Medical Mycology 2012, **Early Online:** 1–6

informa
healthcare

Frequency of fungal isolation and antifungal susceptibility pattern of the fungal isolates from nasal polyps of chronic rhinosinusitis patients at a tertiary care centre in north India

SARIKA JAIN*, SHUKLA DAS*, NEELIMA GUPTA† & JUNAID NASIM MALIK†

*Departments of *Microbiology & †Otorhinolaryngology, University College of Medical Sciences and Guru Tegh Bahadur Hospital, Dilshad Garden, Delhi, India*

Sinonasal polyposis is considered to be the end-result of a chronic inflammatory process in the sinonasal mucosa. Its underlying mechanisms are still unclear, but the involvement of fungi has been suggested for many years. In the present study, we retrospectively evaluated the clinical and mycological profile of 161 patients with chronic rhinosinusitis (CRS) and nasal polyps who were undergoing surgery at our tertiary care facility during 2002 to 2010. CT scan findings and per-operative presence of allergic mucin were provisionally suggestive of fungal rhinosinusitis (FRS) in all the patients. Total serum IgE and peripheral eosinophilia were noted. Histological examination of polyp tissue showed eosinophilic mucin in 100% of the cases and the incidence of allergic fungal rhinosinusitis (AFRS) was 83.9% in the patient population. KOH and/or culture were positive for fungal hyphae or yeast in 93% (150/161) of the patients. *Aspergillus* spp. were the most commonly recovered isolates (70%). MICs of all *A. flavus* and *A. fumigatus* isolates were within the susceptible zone for itraconazole, voriconazole, and amphoterecin B. In conclusion, allergic fungal rhinosinusitis (FRS) is a common disorder in patients with sinonasal polyposis and due to its recurrent and intractable nature, a high degree of clinical suspicion for the presence of FRS in nasal polyposis should be considered.

Keywords chronic rhinosinusitis, nasal polyps, *Aspergillus*, anti-fungal susceptibility

Introduction

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease of the nasal and paranasal sinus mucosa lasting for a duration longer than 3 months. The typical symptoms involve nasal obstruction, posterior nasal drip, a reduction/loss of the ability to smell, facial pressure and/or pain, and in some cases, nasal polyposis. Around 4% of the adult population suffer from a significantly compromised quality of life and huge medical costs as a result of the disease [1–3]. The exact pathogenesis remains unclear but various etiologies have been implicated such as anatomical variants, microbial infection and/or colonization, fungal stimulation, atopic response, acetyl salicylic acid intolerance and a combination of the above. These factors may be possible initial triggers that up-regulate inflammation of the lateral wall of the nose resulting in the development of nasal polyposis [4,5].

It is suggested that there may be a unique immune response to fungal antigen in patients with CRS that induces production of cytokines and drives intense heterogeneous eosinophilic inflammation which is absent in healthy controls. The fungal spore germinates in the mucin and continues to provide an antigenic stimulus which ultimately results in polyps and hyperplastic mucosa formation [6–9]. Since the incidence of fungal colonization has been shown to be similar in healthy controls, the exact relevance of fungi in CRS still remains doubtful and it is suggested that the pathophysiology of disease is probably a mucosal hypersensitivity directed against fungal antigens deposited on sinus mucosa rather than true infections [10,11]. Interestingly IL-17 has recently been implicated in regulating

Received 5 January 2012; Received in final form 21 April 2012; Accepted 14 May 2012

Correspondence: Sarika Jain, Department of Microbiology, University College of Medical Sciences and Guru Tegh Bahadur Hospital, Microbiology, Dilshad Garden, Delhi, 110095 India. E-mail: drsarika6@gmail.com

DOI: 10.3109/13693786.2012.694486

the atopic inflammation in NP by attracting the eosinophils and subsequent tissue reaction [12]. Fungal agents commonly isolated from patients with CRS are *Aspergillus*, *Penicillium*, *Cladosporium*, and *Aternaria* [9].

The management of CRS is difficult, and post-surgery recurrence is very common [13,14]. Although the role of fungi in CRS is controversial, often the condition has been treated with antifungal protocols. Topical intranasal application of amphotericin B (AmB) or long-term oral itraconazole may decrease the fungal load in the sinonasal region, and subsequent eosinophilic inflammatory reaction to fungal antigens [15,16]. In this context, early diagnosis of non-invasive fungal sinusitis would be crucial to prevent multiple surgical procedures and improve long-term outcomes in these patients.

Therefore, the aims of this study were to determine the frequency of fungi isolated in cultures of portions of nasal polyps, the antifungal susceptibilities of the most common of these fungi, and evaluate the clinico-mycological profile of the patients with sinonasal polyposis presented at our tertiary care hospital in north India.

Material and methods

Patients

A total of 161 patients who presented with sinonasal polyposis over a period of 8 years (April 2002–March 2010) who were undergoing functional endoscopic sinus surgery (FESS) were included in the study undertaken at the departments of Otorhinolaryngology and Microbiology of our tertiary care hospital. Twenty percent of these patients had a recurrent episode and were admitted for revision surgery, although none were previously admitted to our hospital. A retrospective review of the medical records was conducted to obtain demographic profiles, clinical presentations including previous history of allergy or complaints of breathlessness, cough or asthma (described as more than two episodes of wheezing clinically suggestive of an allergic episode or patients having received adrenergic stimulants or responded to steroids in the past for such a condition). In addition, data on the duration of nasal polyposis, previous surgery, laboratory findings (total peripheral eosinophil count, IgE) and medical treatment were part of the survey. The diagnosis of CRS was formed according to definition by the European 2007 position paper on chronic rhinosinusitis [1]. The CRS cases were clinically diagnosed as allergic fungal chronic sinusitis in the adult age group of patients based on criteria proposed by Bent and Kuhn [17], which included a strong suspicion of fungal etiology in patients who had undergone sinus surgery. The major criteria included: (1) nasal polyposis, (2) type-1 hypersensitivity by history, skin testing or *in vitro* testing, (3) characteristic computed tomographic findings, (4) eosinophilic mucin without tissue invasion, and (5) positive fungal smear. The minor criteria involved: (i) asthma, (ii) unilateral predominance of disease, (iii) serum eosinophilia, (iv) charcot laden crystals, (v) fungal culture, and (vi) bone erosion.

Diagnostic nasal endoscopic examinations and pre-operative sinus CT scans were performed for each CRS patient to observe the extent of disease involvement (Fig. 1). Per-operatively, polyp tissue was collected from each patient and sent to the laboratory for mycological processing (KOH and fungal culture) in 0.9% normal saline and histological examination in 10% formalin.

Mycological processing of samples

A total of 161 specimens were received in the mycology division of the microbiology laboratory of University College of Medical Sciences & Guru Teg Bahadur Hospital. All samples were processed under laminar flow. Biopsied tissues were cut into small pieces with sterile scissors and homogenized in a mortar by gentle grinding and then inoculated onto Sabouraud dextrose agar (SDA) with antibiotics (chloramphenicol – 0.5 g/l, gentamicin 0.04 g/l) and incubated at 25°C for 4 weeks with weekly monitoring before being considered as negative for fungal growth. Direct microscopic examination of tissue specimen after digestion with 10% potassium hydroxide (KOH) was performed to screen for any fungal elements (hyphae or yeast cells). Identification of different mycelial isolates was based on the macroscopic characteristics and lactophenol cotton blue microscopic examination of the fungal colonies growing on the medium or by slide culture mounts. Yeasts were identified by standard biochemical tests.

Antifungal susceptibility testing was performed for *Aspergillus flavus* and *A. fumigatus* isolates. Minimum inhibitory concentrations (MICs) were determined for

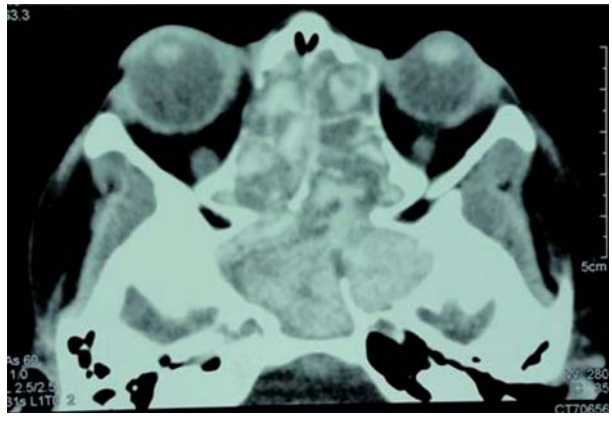

Fig. 1 Computed tomography scan showing nasal polyps along with hyper-densities as seen in allergic fungal sinusitis (AFS).

itraconazole, voriconazole and amphotericin B by broth microdilution method following the standard method M38A of the Clinical and Laboratory Standards Institute (CLSI). This same organization's breakpoint levels are included in Table 3.

Serum total immunoglobulin E (IgE) levels

Serum IgE levels were measured by ELISA, using commercially available kit from Calbiotech Inc., CA, USA. The manufacturer's instructions were followed and IgE values were expressed in IU/ml.

Results

The medical records of 161 patients (108 males and 53 females) included in our study were reviewed which indicated that their mean age was 32 years (range 28–56 years). The patients' frequent clinical manifestations are described in Table 1. Nasal obstruction (100%) and post nasal drip (100%) were the major presenting symptoms. On endoscopic examination, multiple pale grey polypoid masses were revealed in the nasal cavities of all the patients (100%). Per-operatively, thick greenish to brown allergic mucin was found in 60% the patients undergoing functional endoscopic sinus surgery (Table 1). A provisional diagnosis of fungal rhinosinusitis was made based on characteristic findings of computed tomographic (CT) scan of nose and paranasal sinuses. The CT studies showed diffuse heterogeneous masses of hyper-attenuation, with or without intervening areas of lesser density, polypoid or nodular masses, were observed in all the patients. Elevated IgE levels ranged from 830–1,580 IU/ml (normal value of 175 IU/ml in females and 250 IU/ml in males) was observed in 87% of our patients (Table 2).

In 150 of the 161 (93%) patients with clinically suspected FRS there was evidence of fungal infections as indicated by either direct microscopy and/or culture. Of the 161 specimens from these individuals, 110 (68.2%) and 84 (52.1%) were positive for fungi by direct microscopic examination (KOH) and culture, respectively. In 11 of these 161 patients (6.8%), no evidence of fungal elements was seen. *Aspergillus* species (70%) and *Fusarium* spp. (9.8%) were the most common isolates identified in the culture positive samples (Table 3). The drug susceptibility testing of *A. flavus* and *A. fumigatus* showed 100% susceptibility to itraconazole, voriconazole and amphotericin B (Table 4). Based on Bent and Kuhn criteria, the incidence of AFS among 161 patients with sinonasal polyposis was 83.9% (135/161). In 16.1% of the patients (26/161), fungal hyphae were not seen within eosinophilic mucin.

Discussion

Nasal polyps are lesions that originate from any portion of the nasal mucosa or paranasal sinuses, seen more commonly in adults and are the end-result of various inflammatory disease processes involving the paranasal sinuses. Multiple polyps can occur in patients with chronic sinusitis, allergic rhinitis, cystic fibrosis (CF), or allergic fungal sinusitis (AFS). The patients involved in our study were predominantly young males presenting with multiple nasal polyposis, both unilateral, bilateral and multiple sinuses opacification, principally involving the ethmoidal and maxillary sinuses. Similar observations have been reported in other studies [18,19]. The majority of the patients presented with nasal obstruction, nasal discharge, post-nasal

Table 1 Clinical characteristics of patients with chronic rhinosinusitis and nasal polyposis.

Clinical characteristic	*n* (%)
Mean age in years (range)	32 (28–56)
Sex (Male: female)	2:1
Symptoms	
Nasal purulent and thick discharge	150 (93%)
Nasal obstruction	161 (100%)
Posterior secretion	161 (100%)
Headache	122 (76%)
Nasal congestion	130 (81%)
Anosmia	141 (88%)
Nasal polyp	
Multiple	161 (100%)
Unilateral	96 (60%)
Bilateral	65(40%)
CT scan findings	65 (40%)
(Hyperattenuation within sinuses)	161 (100%)
Maxillary sinus	161 (100%)
Ethmoid sinus	161 (100%)
Sphenoid sinus	64 (40%)
Frontal sinus	101 (63%)
Bilateral	
Asthma	17 (28%)

CT, Computed tomography.

Table 2 Laboratory results of patients with sinonasal polyposis.

Laboratory parameters	*n* (%)
Histological findings:	
Eosinophilic mucin	97 (60%)
Fungal hyphae with eosinophilic mucin	135 (83.5%)
Angio-invasion by fungal hyphae	0
Peripheral blood eosinophilia	130 (81%)
Total serum IgE level	140 (87%)
Mycological results:	
Total mycological positive samples	150 (93%)
Positive KOH only	58 (36%)
Positive Fungal culture only	32 (19.9%)
Both KOH and Fungal culture positive	52 (32.2%)

IgE, Immunoglobulin E; KOH, Potassium hydroxide.

Table 3 Isolated fungal species from 84 positive fungal cultures in patients with sinonasal polyposis.

Fungal species	*n* (%)
Aspergillus flavus	47 (55.9%)
Aspergillus fumigatus	12 (14.3%)
Aspergillus terreus	1 (1.2%)
Fusarium	8 (9.8%)
Bipolaris	6 (7.4%)
Alternaria	4 (4.9%)
Cladosporium	2 (2.4%)
Trichosporon	2 (2.4%)
Candida parapsilosis	2 (2.4%)
Total	84 (100%)

drip as the predominant symptoms, which is in agreement with the findings of Baloch *et al*. and Thahim *et al*. [20,21]. However, only a portion of patients with CRS have nasal polyposis (20–33%), they likely represent a disproportionate number of recalcitrant patients [22]. It has been frequently suggested that a fungus-mediated process is the primary cause of CRS with and without polyps wherein fungal colonization is followed by an allergic or a mixed Th-1 and Th-2 type immunologic reaction by the host [23]. Although several fungal species have been associated with CRS and healthy controls, disease manifestation has been consistently found in those previously sensitized to the fungal agent, initiating an eosinophilic reaction [24–26].

Of our patients with CRS and nasal polyposis, 89.3% were diagnosed with AFRS. Characteristic allergic or eosinophilic mucin with greenish brown or black peanut buttery consistency, and consisting of eosinophils, charcot-leyden crystals, in which non-invasive fungal hyphae were seen. Allergic or eosinophilic mucin was found per-operatively in 60% of our patients, and fungal hyphae without tissue invasion in 83.9% cases which was confirmed on histological examination of polyp tissue. Braun *et al*. also reported the presence of fungi and eosinophilic mucin in nasal mucus in majority of CRS patients [27] as the hallmark for diagnosis of AFS. However, hyphae can be easily missed because hyphae are sparse in sinus content, and considerable time is required to visualize with the currently used stains. Thus an AFRS or EFRS (eosinophilic fungal rhinosinusitis) may often be misdiagnosed as EMRS (eosinophilic mucin rhinosinusitis) [28–30]. It may then lead to misdiagnosis of allergic fungal sinusitis or incorrectly classified for the want of increased awareness of the distinct morphologic features of this entity among clinicians, as well as histopathologists [31]. It can also be a possibility that the section of polyp tissue that is histologically examined is not a true representative of the pathological process and may have a poor hyphal content which leads to missed diagnosis. For these reasons, the diagnosis of AFRS cannot be ruled out completely in 16.1% of our patients in whom fungal hyphae were not seen within eosinophilic mucin and the clinical history was suggestive of asthma. Moreover, high IgE levels, characteristic CT scan findings and fungal smear and/or cultures positive for *Aspergillus* species makes it difficult to assume these cases to be EMRS and not AFRS [32]. In such situations, if KOH mount or culture shows fungal hyphae/fungal growth, pathologists should be requested to repeat the section and do a thorough re-examination to ensure that technical errors have not occurred. Ferguson had shown that the polyp occurrence was almost 100% in both AFRS and EMRS but asthma was greater in patients with EMRS (93%) compared to ARFS (41%). Other studies also indicate that asthma is not a significant and a consistent factor in nasal polyposis or AFRS [33–35].

Our study also measured total serum IgE levels to confirm the diagnosis of allergic fungal rhinosinusitis which is found to be higher in 87% of patients. Kuhn and Javer have stated that the total serum IgE levels could be used as a marker to detect disease recurrence [13]. Total IgE may help in differentiating between eosinophilic mucin rhinosinusitis and AFRS, where these are significantly elevated in latter cases [29]. Recently, Elmorsy and colleagues reported that among patients with nasal polyposis, levels of IgE and IL-13 in polyp fluid and sera were significantly higher in allergic groups compared to that in non-allergic groups [36,37]. Shen *et al*. have recently highlighted the role of atopy on Th17/Treg subset of T cell population which can aggravate nasal polyp formation by influencing a defective suppression of Treg on Th1 and Th2 [12].

Table 4 Antifungal susceptibilities of *Aspergillus* strains from patients with sinonasal polyposis.

	Aspergillus flavus (*n* = 47)			*Aspergillus fumigatus* (*n* = 12)		
Antifungal agents	Breakpoint concentration (mcg/ml)	MIC obtained after 48 hours (mcg/ml)	Susceptible strains (%)	Breakpoint concentration (mcg/ml)	MIC obtained after 48 hours (mcg/ml)	Susceptible strains (%)
Itraconazole	0.25–0.5	0.03–0.2	100	0.125–1	0.01–0.2	100
Voriconazole	0.5–4	0.25–1	100	0.25	0.12–0.25	100
Amphotericin B	0.5–4.0	0.25–2	100	0.5–2	0.08–1	100

MIC, Minimum inhibitory concentration; mcg, microgram; ml, millilitre.

Aspergillus flavus was the most common species (55.9%) isolated from samples of our patients with histologically confirmed AFRS. This is in agreement to other studies from India and Middle-East [38,39]. *Fusarium* (9.8%) followed by *Bipolaris* (7.4%) were the other dematecious fungi isolated [40–42]. However, in the USA, particularly in the South and Southwest, the majority of cases of AFRS are associated with dematiaceous fungi such as *Bipolaris*, *Curvularia*, and *Alternaria*. This variable incidence of fungi may be due to differences in geographic distribution or different diagnostic techniques [43]. Keeping the positivity (64–100%) of fungal cultures in mind, a diagnosis of AFRS is possible in the context of negative fungal cultures, and hence should be considered as supportive evidence [28]. In our study, some patients had positive direct microscopic examination but their cultures were negative for fungal growth. Similar finding has also been observed by Hedayati *et al*. [38].

Although surgery has long been a treatment of choice for persistent CRS, surgically excised polyps inevitably recur without aggressive medical management [44–46].

There have been documented variable outcomes in patients with AFRS [47,48]. Gerlinger *et al*. speculated that one of the possible reasons for ineffectiveness of antifungal therapy in patients with CRS and NPs could be due to differences in sensitivity of the various fungi to amphotericin B [49]. Nonetheless, Kumar *et al*. from north India found 100% of the 25 strains of *A. flavus* including 13 from paranasal sinus mycoses were sensitive to amphotericin B and itraconazole [42,50].

In our study, antifungal susceptibility rates for the most common isolates, *A. flavus* and *A. fumigatus*, from our patients with CRS and NP, were 100% to amphoterecin B, itraconazole and voriconazole. An important explanation suggested for the poor response to antifungal was that the amount of drug penetrating the bottom of sinuses filled with mucin and polyps may be insufficient [49]. Topical use amphotericin B in the form of intranasal lavage has no additional benefit to intranasal steroids and irrigations, on nasal polyps [47].

In conclusion, FRS is a common disorder in patients with sinonasal polyposis and in concordance with other studies from warm and humid regions, we also showed that *A. flavus* is the prevalent fungus recovered from these patients. The role of fungi in this condition appears controversial. Although antifungals, particularly Amphotericin B, appear to be a rational choice due to its high susceptibility and safety profile, prospective studies with long-term follow-up are needed in patients with AFS in order to determine if changes in dosage, concentration, treatment duration or route of administration could lead to significant improvement in the outcome.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1 Fokkens W, Lund V, Mullol J, *et al*. European position paper (EPP) on rhinosinusitis and nasal polyps 2007. *Rhinol Suppl* 2007; **20**: 1–136.
2 Kaliner MA, Osguthorpe JD, Fireman P, *et al*. Sinusitis: bench to bedside. Current findings, future directions. *Otolaryngol Head Neck Surg* 1997; **116**: S1–5.
3 Virat K. Update on nasal polyps: etiopathogenesis. *J Med Assoc Thai* 2005; **88**: 1966–1972.
4 Bachert C, Wagenmann M, Rudack C, *et al*. The role of cytokines in infectious sinusitis and nasal polyposis. *Allergy* 1998; **53**: 2–13.
5 Ricchetti A, Landis BN, Maffioli A, *et al*. Effect of anti-fungal nasal lavage with amphotericin B on nasal polyposis. *J Laryngol Otol* 2002; **116**: 261–263.
6 Ferguson BJ. Categorization of eosinophilic chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg* 2004; **12**: 237–242.
7 Sasama J, Sherris DA, Shin SH, *et al*. New paradigm for the role of fungi and eosinophils in chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg* 2005; **13**: 2–8.
8 Marple BF, Mabry RL. The role of fungus in chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg* 2002; **10**: 207–212.
9 Ponikau JU, Sherris DA, Kern EB, *et al*. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999; **74**: 877–884.
10 Karthikeyan P, Nirmal Coumare V. Incidence and presentation of fungal sinusitis in patient diagnosed with chronic rhinosinusitis. *Ind J Otolaryngol Head Neck Surg* 2010; **62**: 381–385.
11 Rozanska-Kudelska M. Mold fungi and the role of allergy on fungi in chronic rhinosinusitis. *Otolaryngol Pol* 2009; **63**: 245–248.
12 Shen Y, Tang X-Y, Yang Y-C, *et al*. Impaired balance of Th17/Treg in patients with nasal polyposis. *Clin Immunol* 2011; **74**: 176–185.
13 Kuhn FA, Javer AR. Allergic fungal sinusitis: a four-year follow-up. *Am J Rhinol* 2000; **14**: 149–156.
14 Kupferberg SB, Bent JP III, Kuhn FA. Prognosis for allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1997; **117**: 35–41.
15 Ponikau JU, Sherris DA, Weaver A, Kita H. Treatment of chronic rhinosinusitis with intranasal amphotericin B: a randomized, placebo-controlled, double-blind pilot trial. *J Allergy Clin Immunol* 2005; **115**: 125–131.
16 Rains BM 3rd, Mineck CW. Treatment of allergic fungal sinusitis with high-dose itraconazole. *Am J Rhinol* 2003; **17**: 1–8.
17 Bent JP 3rd, Kuhn FA. Diagnosis of allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1994; **111**: 580–588.
18 Akhtar MR, Ishaque M, Saadatulah S. Aetiology of nasal polyp: a study of 200 cases at Combined Military Hospital Rawalpindi. *Pak J Otolaryngol* 2004; **20**: 9–11.
19 Tritt S, McMains KC, Kountakis SE. Unilateral nasal polyposis: clinical presentation and pathology. *Am J Otolaryngol* 2008; **29**: 230–232.
20 Baloch ZA, Ahmad NA, Mahmood Z, *et al*. Frequency of allergic fungal sinusitis in patients with nasal polyposis and its causative species. *Pak J Otolaryngol* 2010; **26**: 76–77.
21 Thahim K, Jawaid MA, Marfani MS. Presentation and management of allergic fungal sinusitis. *J Coll Physicians Surg Pak* 2007; **17**: 23–27.
22 DeMarcantonio MA, Han JK. Nasal polyps: pathogenesis and treatment implications. *Otolaryngol Clin N Am* 2011; **44**: 685–695.
23 Hamilos DL, Lund VJ. Etiology of chronic rhinosinusitis: the role of fungus. *Ann Otol Rhinol Laryngol Suppl* 2004; **193**: 27–31.
24 Kim ST, Choi JH, Jeon HG, *et al*. Comparison between polymerase chain reaction and fungal culture for the detection of fungi in patients with chronic sinusitis and normal controls. *Acta Otolaryngol* 2005; **125**: 72–75.

25 Murr AH, Goldberg AN, Vesper S. Fungal speciation using quantitative polymerase chain reaction (QPCR) in patients with and without chronic rhinosinusitis. *Laryngoscope* 2006; 116: 1342–1348.
26 Scheuller MC, Murr AH, Goldberg AN, Mhatre AN, Lalwani AK. Quantitative analysis of fungal DNA in chronic rhinosinusitis. *Laryngoscope* 2004; **114**: 467–471.
27 Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. Eosinophilic fungal rhinosinusitis: a common disorder in Europe? *Laryngoscope* 2003; **113**: 264–269.
28 Glass D, Amedee RG. Allergic fungal rhinosinusitis: a review. *Ochsner J* 2011; **11**: 271–275.
29 Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinicopathological entity. *Laryngoscope* 2000; **110**: 799–813.
30 Chatterjee SS, Chakrabarti A. Epidemiology and medical mycology of fungal rhinosinusitis. *Otolaryngol Clin* 2009; **1**: 1–13.
31 Granville L, Chirala M, Cernoch P, Ostrowski M, Truong LD. Fungal sinusitis: histologic spectrum and correlation with culture. *Hum Pathol* 2004; **35**: 474–481.
32 Saravanan K, Panda NK, Chakrabarti A, Bapuraj RJ. Allergic fungal rhinosinusitis: An attempt to resolve the diagnostic dilemma. *Arch Otolaryngol Head Neck Surg* 2006; **132**: 173–178.
33 Correy JF, Delsupche KG, Ferguson BJ. Allergic fungal sinusitis: allergic, infection or both? *Otolaryngol Head Neck Surg* 1995; **113**: 110–119.
34 Telmesani LM. Prevalence of allergic fungal sinusitis among patients with nasal polyps. *Ann Saudi Med* 2009; **29**: 212–214.
35 Staik□niene J, Vaitkus S, Japertiene LM, Ryskiene S. Association of chronic rhinosinusitis with nasal polyps and asthma: clinical and radiological features, allergy and inflammation markers. *Medicina* (*Kaunas*) 2008; **44**: 257–265.
36 Elmorsy SM, Khafagy YW, Rizk NM, *et al*. IgE and cytokines (IL-6 and IL-13) in sinonasal polyposis. *Insight Immuno* 2011; **1**: 1–5.
37 Hafidh M, Harney M, Kane R, *et al*. The role of fungi in the etiology of chronic rhinosinusitis: a prospective study. *Auris Nasus Larynx* 2007; **34**: 185–189.
38 Hedayati MT, Bahoosh M, Kasiri A, *et al*. Prevalence of fungal rhinosinusitis among patients with chronic rhinosinusitis from Iran. *J Mycol Méd* 2010; **20**: 298–303.
39 Challa S, Uppin SG, Hanumanthu S, *et al*. Fungal rhinosinusitis: a clinicopathological study from South India. *Eur Arch Otorhinolaryngol* 2010; **267**: 1239–1245.
40 Razmpa E, Khajavi M, Hadipour-Jahromi M, Kordbacheh P. The prevalence of fungal infections in nasal polyposis. *Acta Medica Iranica* 2007; **45**: 46–50.
41 Michael RC, Michael JS, Ashbee RH, Mathews MS. Mycological profile of fungal sinusitis: an audit of specimens over a 7-year period in a tertiary care hospital in Tamil Nadu. *Indian J Pathol Microbiol* 2008; **51**: 493–496.
42 Erbek SS, Serefhanoglu K, Erbek S, *et al*. Clinical subgroups and antifungal susceptibilities in fungal culture-positive patients with chronic rhinosinusitis. *Eur Arch Otorhinolaryngol* 2008; **265**: 775–780.
43 Taj-Aldeen SJ, Hilal AA, Schell WA. Allergic fungal rhinosinusitis: A report of 8 cases. *Am J Otolaryngol* 2004; **25**; 213–218.
44 Garrel R, Gardiner Q, Khudjadze M, *et al*. Endoscopic surgical treatment of sino-nasal polyposis-medium term outcomes. *Rhinology* 2003; **41**: 91–96.
45 Poetker D, Mendolia-Loffredo S, Smith T. Outcomes of endoscopic sinus surgery for chronic rhinosinusitis associated with sinonasal polyposis. *Am J Rhinol* 2007; **21**: 84–88.
46 Becker SS. Surgical management of polyps in the treatment of nasal airway obstruction. *Otolaryngol Clin N Am* 2009; **42**: 377–385.
47 Ebbens FA, Scadding GK, Badia L, *et al*. Amphotericin B nasal lavages: not a solution for patient with chronic rhinosinusitis. *J Allergy Clin Immunol* 2006; **118**: 1149–1156.
48 Weschta M, Rimek D, Formanek M, *et al*. Topical antifungal treatment of chronic rhinosinusitis with nasal polyps: a randomized, double-blind clinical trial. *J Allergy Clin Immunol* 2004; **113**: 1122–1128.
49 Gerlinger I, Fittler A, Fónai F, *et al*. Postoperative application of amphotericin B nasal spray in chronic rhinosinusitis with nasal polyposis, with a review of the antifungal therapy. *Eur Arch Otorhinolaryngol* 2009; **266**: 847–855.
50 Kumar R, Shrivastava SK, Chakraborti A. Comparison of broth dilution and disc diffusion method for the antifungal susceptibility testing of *Aspergillus flavus*. *AmJ Biomed Sci* 2010; **2**: 202–208.

This paper was first published online on Early Online on 11 July 2012.