invasive classes		
Allergic Fungal Rhinosinusitis (AFRS)	10	66.66
Fungal Ball (FB)	1	6.66
Invasive classes		
Acute Fulminant Invasive Fungal Rhinosinusitis (AFIFS)	1	6.66
Granulomatous Invasive Fungal Rhinosinusitis (GIFS)	1	6.66
Chronic Invasive Fungal Rhinosinusitis (CIFS)	2	13.66
Total	15	

Table 3: Distribution of fungal agents among different histological classes of FRS

Fungal agents	AFRS (n=10)	FB (n=1)	AFIFS (n=1)	GIFS (n=1)	CIFS (n=2)	Total	Percentage (n=15)
<i>A.flavus</i>	6	1	0	1	0	8	53.33
<i>A.fumigatus</i>	0	0	0	0	1	1	6.66
<i>P.variotii</i>	0	0	0	0	1	1	6.66
<i>R.seeberi</i>	4	0	0	0	0	4	26.66
<i>C.bertholletiae</i>	0	0	1	0	0	1	6.66
Total	10	1	1	1	2	15	

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