Fine Needle Aspiration Cytology: A Useful Technique for Diagnosis of Invasive Fungal Rhinosinusitis

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Abstract Mycotic infections are on the rise globally. Patients with invasive fungal infection of the paranasal sinuses often present with destructive mass lesions and mimic malignancy clinically and radiologically. To assess the utility of Fine needle aspiration cytology for early diagnosis of invasive fungal rhinosinusitis. Fine needle aspiration cytology was performed from the maxillary/ethmoid sinus in patients with a destructive mass lesion in the maxilla. Differential diagnoses were malignancy and fungal rhinosinusitis. In eight cases fungi were detected on initial examination whereas in a single case that was initially reported as giant cell lesion, hyphae could be identified within giant cells, on review. Smears showed inflammatory cells with variable numbers of eosinophils with neutrophils and histiocytes. Foreign body giant cells were seen in all cases. The fungi conformed to morphology of aspergillus in

seven cases (77.78 %); in two cases (22.22 %), typing could not be done. Periodic acid Schiff and Grocott stains highlighted the fungi in all the cases. Fine needle aspiration is a simple technique that can be useful for diagnosis of fungal rhinosinusitis and to exclude malignancy. Search for fungus may be more aggressive in smears with many foreign body giant cells and inflammatory cells and in cases with a high clinical suspicion. Differentiation between aspergillus and mucor can be made with help of special stains. *Aspergillus* is the commonest agent isolated. Preoperative cytological diagnosis obviates the need for biopsy, saves time and helps to plan proper treatment.

Keywords Fine needle aspiration (FNA) · Cytology · Invasive fungal rhinosinusitis · Aspergillosis · Mycotic rhinosinusitis · Paranasal sinus mycoses

The study was conducted on ethical guidelines for biomedical research on human subject as given in “Declaration of Helsinki” and by Central Ethics Committee on Human Research (CECHR) of ICMR, New Delhi.

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Introduction

Mycotic infections of the paranasal sinuses are on the rise globally. They are common in north India, and are now being recognized in other parts of India as well [1, 2]. *Aspergillus* is the commonest agent isolated. The spectrum of fungal diseases of the paranasal sinuses ranges from allergic sinusitis to invasive disease (fungal rhinosinusitis). Early diagnosis is important as the lesions may be rapidly progressive and destructive and may even be fatal. These lesions can mimic malignancy clinically and on radiology. Thus tissue/microbiological diagnosis is required for adequate treatment and proper management [2].

Fine needle aspiration (FNA) is a safe and simple technique widely used for rapid diagnosis of head and neck lesions. It can also be used for diagnosis of fungal etiology in rhino-sinusoidal inflammation. In literature there are

only few case reports which describe diagnosis of rhinosinusoidal mycoses on cytology [3–5]. We present a series of nine such cases diagnosed on FNA.

Materials and Methods

This is a retrospective study over a period of 5 years. Free hand FNA (without any radiological guidance) was performed from palpable swelling in infra or medial-orbital region from maxillary/ethmoid sinus, taking precaution that the needle was directed away from the orbit. Aspiration was performed with a 22 gauge needle attached to 10 cc syringe mounted on a Cameco's handle. Only one to two passes were made in each case, because only small part of the lesion was visible externally. Air dried and wet fixed smears were prepared and stained with May Grunwald Geimsa (MGG) and hematoxylin and eosin (H&E), respectively. Cultures could not be sent at the time of FNA, because the aspirates were relatively scanty and used up for making smears; however the clinicians sent material for culture in two cases at the time of surgical debridement. After examination of the stained slides, the smears in which profiles suspicious for fungus were detected, confirmation was done by employing periodic acid Schiff stain (PAS) and Grocott's stain after destaining the slides or on unstained smears, when available. The results were correlated with the clinical and radiological diagnosis and the features on smears as well as with the histopathological diagnosis, which was available in four cases.

Results

Out of total of nine cases; there were seven males and two females and the age varied from 25 to 67 years. The

patients presented with a palpable swelling in sino-nasal region ranging in size from 1.5 to 2 cm in diameter; with or without proptosis. Patients were presumed to be immuno-competent as they had not received chemotherapy, steroids, or other immunosuppressive agents. None of the patients had a diagnosis of cancer, chronic liver disease, end-stage renal failure, diabetes mellitus, HIV infection, or congenital immunodeficiency. On radiology, the lesions involved the nasal cavity in seven cases, maxillary sinus in eight cases and ethmoid sinuses in five cases, ranging in size from 3.5 to 6 cm, with evidence of bone destruction and soft tissue infiltration. The clinical and radiological suspicion was of either fungal infection or malignancy in four cases, only fungal infection in one case and of malignancy only in four cases (Table 1).

Fine needle aspiration smears were cellular in 8 cases; one had scanty cellularity. Smears showed an eosinophilic infiltrate admixed with neutrophils and histiocytes in six cases (66.7 %); only histiocytes in three cases (33.3 %) and neutrophils and lymphocytes only in a single case. Any allergic mucin was not found in any of the cases. Foreign body giant cells were seen in all cases; many of giant cells also showed presence of fungal profiles within them. The fungal profiles were septate and slender in six cases (66.7 %) and conformed to the morphology of aspergillus (Fig. 1). In a single case with a strong clinical and radiological suspicion of malignancy, there were predominantly giant cells and it was reported out as a giant cell lesion. On review of the case, aspergillus-like fungal profiles were seen within the giant cells, which were highlighted on PAS and Grocott stains (Fig. 2). In two cases, a diagnosis of fungal infection was given on FNA but typing could not be done (Table 2).

Based on FNA diagnoses, the patients were treated with surgical debridement followed by systemic anti-fungal

Table 1 Clinical and radiological features of the patients

Location	Presentation	Impression on CT scan
1 Left infraorbital region	Sinonasal mass with proptosis	Mass lesion ?fungal ?malignant
2 Right cheek	Nasal discharge, obstruction, swelling maxilla	Mass lesion ?fungal ?malignant
3 Right infratemporal fossa	Sinonasal polypoid mass, nasal discharge	Mass lesion ?fungal ?malignant
4 Left infraorbital region	Proptosis, mass lesion	Mass lesion ?malignant
5 Left nasofacial groove	Proptosis	Mass lesion ?malignant
6 Right infraorbital region	Sinonasal mass, nasal obstruction	Mass lesion ?malignant
7 Right infraorbital region	Proptosis, mass lesion	Mass lesion ?fungal ?malignant
8 Right cheek	Hard swelling, epistaxis	Mass lesion ?malignant
9 Right infraorbital region	Proptosis, polypoidal mass	Mass lesion ?fungal

CT scan computerized tomography

Fig. 1 Smear shows cluster of giant cells showing presence of slender septate hyphae conforming to morphology of *Aspergillus* with neutrophilic infiltrate in the background (MGG, $\times 200$)

Fig. 2 The hyphae are highlighted on PAS stain and demonstrate septations characteristic of *Aspergillus*. Background shows dense acute inflammatory infiltrate (PAS, $\times 400$)

therapy (Amphotericin B/Voriconazole) for 6–12 weeks in four cases and with only systemic anti-fungal treatment in five cases. They were subjected to regular follow up with endoscopy. Histopathology confirmed the diagnosis as aspergillus in two cases (Fig. 3). Of the two cases of diagnostic aspirates in which speciation could not be done on cytology; histopathology and cultures were available. One case showed broad aseptate hyphae conforming to morphology of *Mucor* spp. that corroborated with the culture report of *Conidiobolus* spp. In the other case, fungus was not detected on histopathological examination and was reported as chronic sinusitis; however culture of the tissue showed aspergillus.

Discussion and Conclusion

Aspergillosis is seen in warm and dry climatic conditions. It is particularly common in young men from rural background in north India [1]. Invasive aspergillosis of the paranasal sinuses in immunocompetent hosts was reported for the first time by Miloshev et al. [6]. Inhalation is the usual mode of infection but the disease is not contagious. The pathogenesis is attributed to local invasiveness and Types I and III hypersensitivity reactions. Predisposing factors for invasiveness in healthy adults include local obstruction, long duration of disease, improper treatment of non-invasive form and immune status of the patient [7].

In the current series, the patients presented with mass lesions involving nose and paranasal sinuses with and without proptosis, nasal discharge and obstruction, mimicking malignancy. This has been the experience with other centres as well [1–8]. Patients may also complain of facial and dental pain, headache and decreased vision. Ocular and neurological complications are seen in invasive forms and may even be fatal, thus it is imperative to make quick and correct diagnosis [1, 8]. Initial work up of the patients includes radiological assessment. Plain X-rays may show dense shadow akin to metal which is pathognomonic for aspergillosis. Computerised tomography (CT scan) findings that are suspicious for fungal rhino-sinusitis include edema of nasal and facial soft tissues, sinus muco-periosteal thickening, bone erosion, orbital and peri-antral soft tissue infiltration [9]. Magnetic resonance imaging (MRI) is restricted to patients suspected with intracranial extension. However, it is not always possible to differentiate an inflammatory lesion from malignancy. In the current series also, a differential diagnosis of malignancy was kept on CT in 88.88 % cases and of fungal etiology in 65.66 % cases. Thus tissue diagnosis is essential for adequate management.

The current study assesses the role of pre-operative FNA for this purpose. FNA provided a reliable generic diagnosis of inflammation and sparse to moderate inflammatory infiltrate and foreign body giant cells with or without necrosis were seen in all cases. A specific diagnosis can be made when fungal profiles are identified. FNA could correctly diagnose fungal infection in all but one case, in which fungi were detected on review. Fungi were present both intra and extra-cellularly. Recognition of the fungus is important on smears and erythrocytes, leucocytes, overstained naked nuclei, as well as foreign material like organic and non-organic fibres can mimic fungal profiles. Moreover, fungal profiles are not stained well with routine stains and special stains like PAS and silver impregnation stains (Grocott/Gomori methanamine silver—GMS) are necessary to highlight them. The hyphae take magenta color on PAS and are stained black on Grocott/GMS.

Table 2 Character of aspirates and cytological features of all the cases

	Aspirate	Foreign body giant cells	Inflammatory infiltrate	Morphology of fungus	Final impression	Histopathology/culture
1	Hemorrhagic, particulate	Few	Abundant; histiocytes, eosinophils	Slender, septate with branching within giant cells; PAS +ve	Fungal granuloma compatible with Aspergillosis	NA
2	Hemorrhagic	Few	Abundant; neutrophils, lymphocytes	Slender, septate with branching within giant cells; PAS +ve	Fungal granuloma compatible with Aspergillosis	Chronic invasive rhinosinusitis due to <i>Aspergillus</i>
3	Hemorrhagic	Few	Abundant; epithelioid cells	Slender, septate with branching within giant cells; PAS +ve	Fungal granuloma compatible with Aspergillosis	NA
4	Fluidy with whitish specks	Few	Abundant; histiocytes, necrotic debris, cholesterol crystals	Slender, septate with branching within giant cells; PAS +ve	Compatible with fungal granuloma (Aspergillosis)	Chronic invasive rhinosinusitis due to <i>Aspergillus</i>
5	Particulate	Numerous	Scanty; eosinophils, neutrophils	Occasional PAS +ve, Grocott +ve fungal profile within giant cell	Chronic fungal inflammation	Chronic invasive rhinosinusitis due to <i>Mucor</i> ; culture: <i>Conidiobolus</i> spp.
6	Hemorrhagic, particulate	Few	Abundant; neutrophils, lymphocytes, histiocytes	Slender, septate with branching intra- and extra-cellular; PAS +ve	Inflammatory lesion with Aspergillosis	NA
7	Hemorrhagic, particulate	–	Osteoclast like giant cells, few spindle cells	No evidence of malignancy	Giant cell lesion; review cytology: PAS +ve hyphae in giant cells	NA
8	Pus	Few	Abundant; neutrophils, lymphocytes, histiocytes	Occasional fungal profile highlighted on PAS and Grocott stains	Fungal infection	Chronic sinusitis; no fungal profiles detected/culture: <i>Aspergillus</i>
9	Hemorrhagic	Few	Few neutrophils, eosinophils, cell debris	Slender, septate with branching intra- and extra-cellular; PAS +ve	Fungal infection compatible with Aspergillosis	NA

PAS periodic acid Schiff stain, NA not available

Fig. 3 Histopatological section also showed foreign body giant cell granuloma with negatively stained slender fungal hyphae (Haematoxylin and Eosin, ×400)

Demonstration of an inflammatory reaction determines that the organism represents true infection and not contamination.

Due to the presence of many foreign body giant cells; differential diagnoses included other giant cell rich lesions. In nose and paranasal sinuses these include infective conditions like tuberculosis, leprosy, rhinoscleroma, actinomycosis and fungal infections like rhinosporidiosis. Non-infectious causes are Wegener's granulomatosis, sarcoidosis and cocaine abuse [10]. Giant cell reparative granuloma, Langerhans cell histiocytosis, giant cell tumor, brown tumor of hyperparathyroidism and aneurysmal bone cyst are other differential diagnosis of bony destructive lesions with presence of giant cells [10].

Further it is essential to distinguish between the different fungi, as the therapy is different for them. The slender septate hyphae 3–4 microns wide, with acute angled

branching are characteristic of aspergillus whereas broad, aseptate hyphae with irregular obtuse-angled branching are seen in phycomycetes (mucor and rhizopus). Another chronic granulomatous infective disorder commonly seen in this location is rhinosporidiosis, which is caused by *Rhinosporidium seeberi*, but is no longer considered as a classic fungus. Cytological smears show basophilic endospores and amorphous eosinophilic debris. Treatment is mainly surgical and histopathology shows sporangia full of endospores in different stages of development [11].

In the current study, accuracy of fungal identification on microscopy in cytologic specimens was 79 %; in two cases it was not possible to type the fungus on smears alone. *Aspergillus* infection was most common (eight cases; 88.89 %). At times, fragmented or scanty fungal hyphae and extensive necrosis distorts the morphology may make it impossible to subtype. Certain fungi like *Zygomycetes*, *Pseudallescheria boydii*, and *Fusarium* spp. cause confusion with aspergillus on cytology and histopathology [12]. Thus, cultures are required for exact speciation. However, fungal cultures take time and may be negative. In a study they were negative in 36.6 % cases of invasive fungal rhinosinusitis.

Aspergillus rhinosinusitis occurs exclusively with *Aspergillus flavus* and can be divided into non-invasive (allergic fungal rhinosinusitis and fungal balls) and chronic invasive fungal rhinosinusitis (with and without granulomatous reaction) and acute fulminant rhinosinusitis [13]. In chronic invasive cases, histopathology shows fungal hyphae with Splendor Hoeppli reaction and acute inflammatory cells, necrosis and scattered giant cells. There may be a granulomatous reaction with hyphae present within the giant cells. The surrounding tissue shows fibrosis and mild inflammation rich in eosinophils and lymphocytes. Acute fulminant cases show extensive necrosis, scant inflammation and evidence of angioinvasion. It is also associated with zygomycetes fungi. Acute form of the disease is increasing due to increase in number of patients on chemotherapy, steroids or immunosuppressive treatment, transplant recipients, and patients with diabetes [8].

Treatment includes surgical debridement and sinus ventilation followed by Amphotericin B and Voriconazole or combination of both followed by long term Itraconazole [14]. In this study, surgery was required in only 44.44 % cases and the rest were treated by systemic anti-fungal regimens with close follow-up of the patients.

To summarize, fungal rhinosinusitis can closely mimic malignancy, clinically. FNA is a reliable, simple and quick technique for its diagnosis. The search for fungal profiles should be more aggressive in smears rich in inflammatory and foreign body giant cells, or in cases with a strong

clinical or radiological suspicion. In most cases, differentiation between the two most common offending fungi i.e. aspergillus and mucor can be made on cytology with the help of special stains like PAS and Grocott's. Thus, pre-operative FNA diagnosis obviates the need for a diagnostic biopsy, allows rapid diagnosis as cultures take time and helps to plan proper treatment to suit individual patients.

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References

1. Chakrabarti A, Sharma SC. Paranasal sinus mycoses. Indian J Chest Dis Allied Sci. 2000;42:293–304.
2. Panda NK, Sharma SC, Chakrabarti A, Mann SBS. Paranasal sinus mycoses in north India. Mycoses. 1998;41:281–6.
3. Sharma D, Mahajan N, Rao S, Khurana N, Jain S. Invasive maxillary aspergillosis masquerading as malignancy in two cases: utility of cytology as a rapid diagnostic tool. J Cytol. 2012; 29:194–6.
4. Kumar BS, Patro M, Mishra D, Bal A, Behera B, Sahoo S. Fine needle aspiration in aspergilloma of frontal sinus: a case report. Acta Cytol. 2008;52:500–4.
5. Singh N, Siddharaju N, Kumar S, Muniraj F, Bakshi S, Gopal-krishnan S. Fine needle aspiration as an initial diagnostic modality in a clinically unsuspected case of invasive maxillary fungal sinusitis. Diagn Cytopathol. 2010;38:290–3.
6. Miloshev B, Davidson CM, Gentles JC, Sandison AT. Aspergilloma of paranasal sinuses and orbit in Northern Sudanese. Lancet. 1966;1:746–7.
7. Berdana EJ Jr. The clinical spectrum of aspergillosis. CRC Crit Rev Clin Lab Sci. 1980;13:85–159.
8. Singhal SK, Dass A, Singh GB, Punia RPS, Nagarkar N. Destructive aspergillosis. Indian J Otolaryngol Head Neck Surg. 2005;57:244–6.
9. DelGaudio JM, Swain RE Jr, Kingdom TT, et al. Computed tomographic findings in patients with invasive fungal sinusitis. Arch Otolaryngol Head Neck Surg. 2003;129(2):236–40.
10. Lund VJ. Granulomatous diseases and tumors of nose and paranasal sinuses. In: Kennedy DW, Bolger WE, Zinreich SJ, editors. Diseases of the sinuses: diagnosis and management. London: B.C. Decker Inc.; 2011. p. 85–105.
11. Sinha A, Phukan JP, Bandyopadhyay G, et al. Clinicopathological study of rhinosporidiosis with special reference to cytodiagnosis. J Cytol. 2012;29:246–9.
12. Sangoi AR, Rogers WM, Longacre TA, et al. Challenges and pitfalls of morphologic identification of fungal infections in histologic and cytologic specimens. A ten-year retrospective review at a single institution. Am J Clin Pathol. 2001;131:364–75.
13. Das A, Bal A, Chakrabarti A, Panda N, Joshi K. Spectrum of fungal rhinosinusitis; A histopathologist's perspective. Histopathology. 2009;54:854–9.
14. Han DH, An SY, Kim SW, et al. Primary and secondary fungal infections of the paranasal sinuses: clinical features and treatment outcomes. Acta Otolaryngol. 2007;558:78–82.