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ISSN 1462 2815

UNVEILING THE MICROBIOLOGICAL PROFILE AND CLINICAL FEATURES OF ALLERGIC FUNGAL RHINOSINUSITIS

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DOI: 10.5281/zenodo.11571903

Abstract

As a result of an allergic reaction to fungal spores, a complicated condition known as Allergic Fungal Rhino sinusitis (AFRS) causes inflammation of the sinuses and nasal passageways. The precise endotype, or subtype, of AFRS must be identified in order to choose the best course of treatment and enhance patient outcomes. The purpose of the study was to describe and connect the microbiological profile with the incidence and clinical presentation of AFRS. Depending on their medical history, nasal endoscopy, and neuroimaging evidence, patients with fungal Rhinosinusitis that were clinically suspected were included. Relevant clinical samples were gathered, and they underwent histological analysis, direct microscopy, and culture. Results revealed significant relations among certain symptoms (e.g., sneezing, nasal obstruction, and disturbance in smell) and AFRS diagnosis ($p < 0.05$). Polymerase chain reaction (PCR) findings indicated the presence of specific fungal DNA in clinical samples, confirming the diagnosis of AFRS and Immunohistochemistry revealed marked differences in antigen detection between AFRS and non-AFRS cases, emphasizing the role of fungal antigens and eosinophilic markers in AFRS pathogenesis. These findings underscore the importance of recognizing distinct clinical manifestations and utilizing microbiological techniques for AFRS diagnosis and management. The findings of the study emphasize the significance of recognizing specific clinical manifestations and utilizing microbiological techniques in diagnosing AFRS. To validate these findings and enhance diagnostic approaches further exploration in this area is needed.

Keywords: Allergic Fungal Rhinosinusitis, P-value, Polymerase Chain Reaction, Microbiology, Immunohistochemistry, Radiography.

1. INTRODUCTION

A subtype of polypoid chronic rhinosinusitis known as AFRS is distinguished by a type I hypersensitivity to fungi and the presence of eosinophilic mucus with fungal hyphae inside the sinuses. In patients with chronic rhinosinusitis, allergic fungal sinusitis is observed in a wide range of percentages, varying from 5 to 10 percent in certain research to a substantially greater proportion in others[1].

Originally thought to be common exclusively in India's north, reports of the disease have recently come from other areas of the nation. It could indicate a non-invasive condition that manifests as an allergic hypersensitivity reaction to extra mucosal fungus present in the sinus cavity; this condition could be similar to allergic Broncho pulmonary aspergillosis[2].

IgE concentrations that are higher overall and specific to certain fungi, eosinophilia, asthma, and allergic rhinitis are common in patients. The affected sinuses include cellular debris, eosinophil that are intact and degenerating, scattered fungal hyphae, and a dark or greenish-black substance known as allergic mucin[3].

Even though there are few hyphae in the sinus fluid, the identification of fungus in allergic mucin is thought to be significant in the diagnosis of AFRS[4]. This causes uncertainty when classifying this item, particularly Eosinophilic Fungal Rhinosinusitis (EFRS)[5] and Eosinophilic Mucin Rhinosinusitis (EMRS)[6] two more closely related conditions, are described[7].

The core technique for diagnosing infectious diseases is the isolation of the organism that is causing the illness through culture, and this approach continues to be the gold standard for diagnosing infectious diseases in laboratories. The microorganism to be identified, the sample's characteristics, and the processing and storage conditions all influence the methods chosen for isolation by culture.

However, there are situations when it may be difficult to make a serological diagnosis and the rate of isolation of bacteria from cultures in tissue biopsies may be poor[8]. To diagnose allergic FRS, there are no precise diagnostic standards. Setting diagnostic criteria has grown more challenging with the emergence of more recent categories. There is controversy around the highly inconsistent laboratory results in the potential AFRS groups. Thus, the primary goal of this prospective investigation was to examine the incidence and medical manifestation of AFRS, describe the condition, and establish a correlation with the microbiological profile.

To identify a specific infectious agent, immunohistochemistry needs quality control, repeatability, and sensitivity, much like any other diagnostics technique. Thus, a number of parameters, including tissue fixation, tissue processing, and antigen retrieval, must be considered in order to prevent fluctuations in immunostaining and to retain the immunoreactivity of specific antigens[9].

PCR has become a viable technique for the quick and accurate diagnosis of medical mycoses[10]. The challenges of executing nucleic amplification in fungi have been lessened by recent technological developments, which have increased the sensitivity and specificity of PCR-based tests. This molecular method helps with precise diagnosis and focused treatment plans by offering insightful information about the existence and frequency of fungal species. When combined, PCR and IHC improve AFRS therapeutic management and diagnostic accuracy, directing individualized patient care strategies and enhancing clinical outcomes.

The followings are the study’s main contributions

- ✓ To examine in a group of patients suspected of having FRS the prevalence, clinical presentation, and microbiological profile of AFRS.
- ✓ To evaluate the diagnostic efficacy of IHC and PCR methods in identifying inflammatory markers and fungal pathogens linked to AFRS, and to correlate these results with radiological findings and clinical symptoms.
- ✓ To investigate the potential diagnostic and therapeutic implications of these markers in guiding individualized treatment strategies for AFRS patients. To clarify the immunopathogenesis of AFRS by identifying specific fungal antigens, eosinophilic markers, and inflammatory markers in Sino nasal tissues using IHC.

The following components of the study are required to be completed: Section 2 offers Materials and Methods used in this study. Section 3 looks at the Results and Discussion of the proposed study, while Section 4 provides the Conclusion and Future Scope.

2. MATERIAL AND METHODS

The method started with the enrollment of seventy-five patients who were suspected of having FRS. A detailed record of each patient's clinical history, nasal endoscopy, and radiological evidence was made. After the patients were enrolled, a thorough data collection strategy that included pathological and microbiological analyses was implemented. Clinical samples were systematically collected for histological examination, direct microscopy, culture, PCR, and IHC. These samples included allergic mucin, nasal lavage, exudate, tissue biopsy, nasal mucosa, and venous blood. A statistical analysis was then performed to assess the significance and correlations between the immunohistochemistry results, diagnostic profiles, and clinical manifestations. Methods used in this analysis included Fisher's exact test, chi-square test, and kappa coefficient. Following an interpretation and discussion of the data, were made. It is depicted in the Figure 1 below.

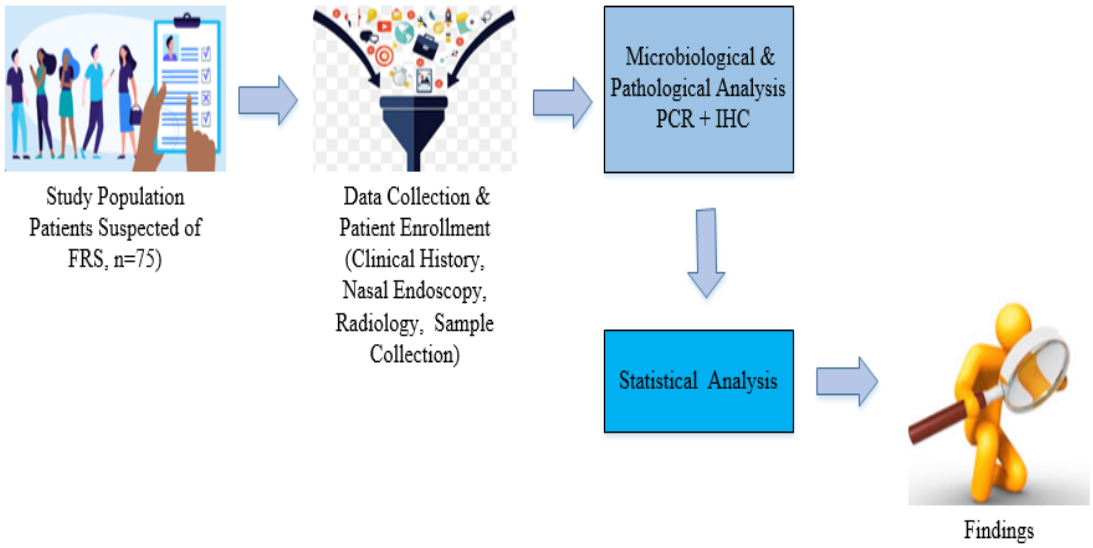

Figure 1: Conceptual Framework of Investigating AFRS

2.1 Data Collection

The data for this research is taken from the secondary source[11]. The prevalence and clinical presentation of AFRS were investigated, described, and correlated with the microbiological profile of patients suspected of having Fungal Rhinosinusitis in a prospective research.

After receiving their informed agreement, 75 clinically suspected FRS patients were enrolled in this prospective study of observation based on their clinical condition, nasal endoscopy, and radiological evidence from our hospital's wards and outpatient departments. Relevant results from radiography, nasal endoscopy, and the clinical history were recorded.

The Department of Microbiology and Pathology collected clinical samples, such as allergic mucin, nasal lavage, exudate, tissue biopsy, nasal mucosa, and venous blood, from suspected FRS patients. Table 1 provides the clinical manifestations in AFRS patients.

Table 1: Clinical Manifestations in AFRS Patients (p = 35)

Symptoms	Non-AFRS n (%)	AFRS n (%)	P-Value
Duration	1.2	1.6	
Headache	19(54.2)	21(60)	0.806
Vision Loss	11(31.4)	4(11.4)	0.03
Sneezing	1(2.8)	11(31.4)	0.002
Fever	8(22.8)	0	-
Diplopia	2(5.7)	4(11.4)	0.67
Pain in face	2(5.7)	0	-
Itching in nose	0	1(2.8)	0.99
Nasal Obstruction	24(68.5)	35(100)	0.0003
Epistaxis	0	3(8.6)	0.23
CNS Symptoms	6(17.1)	0	-
Disturbance in Smell	3(8.6)	18(51.4)	Less ham 0.0001
Proptosis	4(11.4)	7(20)	0.51
Swelling in Face	6(17.1)	0	-
Postnasal drip	1(2.8)	6(17.1)	0.11

Table 2: Characteristics of Study Population

Characteristics	Value
Mean	50
Median	50
Standard Deviation	20.56
Minimum	25
Maximum	75
Male (%)	60
Female (%)	40
Prevalence of AFRS (%)	35

Table 2 gives the demographic characteristics of patients suspected of FRS. The age and median of 50, 50 years, and a standard deviation of about 20.56, indicating a mild dispersion around the mean. The gender distribution skewed towards men, constituting 60% of the populace, whilst women accounted for 40%. Notably, the prevalence of AFRS turned into discovered to be 35% a few of the enrolled patients. These findings provide treasured insights into the demographic profile of people providing with suspected fungal rhinosinusitis and underscore the medical significance of AFRS as a subtype of persistent rhinosinusitis. Further exploration into the factors influencing prevalence charges and demographic styles may additionally provide insights for advanced management and treatment techniques.

2.2 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a powerful molecular technique used in the detection and identification of fungal pathogens, which can be particularly beneficial for fungal rhinosinusitis[12]. PCR amplifies specific regions of fungal DNA present in clinical samples, allowing for the rapid and sensitive detection of fungal species. This technique involves multiple cycles of heating and cooling to denature DNA strands, anneal primers to target sequences, and extend new DNA strands using a DNA polymerase enzyme. PCR has emerged as a crucial diagnostic method for pneumonia, even if direct visualization is still considered the gold standard. Numerous arrays, such as real-time quantitative PCR and qualitative traditional and nested PCR, have been created for usage. PCR primers are designed to target specific sequences within the genomes of fungal pathogens[13]. This specificity ensures that only DNA from the target fungi is amplified, reducing the likelihood of false-positive results. PCR

can accurately identify the particular fungal species presence in medical samples. Real-time PCR is the suggested testing method among the arrays available because of its quantitative character, which may be utilized to distinguish between infection and airway colonization. The identification of certain fungus species found in clinical samples may also be accomplished by PCR. This can be done by sequencing the amplified DNA segments to determine the fungal species through genetic markers or by using PCR tests that target DNA sequences specific to a given species[14]. A useful molecular technique, the multiplex PCR allows for the simultaneous amplification of multiple DNA amplicons of various sizes in a single PCR reaction. This makes it possible to effectively screen for a variety of pathogenic fungi, which is particularly helpful when dealing with multi-organism fungal infections.

2.3 Immunohistochemistry

In addition to direct diagnostic testing for infectious disorders by PCR and culture on fresh tissues, immunohistochemistry (IHC) employing monoclonal as well as polyclonal antibodies is a valuable diagnostic technique for identifying pathogen antigens in preserved tissues. Albert Coons initially used it in a ground-breaking work published in 1941[15].

Several procedures in the technical handling of tissues during immunohistochemical staining might affect how the results are correctly interpreted. The fixation, dehydration, and paraffin embedding processes are these. Two types of fixatives are frequently employed in fixation: coagulation fixing agents (alcohol solutions such as ethanol, methanol, and acetone) and reticulation fixatives (such as formalin).

Trypsin treatment for two hours is all that is needed for antigen extraction by digestion with enzymes from tissue slices in order to identify specific immunoreactivity locations[16]. Except for cytokeratins and desmin, several antigens are not disclosed by trypsin digestion. Formalin-fixed tissue slices are now heated in a microwave instead of using this procedure. The techniques that follow include heating paraffin tissue slices in a microwave to a maximum of 100°C while metallic solutions are present. Because of this pre-treatment, immunohistochemical operations are enhanced, even in long-term formalin-fixed tissues, since antigen recovery is significantly improved. Following many antibody incubation processes, antigens of interest are subsequently exposed using chromogenic substrate, as detailed in the supplementary Materials. Immunohistochemistry can be a helpful approach for the precise and successful identification of a number of prominent human mycoses as well as for identifying the location of typical or atypical fungal constituents in lesions from fixed tissues slices. An immunohistochemical diagnosis of fungal sinusitis is made.

2.4 Statistical Analysis

Categorical variables are shown as exact numbers and percentages, whereas continuous variables are displayed as mean ± SD. When applicable, the Fisher's exact test or the chi-square test were used to analyze the categorical variables. The kappa coefficient was also used to assess the level of agreement among HPE, direct microscopy, and culture variables. For all statistical tests, a difference was considered significant if $P < 0.05$. There are two tails to every statistical significance test.

The test became probable applied to evaluate the frequency distributions of specific variables between groups (AFRS vs. Non-AFRS sufferers). It allows decide whether

or not there's an enormous association between the existence of signs and symptoms and the diagnosis of AFRS.

The pronounced p-values inside the table provided suggest the effects of statistical exams assessing the association among symptoms and AFRS diagnosis. These p-values assist decide whether the discovered variations in symptom occurrence among AFRS and non-AFRS patients are statistically great.

A significance stage (commonly set at $\alpha = 0.05$) is used to interpret the p-values. If the p value is less than the significance stage, it shows that there may be evidence to reject the null hypothesis and finish that there is a statistically considerable association among the symptom and AFRS diagnosis.

3. RESULTS AND DISCUSSION

3.1 Findings of the Research

With the 75 instances with suspected FRS, 50 patients were found to have allergic FRS. Within the range of 18 to 48 years, the average age was 28.4. The male to female sex ratios was observed to be 1.18:1. 94% of the patients were reported to be educated, and 82% of the patients were from metropolitan areas. Fall brought in the greatest number of cases to the hospital, averaging 2.75 cases per month, followed by winter, which averaged 1.83 patients. On average, the symptoms lingered for 1.64 years. Every AFRS patient had nasal obstruction, and there was nasal discharge in 62.8% of instances, which had a statistically significant connection. Table 1 shows that sneezing (31.42%), scent disturbances (51.42%), and vision loss (11.42%) were additional statistically significant related symptoms. Table 3 and 4 given below shows the diagnostic profile and IHC findings in AFRS cases.

Table 3: Diagnostic Profile in AFRS Cases (n = 50)

Findings	Non-AFRS (n=25)	AFRS (n=25)	P-Value
Findings of Computed Tomography			
Heterogeneous opacities, unilateral	6 (24%)	12(48%)	0.11
Heterogeneous opacities, bilateral	5(20%)	15(60%)	0.04
Homogeneous opacities, unilateral	2(8%)	1(4%)	0.65
Homogeneous opacities, bilateral	1(4%)	0	-
Mucosal thickening	10(40%)	6(24%)	0.21
Bone erosion	12(48%)	8(32%)	0.29
Intracranial/ intraorbital extension	11(44%)	6(24%)	0.12
Calcification	3(12%)	1(4%)	0.39
Nasal Endoscopic Examination			
Polyp, unilateral	5(20%)	14(56%)	0.03
Polyp, bilateral	4(16%)	13(52%)	0.02
Deviated nasal septum	3(12%)	9(36%)	0.08
Secretions, greenish yellow	3(12%)	5(20%)	0.47
Inferior turbinate hypertrophy	2(8%)	3(12%)	0.67
Middle turbinate hypertrophy	0	3(12%)	0.09

Table 4: IHC Findings in AFRS Cases (n = 50)

Antigen Detected	Non-AFRS (n=25)	AFRS (n=25)	Positive (%)	P-value
Fungal Antigen A	2 (8%)	20(80%)	80	<0.001
Fungal Antigen B	3(12%)	18(72%)	72	0.003
Eosinophil Marker C	4(16%)	23(92%)	92	<0.0001
Inflammatory Marker D	7(28%)	15(60%)	60	<0.025

The outcomes of the immunohistochemistry (IHC) examination found out big variations in antigen detection among allergic fungal rhinosinusitis (AFRS) instances and non-AFRS instances, providing valuable insights into the pathophysiology of AFRS.

Firstly, the presence of Fungal Antigen A and Fungal Antigen B was markedly higher in AFRS cases in comparison to non-AFRS instances, with wonderful rates of 80% and 72%, respectively, within the AFRS institution. These findings advise a sturdy affiliation between the detection of those fungal antigens and the prognosis of AFRS ($p < 0.001$ for Fungal Antigen A and $p = 0.003$ for Fungal Antigen B), highlighting the role of fungal factors inside the pathogenesis of AFRS.

Furthermore, the Eosinophil Marker C and Inflammatory Marker D have been appreciably more prevalent in AFRS cases, with effective charges of 92% and 60%, respectively. This indicates a prominent eosinophilic and inflammatory factor in the Sino nasal tissue of AFRS patients, reflecting the allergic nature of the disease method. The detection of those markers may additionally serve as useful diagnostic signs for AFRS ($p < 0.0001$ for Eosinophil Marker C and $p < 0.025$for Inflammatory Marker D).

These findings underscore the importance of immunohistochemical evaluation in figuring out specific antigens and markers associated with AFRS, providing valuable diagnostic records and insights into the underlying mechanisms of the disorder. The massive differences discovered among AFRS and non-AFRS cases in antigen detection highlight the capability application of IHC in distinguishing AFRS from other Sino nasal situations and guiding appropriate healing interventions. Further research exploring the diagnostic and prognostic implications of these antigenic markers is warranted to enhance our knowledge and management of AFRS.

3.2 Discussion

An example of a noninvasive FRS is allergic fungal rhinosinusitis. Adolescents and young adults frequently suffer from allergic fungal sinusitis, which is more prevalent in places with high humidity levels. Half of the patients have asthma, while the other two thirds are atopic. Ninety percent of patients with allergic fungal sinusitis have elevated levels in the blood of immunoglobulin E (IgE). In this study investigation, where 80% of instances with AFRS had elevated serum IgE levels. Allergy rhinitis is present in two thirds of individuals with allergic fungal sinusitis.

Based on the existence of allergic mucin during the medical evaluation, the clinical and radiological signs of the illness, and the findings of the immunohistochemistry investigation, 35 patients in the study were diagnosed with AFRS. The mean age of the patients was 28.45 years, with a range of 18 to 48 years. This is very comparable to a research conducted in Chandigarh in 2002–2003, where the mean age of their AFRS cases was 28 years [1].

To diagnose allergic FRS, there are no precise diagnostic standards. Setting diagnostic criteria has grown more challenging with the emergence of more recent categories, such as eosinophilic fungal rhino sinusitis and eosinophilic mucin rhino sinusitis. There is controversy around the highly inconsistent laboratory results in the potential AFR groups[17]. Thus, the primary goals of this prospective investigation were to identify, define, and establish a correlation between the microbiological profile and the incidence and clinical manifestation of allergic fungal rhino sinusitis.

4. CONCLUSION AND FUTURE SCOPE

In summary, this study offers a thorough analysis of the microbiological profile and clinical features of AFRS with the goal of improving diagnostic accuracy and treatment efficacy. By carefully collecting samples and using reliable laboratory techniques like PCR and IHC, the study clarified the existence of fungal antigens and inflammatory markers specific to AFRS.

The results highlight the importance of identifying specific clinical symptoms, such as nasal obstruction, olfactory disturbances, and sneezing, as these showed statistically significant correlations with AFRS analysis. The combination of molecular and immunohistochemical analyses improved diagnostic precision and guided individualized treatment plans by making it easier to detect fungal pathogens and eosinophilic markers. By leveraging PCR and IHC, clinicians can better delineate the underlying immunopathogenesis of AFRS, paving the way for targeted therapeutic interventions tailored to individual patient profiles.

To enhance the molecular foundations of AFRS and investigate potential treatment approaches targeted at modifying the immune response to fungal antigens, more confirmation of these results and investigation of modern diagnostic methods are required. As a result, the study adds insightful knowledge on the microbiological landscape and clinical presentation of AFRS. Through clarifying these complex aspects, the study aims to improve patient treatment for those suffering with AFRS and to contribute to evidence-based therapeutic practices.

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