

Role of Histopathology in the Diagnosis of Paranasal Fungal Sinusitis

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Abstract: Acute or chronic rhinosinusitis is a common condition affecting upto 20% o the population. Acute rhinosinusitis is associated with upper respiratory tract infections such as bacterial /viral and is self limited. Chronic rhinosinusitis has a slow protracted course, and has different aetiologies, fungal infection being the major cause. Fungal organisms are one of the proposed aetiological agents and are seen in 6-12 % of these patients. A retrospective analysis of 30 cases of suspected fungal sinusitis were studied in a 7yrs duration. On histopathological evaluation the cases were classified as Non invasive and invasive fungal rhinosinusitis (FRS) depending on tissue invasion. The morphology of the fungus, H&E visibility of fungus and tissue reactions associated with fungus were assessed. Four histologic categories of FRS were identified:1) Mycetoma/fungal ball (53%). 2)chronic invasive FRS (20%). 3) chronic granulomatous invasive FRS (20%). 4) Acute fulminant FRS (7%). Opportunistic infections like DM was present in 33% and Hodgkin lymphoma in 7%, remaining cases were immunocompetant. Aspergillus was most common fungal species seen in fungal ball and chronic invasive FRS, and mucormycosis was common in granulomatous invasive and acute fulminant cases. Tissue necrosis was abundant in 8 cases of mucormycosis fungal infections. Intracranial extension and angioinvasion was present in 2 cases of mucormycosis and mortality was 100% in these cases.

Keywords: Fungal Rhinosinusitis, classification, histopathology.

I. Introduction

Fungal Rhinosinusitis (FRS) has been a known medical entity for several hundred years but only in more recent times, the entity has been further defined. Disease is most commonly classified as benign non-invasive or invasive based on whether the fungi have invaded into the submucosal tissue resulting in necrosis and tissue destruction.¹⁻³

Non invasive can be further divided into two forms: allergic fungal sinusitis (AFS) and Sinus mycetoma/ fungal ball with occurs in immunocompetent patients. AFS should be suspected in individuals with intractable sinusitis and recurrent nasal polyposis. These patients usually have atopy and have had multiple sinus surgeries by the time of diagnosis. Computerised Tomography (CT) scans of the sinuses reveal opacification with concretions and/or calcification.⁴

Invasive disease is characterised as either acute or chronic based on the length the time symptoms are present before presentation. Patients with acute invasive disease (AIFRS) are usually immunosuppressed and, by definition, present with symptoms of less than one-month duration. This entity is characterized by the presence of fungal forms invading into the sinonasal submucosal tissue with frequent angioinvasion and rapid intervention is necessary. Patients with chronic invasive disease present with symptoms of greater than three months duration. Two forms of chronic invasive disease, chronic invasive FRS (CIFRS) and chronic granulomatous FRS (CGFRS), have been described and like AIFRS both are serious, often requiring surgical and medical therapy.⁵

Accurate classification of FRS is important because prognosis and treatment varies among FS diagnosis. Although the clinical presentation may provide diagnostic clue for each category, only tissue examination can provide accurate classification.⁶

This study aims at categorising the fungal sinusitis according to the classification, studying the histomorphology of fungus and associated tissue reaction to the fungus.

Table 1: Classification of fungal rhinosinusitis based on Histopathologic criteria⁵

Non invasive FRS	
Fungal ball	An entangled mass on fungi with Minimal surrounding inflammatory reaction or surrounding fibrinous necrotic exudates containing fungal forms; No tissue invasion or granulomatous reaction is present
Allergic fungal rhinosinusitis (AFRS)	The presence of eosinophilic mucin (mucinous material admixed with eosinophils, acute inflammatory cells, eosinophilic debris, and charcot leyden crystals; sparse fungi or positive fungal cultures; no tissue invasion present)
Mixed FB/AFRS	The presence of features of both AFRS and FB
Invasive FRS	
Acute (AIFRS)	Invasion of fungal forms into submucosal with frequent angioinvasion and necrosis in a patient with symptoms of less than one month duration.
Chronic (CIFRS)	Invasion of fungal forms into submucosa often with surrounding chronic

	inflammation and fibrosis in a patient with long standing symptoms (>3months duration)
Chronic granulomatous (CGFRS)	Invasion of fungal forms into submucosal often with surrounding chronic inflammation, fibrosis, and granuloma production in patients with long standing symptoms (>3months duration)
Mixed non invasive / invasive FRS	A mixture of either of the invasive and non invasive categories

II. Materials And Methods

A retrospective analysis of 30 cases of suspected fungal sinusitis was included in the study in 7yrs duration. Relevant clinical details, radiological details and culture reports, if any, were analysed. Conventional haematoxylin and eosin stained sections along with special stains Grocotts methenamine silver (GMS) stain were examined. The cases were classified as Non invasive and invasive fungal sinusitis depending on tissue invasion. The morphology of the fungus and H&E visibility of fungus were studied. Tissue reactions like degree of inflammatory infiltrate, granulomatous response and tissue necrosis associated with fungus were assessed.

III. Observations

Of the 30 cases of FRS, ages of the patients ranged from 2 to 80 yrs (mean 30 yrs). There was predominance of FRS in male patients with male:female ratio of 1.8:1.

Oppurtunistic infections like Diabetes mellitus was present in 10 cases (33%) and Hodgkins lymphoma in 2 cases (7%). Remaining cases were immunocompetent.

On Histopathological examination, cases were broadly categorised as (I) Non invasive FRS (n=16 cases-53%) all of which were diagnosed as fungal ball (53%), none of the cases were allergic fungal sinusitis in our study. (II) Invasive FRS (n=14 cases, 47%) includes chronic invasive fungal sinusitis in 6 cases (20%), chronic granulomatous invasive FRS in 6 cases (20 %) and Acute fulminant (acute invasive FRS) in 2 cases (7%)

Fungal Ball (Mycetoma) (16 cases, 53%)

This was characterised by tightly packed fungal hyphae appearing pale in the centre with morphology more apparent on the periphery. The adjoining mucosa showed a mild mixed inflammatory infiltrate. All cases of mycetoma were associated with possibly *Aspergillus*, having thin septate acute-angle branching hyphae and were easily identified on H&E stain. (Figure 1)

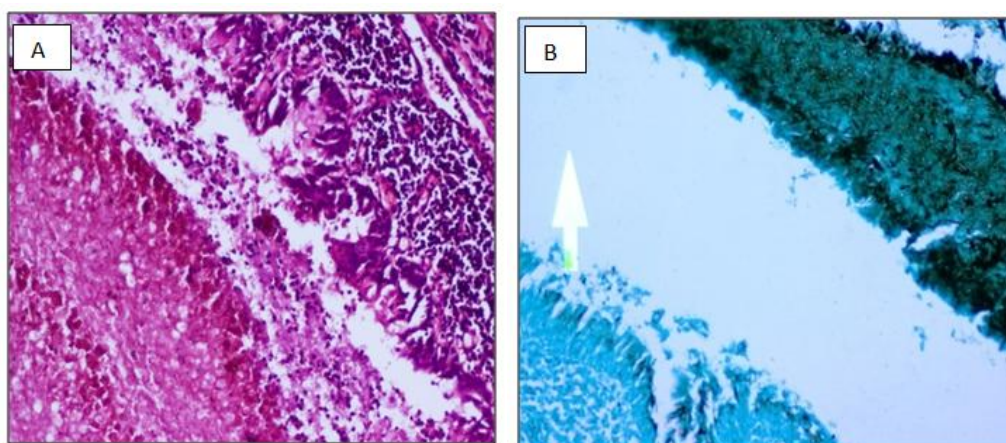


Figure 1. Photomicrograph showing A)Tightly packed hyphae of Fungal ball (non invasive) . Fungi appears as homogenous eosinophilic masses .The subepithelium showing mixed inflammatory reaction . (H & E stain) B) GMS stain showing positivity of fungus(*Aspergillus*).

Chronic Invasive FRS (6cases, 20%)

This showed tissue invasion of fungal hyphae and severe acute inflammatory infiltrate, foci of necrosis and scattered giant cells. *Aspergillosis* was identified in 4 cases (Figure 2A & B) and in remaining 2 cases mucormycosis was seen. Of the 6 cases, fungi was visibly identified in 2 cases on H&E stain and in the remaining cases, fungi was identified on special GMS stains.

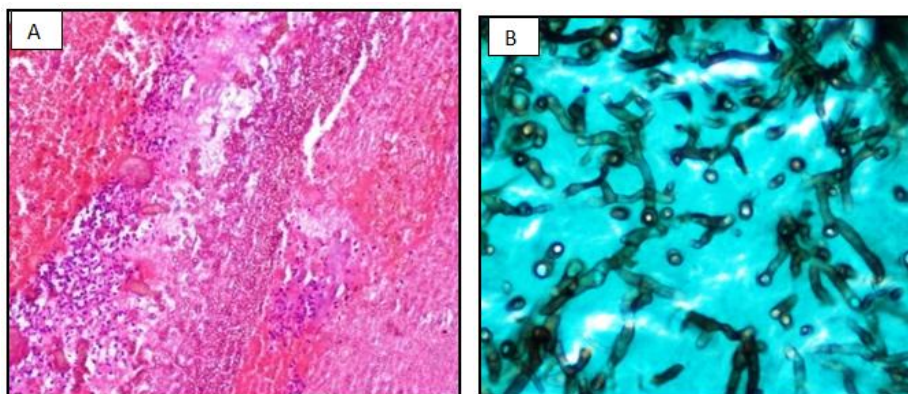


Figure 2. Photomicrograph showing A) Chronic invasive FS by acute angle branching septate fungal hyphae (Aspergillus) . areas of hemorrhage was seen. (H & E stain), B) GMS stain showing by acute angle branching fungal hyphae.

Chronic Granulomatous invasive FRS (6cases- 20%)

This condition showed multiple granulomatous inflammation involving the mucosa with fungal hyphae seen within the splendore-hoepli reaction .(Figure 3) Four out of the 6 cases showed splendore-hoepli reaction. The surrounding tissue showed moderate inflammatory infiltrate. Mucormycosis was identified in all 6 cases . Only in 2 cases fungi was visible on H&E and remaining cases were diagnosed on special stains.

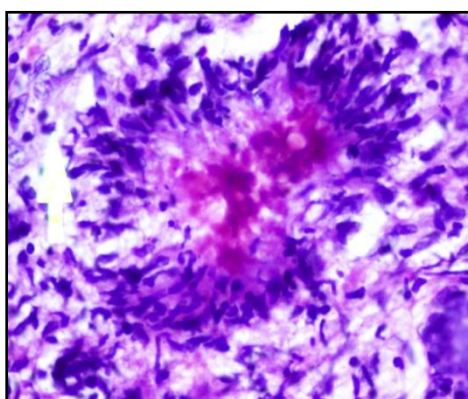


Figure 3) Photomicrograph showing Chronic Granulomatous invasive FS . Fungi seen within the splendore hoeplei reaction surrounding which granulomas are seen (H & E stain)

Acute Fulminant FRS (n=2cases-7%)

Both the cases presented with external ophthalmoplegia, periorbital swelling , altered mental status, stupor and coma. There was extensive areas of coagulative necrosis, mild inflammatory infiltrate and evidence of angioinvasion and invasion into the brain by fungal hyphae(Figure 4) .Both the cases were associated with zygomycetes (mucormycosis) having broad aseptate thin walled right angled branching hyphae (Figure 5). Fungi were readily visible on H&E stain in both the cases and mortality was 100%.

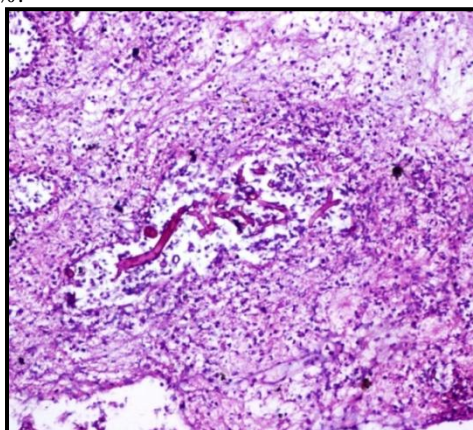


Figure 4: Acute Fulminant FS – Angioinvasion by the fungus . areas of necrosis and acute inflammatory infiltrate was seen. (H & E stain)

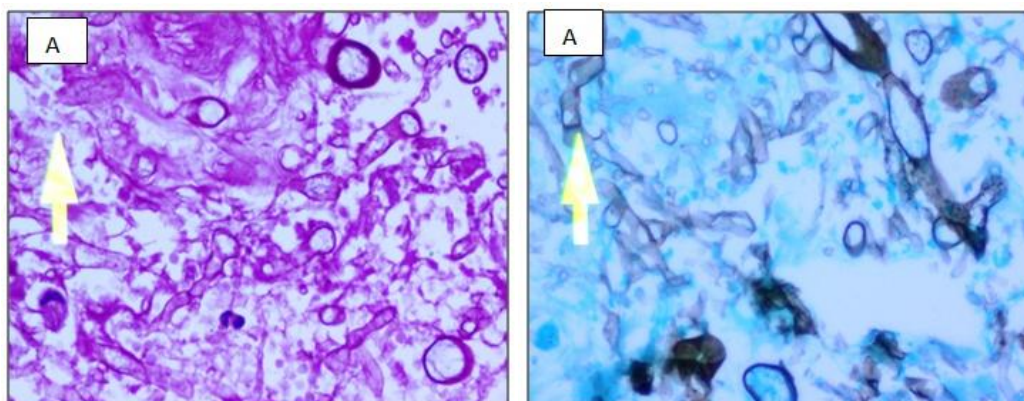


Figure 5) Photomicrograph A) showing mucormycosis - Broad aseptate thin walled right angled branching hyphae.(H & E stain) B) GMS stain.

Table 2: Classification of FRS in 30 cases

Classification of FRS	No of patients	Immune status	Fungi identified
Fungal Ball	16 cases (53%)	Immunocompetent-16(53%)	Aspergillus-16 cases (53%)
Chronic Invasive FRS	6 cases (20%)	Diabetes Mellitus-4(13%) Hodgkins Lymphoma-1(3.3%) Immunocompetent-1(3.3%)	Aspergillus- 4(13%) Mucormycosis-2(6.6%)
Chronic Granulomatous FRS	6 cases (20%)	Diabetes Mellitus-4(13%) Hodgkins Lymphoma- 1(3.3%) Immunocompetent-1(3.3%)	Mucormycosis- 6(20%)
Acute Fulminant FRS	2 cases	Diabetes Mellitus- 2 (6.6%)	Mucormycosis- 2(6.6%)

Correlation with Culture reports

Culture reports were available only for 20 cases. Most of the cases of fungal ball and chronic invasive FRS were associated with *Aspergillus flavus* in 15 cases. Other types of fungi grown were *Aspergillus fumigatus* (4cases). In Acute fulminant FRS (1 case) *rhizopus* was most commonly isolated fungus.

IV. Discussion

FRS, previously considered rare, is now being reported with increasing frequency worldwide. In India, this disease was initially considered to be prevalent only in the northern regions, but is now reported from other parts of the country also.⁷

Fungal sinusitis should be considered in all patients with chronic sinusitis, especially in association with certain clinical features like intractable symptoms despite adequate treatment for bacterial sinusitis, allergic rhinitis, asthma, nasal polyposis, (non invasive types) or fever, headache, epistaxis, diabetes, nasal mucosal ulcer, orbital apex syndrome, proptosis (invasive types).¹³ However the diagnosis of fungal sinusitis depends on direct microscopical examination, culture and histopathology of tissue or the cheesy material obtained from sinuses. 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. But when the lesion is restricted to the sinus, demonstration of histopathological invasion of mucous membrane is the only criterion to rely on.¹⁴

Based upon histopathological findings, FRS is categorised as (1) non invasive FRS, which includes AFRS and Fungal ball and (2) Invasive FRS, which include chronic invasive FRS, chronic invasive granulomatous FRS and acute fulminant FRS.⁸

AFRS constitutes 5-10% of all cases of CRS.⁹ These patients present with atopy, chronic intractable sinusitis with recurrent polyposis. Demonstration of fungal hyphae is important for diagnosis and differentiating allergic mucin from a newly described entity eosinophilic mucinous rhinosininitis in which allergic mucin resembles AFRS but no fungus is demonstrated on histopathology or culture.^{10,11} However in the present study none of the cases were diagnosed as AFRS. This is probably due to different climate and environmental factors.

In our study, Fungal ball was the most common type of FRS constituting 53% which was similar to Panda et al where in the incidence of fungal ball was 60%.¹² Patients with fungal ball present with nasal obstruction, chronic sinusitis with foetid smell. Diagnosing fungal ball/mycetoma is less challenging than other histologic categories of FS as fungal organisms were often abundant and easily seen on routine H&E stain. However, they can be mistaken for mucin or necrotic debris as they appear homogenous eosinophilic masses on

low power examination and hence higher magnification is mandatory for accuracy. Special stains such as GMS are required for confirmation.^{6,8} *Aspergillus flavus* was the common fungal organism isolated in all the cases.

The distinction between mycetoma with fungal growth adjacent to tissue and chronic invasive FS may be problematic, but the latter condition is characterised by hyphae actually within tissue, absence of fungal ball and presence of granulomatous inflammation.¹⁵ Inflammatory exudates adherent to periphery of fungal ball should not be considered tissue invasion.

Chronic invasive (Granulomatous and Non Granulomatous) FRS has a chronic course and is characterised by dense accumulation of fungal hyphae in the mucosal tissue surrounded by acute inflammatory infiltrate, occasional giant cells and in some cases demonstrate splendore- Hoepelli phenomenon. In the current study, four out of six cases of chronic granulomatous invasive FRS showed splendore-Hoepelli rection which is one of the diagnostic clue to identify the fungus.

Acute Fulminant/ invasive Fungal sinusitis is a life threatening systemic illness largely attributed to mucormycosis or aspergillosis in immunocompromised or diabetic patients.^{8,16} In the current study, the commonly involved fungus was mucormycosis. The term Rhino-Orbito-Cerebral zygomycosis (ROCM) refers to the entire spectrum of disease which usually starts in the sinonasal tissue (limited sinonasal disease), progresses to the orbits (limited rhino-orbital disease) and finally affects central nervous system (rhino-cerebral disease).¹⁷ Similar presentation was seen in 2 cases of our study and mortality was 100%.

Although both acute fulminant and chronic invasive FS are characterised by tissue invasion, they display many histologic differences. Acute fulminant FRS is characterised by necrosis, scant inflammation and vascular invasion. Pathological examination of peripheral areas sampled by surgeons is essential to assess adequacy of debridement.¹⁸

Management differs with the classification of fungal sinusitis. Acute fulminant FRS requires aggressive surgery and antifungal treatment. Chronic invasive/ granulomatous FRS requires surgical removal and antifungal therapy. Non invasive FRS(Fungal ball) requires surgery alone and allergic FRS requires steroids.

V. Conclusion

Though clinical presentation and radiological findings may provide diagnostic clue for each fungal sinusitis category, histopathological examination and classification of FRS into invasive or non invasive disease is important with regards to treatment.

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