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Increasing diagnostic yield in allergic fungal sinusitis

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Abstract
Diagnosis of allergic fungal sinusitis (AFS) in patients who present with rhinosinusitis and polyposis is based upon certain clinical, histopathological and mycological criteria. Specific diagnostic histopathological criteria are the demonstration of fungal hyphae in allergic mucin and absence of tissue invasion in the excised polyps. Previous reports have indicated difficulty in demonstrating fungal hyphae on histological examination in up to 75 per cent of cases. Analysis of a series of 25 patients with AFS, suggested methods to ensure demonstration of fungal hyphae and thus increase diagnostic yield in cases with suspected AFS.

Key words: Paranasal Sinus Diseases; Fungi; Diagnostic Techniques and Procedures

Introduction

Allergic fungal sinusitis (AFS), a disease characterized by fungal colonization of the nose and paranasal sinuses, has become an increasingly recognized entity over the past decade. Although first described in the early 1980s by Millar *et al.*[1] and Katzenstein *et al.*,[2] acceptance of the disease as an entity distinct from fungal infections of the sinuses has been slow in clinical practice. One of the reasons for this is the absence of standard diagnostic criteria. A review of the literature shows that an entire gamut of diagnostic criteria have been suggested by various authors over the years. However, four main criteria are universally found in patients with AFS (Table I).

The presence of allergic mucin either intraoperatively or at pre-operative endoscopy is diagnostic. Grossly, it consists of brownish-green, thick, viscid material of a peanut butter-like consistency lying interspersed with the nasal or sinus polyps. Histologically, it consists of layered mucus, clumps of cells, chiefly eosinophils (many of which are necrotic), Charcot Leyden crystals and fungal hyphae lying amidst the mucus layers (Figure 1). The nasal and sinus polyps are found to consist of oedematous mucosa with acute or chronic inflammatory infiltrate without any evidence of tissue invasion by fungus.

TABLE I
DIAGNOSTIC CRITERIA FOR ALLERGIC FUNGAL SINUSITIS

Rhinosinusitis with nasal polyposis
Presence of allergic mucin
Demonstration of fungal hyphae in allergic mucin by direct microscopy or special stains
Absence of tissue invasion histologically

While the assumption in most publications on AFS is that all the histological criteria are satisfied when the diagnosis is made, some authors have made a special mention of the absence of one or more histological features. Katzenstein *et al.*,[2] when first describing the entity, pointed out that in five of seven patients, fungal hyphae were not demonstrable on histopathological examination. They attributed this phenomenon to either sampling difficulties or the presence of an allergen other than *Aspergillus*. Diagnosis was confirmed by positive fungal culture. Similarly, Allphin *et al.*,[3] reported that, in 11 patients, no fungal hyphae were seen; one of these patients also had negative fungal culture.

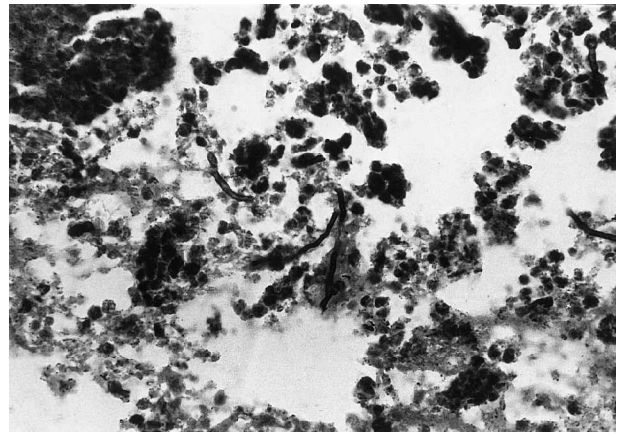

Fig. 1
Allergic mucin specimen showing fungal hyphae lying scattered in mucus between clumps of eosinophils and necrotic cells (GMS ×400).

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What is the reason for the inability to demonstrate fungus on histopathology? It is our hypothesis that the manner of specimen processing after excision is critical to the demonstration of fungal hyphae on histopathological examination once allergic mucin has been identified. The present study aims to analyse the role of specimen processing in successful demonstration of fungal hyphae and consequent establishment of a valid diagnosis in patients with suspected AFS.

Materials and methods

During the period July 1997 to July 2000, 33 nasal and sinus surgical procedures were performed in 25 patients with suspected AFS in the ENT Department of the Christian Medical College and Hospital, Vellore, India. All patients were found to have allergic mucin at surgery. The excised specimen consisting of polypoid tissue and allergic mucin was sent in formalin as a single specimen in 16 cases and as two specimens (polyps and allergic mucin separately) in 19 cases. Polyps and mucin were processed together as a single specimen in the first 16 cases. However, in the other 19 cases, polyps and mucin were processed as two separate specimens. Excised material was sent as a single specimen to the microbiology laboratory for fungal culture and direct microscopy for all 35 cases.

The slides were stained with haemotoxylin and eosin stain which demonstrated the various features of allergic mucin and polyps. Periodic-acid-Schiff (PAS) stain and Gomori-methenamine-silver (GMS) stain were used in all 35 cases to demonstrate fungal hyphae. All slides that were negative for fungus on initial examination were reviewed retrospectively. Each slide was examined several times before a pronouncement on the absence of fungal hyphae was made.

In the microbiology laboratory, several areas of each specimen were examined by direct microscopy after treatment of a 'crush' preparation with potassium hydroxide (KOH). The tissue was minced and cultured primarily on plates of Sabouraud's dextrose agar and incubated at both 28°C and 30°C.

Results

Fungal hyphae were demonstrated in all 19 cases in which allergic mucin and polyp was sent as two separate specimens, and in only eight of 16 cases in which a single specimen was sent. Fungal culture was positive in 24 of 25 patients. In the single patient in whom fungal culture was negative, fungal hyphae were seen on histology. In all patients, fungal hyphae were seen to lie loosely in mucus and there was no evidence of tissue invasion. *Aspergillus* species was the commonest fungal isolate. One case of *Curvularia* species and one case of *Dreschlera* species were also identified.

Discussion

Our hypothesis that the manner in which the excised specimen is submitted for histopathological examination is critical for demonstration of fungal hyphae in samples of allergic mucin, is well substantiated by the cent per cent positivity in those cases in which two specimens were sent. It is quite possible that the polyps are processed and allergic mucin not separately grossed and processed in a routine general pathology laboratory. Further, unless the processing of specimens is done by the same individual who examines the stained slide, emphasis on processing the allergic mucin separately and then staining it will not be given. In most centres where a large volume of specimens are being processed from various sites, this is likely to be the situation.

Allphin *et al.*,[3] when commenting on the large number of allergic mucin specimens which were negative for fungal hyphae in their series, suggested that these cases could be called cases of 'allergic mucin sinusitis without fungus'. Similarly, Cody *et al.*[4] found that 20 of 51 cases of rhinosinusitis with polyposis in whom fungus could not be isolated, had an 'allergic fungal sinusitis-like syndrome'. Concurring with both these reports, Ramadan *et al.*[5] went on to describe 12 patients with allergic mucin, eight being diagnosed as having classical allergic fungal sinusitis and four as having allergic mucin sinusitis without fungus. Comparison of these two small groups of patients, interestingly, revealed a marked similarity in the manner of presentation and laboratory results, leading the authors to conclude that allergic mucin sinusitis may not be a separate disease entity at all. Ramadan *et al.*[5] suggested that the fungus was not detected either on histopathology or by fungal culture because it was present in minimal amounts. The findings of our study show that separate examination of allergic mucin (in which fungal hyphae are concentrated) using special fungal stains will ensure detection of the elusive fungal hyphae. In four of our patients in whom a single specimen was sent, fungal hyphae were not detected on initial examination. A detailed search of the scanty areas of allergic mucin subsequently revealed a few hyphae. In contrast, in patients for whom two separate specimens were sent, a conclusive diagnosis was obtained at the very first instance. Close coordination between the attending clinician and the pathologist is vital in these cases.

As far as fungal culture is concerned, early processing and examination of several areas of adequate amounts of specimen is essential for a positive fungal culture. Previous reports have shown that up to 25 per cent of cases have negative fungal culture.[6] Delay in processing of the specimen more than six to eight hrs after excision leads to degradation of fungal elements.[7] Obtaining a positive KOH smear and/or fungal culture is of particular importance in cases of AFS in which histopathological examination is negative for fungal hyphae.

In conclusion, we emphasize that one of the important factors in being able to demonstrate fungal hyphae in allergic mucin in patients with suspected allergic fungal sinusitis, is separation of specimens into polyps and allergic mucin. Detailed search of the former for evidence of absence of tissue

invasion and of the latter for fungal hyphae lying loosely in the layered mucus, facilitates a conclusive diagnosis of allergic fungal sinusitis. This important step may prevent mischaracterization of these cases as allergic mucin sinusitis without fungus.

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