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Microbiology Section

Fungal Rhinosinusitis: Microbiological and Histopathological Perspective

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ABSTRACT

Introduction: On the basis of histopathology Fungal Rhinosinusitis (FRS) is categorized into non-invasive (allergic fungal rhinosinusitis, fungal ball) and invasive (acute invasive, chronic invasive and granulomatous invasive fungal sinusitis). This differentiation helps to decide the treatment. Role of latest molecular methods such as PCR and conventional methods such as KOH microscopy and culture also needs to be evaluated. Therefore, in this study we planned to categorise fungal rhinosinusitis on the basis of histopathology and compare it with other methods such as PCR, culture and KOH microscopy.

Aim: To analyse fungal rhinosinusitis cases by both histopathologically and microbiologically.

Materials and Methods: A total of 76 clinically suspected fungal rhinosinusitis cases were included in the study. The tissue of suspected cases were processed and examined by KOH microscopy, histopathologically, culture and PCR. Histopathological examination was done by PAS, GMS and H&E stain.

Results: FRS was diagnosed in 37 (48.68%) cases out of 76 clinically suspected cases of FRS. In which 17 (22.3%) cases were positive by direct microscopy, 21 (27.6%) by culture, 27 (35.5%) by PCR and 14 (18.42%) by histopathology. Approximately 14 cases of FRS were classified according to histopathology; 10 (71.3%) as non-invasive FRS. Out of these 10, 9 (64.2%) were classified as AFRS and 1 (7.14%) as fungal ball. Only 4 cases (28.5%) were diagnosed with invasive FRS. Out of these 4 cases, 2 (14.2%) were of chronic invasive fungal rhinosinusitis, 1 (7.14%) was of granulomatous invasive fungal rhinosinusitis and 1 (7.14%) was of acute fulminant invasive fungal rhinosinusitis. Allergic Fungal Rhinosinusitis (AFRS) is the most common type of FRS. *Aspergillus flavus* was found to be the most common fungi causing FRS.

Conclusion: Diagnosis should not be based on the single method. It should be done by both histopathological and microbiological methods, especially for those cases which are difficult to diagnose.

Keywords: Allergy, Culture, Fungal ball, Histopathology

INTRODUCTION

Colonisation of fungus in nose and paranasal sinuses is a common finding in diseased and healthy individuals. In recent years FRS has increased in prevalence. This prevalence is even greater in tropical countries like India [1-3]. *Aspergillus* species are the major aetiological agents of FRS but other fungi like *Schizophyllum commune*, *Alternaria*, *Curvularia* and *Bipolaris* are also not uncommon [4,5]. Histopathology is important to distinguish the invasive from the non-invasive type as this differentiation helps to decide the treatment [4]. On the basis of histopathology, FRS is categorized into non-invasive (allergic fungal rhinosinusitis, fungal ball) and invasive (acute invasive, chronic invasive and granulomatous invasive fungal sinusitis) [6,7]. Histopathology needs biopsy sample and is thus, an invasive procedure. But this procedure carries importance in categorising FRS. Although there are other methods which can help in early diagnosis, but these methods do not tell about tissue invasion and tissue reaction. In this study, we have planned to categorise fungal rhinosinusitis on the basis of histopathology and compare it with other methods such as PCR, culture and KOH microscopy.

MATERIALS AND METHODS

A prospective cohort study was undertaken in which 76 clinically suspected cases were included on the basis of following inclusion and exclusion criteria.

Inclusion Criteria

Patients with at least two major or one major and two minor criteria were considered for inclusion as described by Lanza and Kennedy (1997) [8]. In short, major criteria were: Facial pain/fullness, nasal obstruction, postnasal discharge, hyposmia/anosmia and fever; Minor criteria were: Headache, halitosis, fatigue, dental pain, cough, ear pain/fullness.

Exclusion Criteria

Patients suffering from other diseases like congenital mucocilliary disorder, atrophic rhinitis were excluded from the study.

Sample Collection

Tissue samples from suspected cases of FRS admitted to the ENT Department of King George's Medical University, Lucknow, India, were obtained from the sinuses following Functional Endoscopic Sinus Surgery (FESS). Samples were collected during the study period of two years starting from February 2013 till February 2015. Samples were obtained in two vials each; normal saline and 10% formalin. Saline sample was processed in mycology lab for KOH microscopy, culture and PCR. The sample in formalin was used to prepare histopathology sections.

KOH Microscopy and Csulture: Tissue was examined in 20% KOH. Culture was done on Sabouraud Dextrose Agar (SDA) with chloramphenicol and incubated at 25°C and 37°C respectively and were examined until 28 days.

Histopathology: All histological sections were stained by Haematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS) and Gomori Methamine Silver (GMS) stains.

DNA Extraction

DNA extraction was performed by using ZR Fungal/Bacterial DNA Mini Prep (Zymo Research), as per manufacturer's instructions.

PCR

As previously described by Sandhu G et al., universal primers for the 28S rDNA were used to amplify a DNA sequence of 260 bp (primers U1 {5′-GTG AAA TTG TTG AAA GGG AA-3′} and U2 {5′-GAC TCC TTG GTC CGT GTT-3′}) [9]. PCR amplification was carried out in 50-µl reaction volumes at following condition: Initial denaturation at 94°C for seven minutes followed by 35 cycles of denaturation at 94°C for one minute, annealing at 45°C for one minute and extension at 72°C for one minute followed by a final extension phase at 72°C for 10 minutes. Amplification products were analysed by agarose gel electrophoresis. PCR product of 260 bp was considered as evidence of successful target amplification.

Ethical Approval: Ethical approval was taken from Institutional Ethical Committee, King Georges Medical University, Lucknow, Uttar Pradesh, India.

RESULTS

Out of 76 patient 37 (48.68%) cases were found to be positive based on direct microscopy, culture, histopathology and PCR. Among these 17 (22.3%) cases were found to be positive by direct microscopy, 21 (27.6%) by culture, 27 (35.5%) by PCR and 14 (18.42%) by histopathology [Table/Fig-1]. Among 14 cases which were positive by histopathology, 13 cases came out to be positive by PCR, 11 cases came out to be positive by culture and eight cases came out to be positive by KOH microscopy [Table/Fig-2]. Considering histopathology as a gold standard sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for KOH microscopy, culture and PCR were found to be 57%, 85%, 47%, 88%; 72%, 85%, 55%, 93%; 87%, 77%, 48%, 96% respectively.

The gender ratio of male to female patients in our study was 1.2:1. The patient population age ranged from nine years to 74 years. Majority of cases were found in the month of November (18.42%) and December (14.47%) and most of these cases 44 (57.89%) were from urban area.

As shown in [Table/Fig-3] on the basis of histopathology 14 patients of FRS was classified into five categories: 9 (64.2%) as Allergic Fungal Rhinosinusitus (AFRS), 1 (7.14%) as fungal ball, 2 (14.2%) as Chronic Invasive Fungal Rhinosinusitis (CIFRS), 1 (7.14%) as Granulomatous Invasive Fungal Rhinosinusitus (GIFRS) and 1(7.14%) as Acute Fulminant Invasive Fungal Rhinosinusitis (AFIFRS).

Patients with Chronic Rhinosinusitis (CRS) presented with following clinical sign and symptoms: 96% (73) had nasal obstruction, 88.16% (67) had purulent nasal discharge, facial congestion and other complaints.

Among the fungi *Aspergillus flavus* (75%) was the most common isolate followed by *Aspergillus niger* (10%), *Schizophyllum commune* (10%) *and Alternaria* (5%) in among seventy six cases.

Total cases n= 76	Direct Microscopy	Culture	PCR	Histopathology
Positive	17	21	27	14
Negative	59	55	49	62

[Table/Fig-1]: Results of 76 clinically suspected cases of chronic fungal rhinosinusitis by different methods.

Histopathology Positive	PCR Positive	Culture Positive	KOH microscopy Positive
14	13	11	8

[Table/Fig-2]: Comparison of histopathology with PCR, culture and KOH microscopy.

Histopathological Findings	Number of patients	Percentage (%)
Allergic fungal rhinosinuistis	9	64.2%
Fungal ball	1	7.14%
Acute Fulminant Invasive FRS (AFIFRS)	1	7.14%
Granulomatous Invasive FRS (GIFRS)	1	7.14%
Chronic Invasive FRS (CIFRS)	2	14.2%

[Table/ Fig-3]: Characterization of FRS on the basis of histopathology (n=14).

DISCUSSION

FRS previously considered as rare, is now being recognized and is being reported with increasing frequency worldwide. In India, the disease was reported earlier only from Northern region of the country, but now it is increasingly being recognised from other parts as well [10]. In the present study the incidence of FRS was 48.7%. Other studies from North and South Indian regions of the country have also reported similar incidence ranging from 21% to 46.7% [4,11,12]. Study from Delhi by Kaur R et al., had reported FRS in patients from 18 to 48 years with male to female ratio 1.18:1 [11]. In a previous study from Lucknow, Uttar Pradesh by Prateek S et al., the cases of FRS ranged from 22-63 years and male to female ratio was 1.33:1 [12]. In our study, the patient population age ranged from nine years to 74 years and ratio of male to female was 1.2:1.

Among the laboratory methods, PCR was found to be the most sensitive method. However, its specificity was lower in comparison to KOH microscopy and culture. Considering its high NPV, PCR can also be used to rule out the disease.

The disease is most common in winter season (November; 18.2%). However, we observed that whenever there is a change in the season, the cases of FRS rises. They rise in November (18.4%) and December (14.5%). Fungi obtained in culture were season dependent, most commonly found during summers and winters. On the basis of histology, nasal polyps are associated with fungal culture rate, organism type and seasonal variations [13]. In our study, *Aspergillus flavus* was found to be the most common species causing FRS. This is confirmed with other study from India, in which *A. flavus* was found to be the predominant species [14]. Ashraf MJ et al., reviewed and found that in Sudan, Saudi Arabia, Northern India and Atlantic coast of USA, *Aspergillus* as the most common fungi causing FRS. However, in North America, *Alternaria* and *Bipolaris* are the most common species causing FRS. Among the species of *Aspergillus*, *A. flavus* is more common in India and Arabian countries while in USA *A. fumigatus* is the most common. This difference in the species causing FRS could be climate and geography related [15].

Based on histopathology, FRS was categorized as non-invasive (71.3%) which included AFRS and fungal ball and as invasive (28.5%) which included chronic invasive FRS, chronic invasive granulomatous FRS and acute fulminant FRS [Table/Fig-3].

Allergic Fungal Rhinosinuistis (AFRS) patients mostly present with pan-sinusitis and nasal polyposis. These manifestations are mainly an allergic response to the fungus colonizing the mucosa of the cavity. Demonstration of fungal hyphae in histopathology with characteristic cellular response or a positive fungal culture from a suspected case of AFRS is an important diagnostic criteria [3,16].

Invasive Fungal Rhinosinusitis (IFRS) patients presents with diagnostic and therapeutic challenges. The histopathology of surgical sinus specimen plays a major role in categorizing IFRS patients [3,17]. Chronic Granulomatous FRS (CGFRS) is a histopathological diagnosis which includes granulomatous response and fibrosis. It is primarily caused by *Aspergillus* spp. and is mainly found in Africa and Southeast Asia [4,18,19]. Many times Chronic Invasive FRS (CIFRS) presents with mass involving nose and paranasal sinuses and proptosis which mimics malignancy [4]. On histopathology there is granulomatous response along with fibrosis and inflammation. We

think this could also be because of same histomorphological picture of central or peripheral giant cell granuloma.

Prognosis and treatment of FRS is different for invasive and non-invasive. It therefore, depends on the accurate classification of FRS. Clinical presentation of the disease may provide some clue for each category, but only histopathology provides the accurate and confirmed diagnosis [20]. Though other modalities may be used either for supportive diagnosis or when identification of fungus is clinically relevant such as when patient is not responding to antifungals.

As shown in [Table/Fig-1], of all the methods used PCR and culture were found to be the most sensitive. This was followed by KOH microscopy and histopathology. There were eight such cases which were positive by PCR but were negative by other methods. Possible reason for this could be that either laboratory diagnosis of PCR was correct or there was some cross contamination due to amplicon carryover [21]. This may be the major limitation of the use of PCR in diagnosis of fungal infections. Also, there were six such cases which were positive for fungi only in culture. Among these four had grown *A. flavus*, one *A. niger* and one *Alternaria* spp. In such cases possibility of laboratory contamination cannot be ruled out. However, histopathology is the gold standard [4]. No such case was found in which only histopathology was positive. They were also positive by some or the other method. Although less positive results by histopathology in comparison to other methods used in this study may be due to following reasons: (1) tissue was not taken from the proper site i.e., was only taken from the surrounding inflamed area; (2) the fungal elements were less in amount, so were not taken up by special stains such as PAS and GMS; (3) Sparse fungal elements were missed out during formalin/wax processing of histopathology; (4) Inter observer variability.

When we compared histopathology with other methods we observed that in some cases fungal hyphae were demonstrated by histopathology but not by KOH microscopy, culture and PCR. The possible reason for this could be that either fungal hyphae were sparse or were entrapped in the mucus which prevented fungal contact with the culture media or the tissue portion which was used for microscopy or PCR were not having any fungal elements. However, this happened less in case of PCR than culture and KOH microscopy [Table/Fig-2]. Other possible causes of less positive result by KOH microscopy in our study could be inter-observer variability or sample not taken from the proper site i.e., was only taken from surrounding inflamed area. PCR negative result could be because of improper DNA extraction. Few studies have also reported that use of mucolytic agents before inoculation may increase the yield in culture by up to 96% [22]. Therefore, to minimise such issues proper processing of the tissue sample is very important.

LIMITATION

In present study, sample size was small which was not sufficient for significant results. However, larger sample size studies are further required to reaffirm our findings.

CONCLUSION

Diagnosis of FRS should not be based on the single method as every method has its own advantage. Histopathology is important in classifying the disease but it may lack the sensitivity. Therefore, all the tests such as KOH microscopy, fungal culture and PCR must be used in conjunction with histopathology, especially for those cases which are difficult to diagnose.

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