Original Article / Çalışma - Araştırma

Prevalence of allergic fungal rhinosinusitis among patients with chronic rhinosinusitis with or without nasal polyposis who underwent endonasal sinus surgery in Northwestern Turkey

Türkiye'nin Kuzeybatısında endoskopik sinüs cerrahisi uygulanan nazal polipli ya da polipsiz kronik rinosinüzit hastalarında alerjik fungal sinüzit yaygınlığı

Deniz Demir, M.D.,[1] Yusufhan Süoğlu, M.D.,[2] Mehmet Güven, M.D.,[1] Mahmut Sinan Yılmaz, M.D.[1]

[1]*Department of Otolaryngology, Sakarya University Training and Research Hospital, Sakarya, Turkey*
[2]*Department of Otolaryngology, Medicine Faculty of İstanbul University, İstanbul, Turkey*

Objectives: This study aims to evaluate the prevalence of allergic fungal rhinosinusitis in chronic rhinosinusitis patients in the northwest of Turkey.

Patients and Methods: Eighty-seven patients diagnosed with chronic rhinosinusitis and who were planned endonasal sinus surgery were enrolled in this prospective study. Patients were evaluated by detailed clinical examination, computed tomography, skin test against allergens, total serum immunoglobulin E (IgE) antibody, fungal-specific IgE antibody, and histopathologic and mycologic monitoring.

Results: Fungal elements showing tissue invasiveness were detected in only four patients. These patients had specific IgE against *Aspergillus fumigatus* (4.6%). Skin tests were positive for at least one allergen in 16 patients (18.3%).

Conclusion: None of the patients met diagnostic criteria for allergic fungal rhinosinusitis. This result may be due to the diversity in disease prevalence based on geographical location or diagnostic methods.

Keywords: Allergy; fungus; nasal polyp; prevalence; sinusitis.

Amaç: Bu çalışmada Türkiye'nin kuzeybatısındaki kronik rinosinüzit hastalarında alerjik fungal rinosinüzit yaygınlığı araştırıldı.

Hastalar ve Yöntemler: Bu prospektif çalışmaya kronik rinosinüzit tanısı konulup endonazal sinüs cerrahisi planlanan 87 hasta dahil edildi. Hastalar ayrıntılı klinik muayene, bilgisayarlı tomografi, alerjenlere ve total serum immünoglobulin E (IgE) ve mantar spesifik IgE antikora karşı deri testi ve histopatolojik ve mikolojik izlem ile değerlendirildi.

Bulgular: Doku invazyonu gösteren fungal elementler yalnız dört hastada tespit edildi. Bu hastalarda *Aspergillus fumigatus*a karşı spesifik IgE vardı (%4.6). On altı hastada deri testleri en az bir alerjen için pozitifti (%18.3).

Sonuç: Hastaların hiçbiri alerjik fungal rinosinüzit tanı kriterlerini karşılamadı. Bu sonuç, hastalığın yaygınlığının coğrafi konuma göre farklılaşmasından ya da tanı yöntemlerinden kaynaklanabilir.

Anahtar Sözcükler: Alerji; mantar; nazal polip; yaygınlık; sinüzit.

Available online at
www.kbbihtisas.org
doi: 10.5606/kbbihtisas.2014.24382
QR (Quick Response) Code

Received / *Geliş tarihi:* February 10, 2014 Accepted / *Kabul tarihi:* April 15, 2014

Correspondence / İletişim adresi: Deniz Demir, M.D. Sakarya Üniversitesi Eğitim ve Araştırma Hastanesi Kulak Burun Hastalıkları Kliniği, 54100 Sakarya, Turkey.
Tel: +90 505 - 578 41 26 e-mail *(e-posta):* drdenizdemir@hotmail.com

Fungal rhinosinusitis is classified into two subgroups: three invasive forms (acute necrotizing, chronic invasive, granulomatous invasive), and two noninvasive forms (fungal ball and allergic fungal).[1] Allergic fungal rhinosinusitis (AFRS) is a noninvasive form that can be distinguished clinically and histopathologically and there are current strategies for treatment compared to other forms of fungal rhinosinusitis. It may represent an allergic hypersensitivity response to extramucosal fungi within the sinus cavity. Affected patients are usually young, immunocompetent, atopic and initially present with nasal polyps.[2] Patients often have asthma, aspirin-sensitivity, allergic rhinitis, and eosinophilia.[2] The pathogenesis of AFRS, particularly its similarity to allergic bronchopulmonary aspergillosis (ABPA), has been presumed to be a combination of types 1 and 3 hypersensitivities to fungal allergens.[3] This supposition was enforced by elevated fungal-specific immunoglobuline E (IgE) and IgG antibodies.[4] However, the overall immunological mechanisms of AFRS are currently considered to be more complicated.

The incidence of AFRS is estimated at 5 to 10% of all chronic rhinosinusitis (CRS) who undergo sinus surgery.[5,6] In India, the incidence was 83.9% in patients with nasal polyps (NP).[7] The incidence variability may depend on geographical variation and problems in diagnosis of the disease.[8] Although there are physical examination findings, laboratory test results, and computed tomography (CT) showing evidence for CRS, the histopathology from surgical sinus specimens is diagnostically defined for AFRS.[2,9] Histopathology shows inflammatory tissue, frequently accompanied by eosinophilia and extramucosal allergic mucin. Fungal stains are positive for hyphae within the allergic mucin but not in the mucosa. Fungus may be hard to find within the mucin and the tissue. Therefore, this led to the definition of a new entity: Eosinophilic mucin rhinosinusitis (EMRS).[10] Previous reports in the literature have alluded to this entity and have described it variously as "AFS-like syndrome"[11] or "allergic mucin sinusitis without fungus".[12]

The aim of this study was to investigate the prevalence of AFRS among patients with CRS with or without NP who underwent endonasal sinus surgery (ESS) in our region.

PATIENTS AND METHODS

Eighty-seven patients (56 males, 31 females; mean age 38.7 years; range 9 to 78 years) with CRS with or without NP who underwent ESS at the Department of Otorhinolaryngology, Istanbul School of Medicine at the University of Istanbul were included in this study. The diagnostic criteria for CRS were defined by clinical symptoms for more than 12 weeks, CT scanning and endoscopic examination. These patients had two or more symptoms: nasal obstruction, anterior/posterior discolored discharge, reduction/loss of smell, facial pain/pressure, itching. Surgery was planned for these patients after ineffective medication for several months. Additional information was obtained on age, sex, aspirin sensitivity, asthma, and disease duration. Aspirin sensitivity was considered from history alone. Patients with sinonasal benign or malignant pathology were excluded.

Endoscopy, CT of the paranasal sinuses in axial and coronal planes, blood analysis, and skin tests were performed on all these patients. The presence or absence of NP, nasal mucosa, and deviation of septum were determined visually using a 0 degree nasal endoscope. Atopy was confirmed by intradermal skin testing and serum total immunoglobulin E and fungal-specific IgE antibody. Intradermal skin test was performed using 0.1 ml of antigen: *Aspergillus fumigatus (A. fumigatus), Alternaria alternata, Cladosporium herbarum, Curvularia lunata, Mucor mucedo,* trees, grasses, house-dust mite, cats, and dogs. Blood was drawn for in vitro analysis of serum total IgE and fungal-specific IgE antibodies. Radioallergosorbent test (RAST) was performed on five fungi: *A. fumigatus, Alternaria alternata, Cladosporium herbarum, Curvularia lunata, and Penisillium notatum*. In the analysis, an IgE count higher than 100 IU/ml was considered to indicate atopy.

Tissues and intrasinus debris were endoscopically removed. The material obtained was covered and sent to our mycology and pathology laboratory within one hour. Direct microscopy under 10% potassium hydroxide wet mount was performed on tissue for mycologic examination. The tissue was cultured by inoculation on Sabouraud dexrose agar and brain-heart infusion agar. All these procedures were performed to prevent contamination of airborne

microorganisms in a class 2 safety cabinet. One petri dish of each was incubated at 27 °C and at 37 °C for a period up to five weeks. All positive cultures were examined to identify pathogens microscopically and macroscopically. Reproducing fungi were stained with lactophenol cotton blue. In addition, direct microscopic examination of material sent for Gram and Giemsa preparations were prepared.

For histopathologic analysis, the tissue with mucus was manually removed and not placed directly on a surgical towel or gauze to prevent absorption. The material obtained was fixed in 10% formalin, and 0.5-0.7 micron thick sections were cut from paraffin blocks and stained with periodic Asid schiff (PAS), hematoxylin-eosin (H-E) and Gomori methanamin silver (GMS) stains. Extramucosal allergic mucin with or without fungus, Charcot-Leyden crystals, fungal hyphae, eosinophils and mucosal invasion by fungal hyphae were investigated at our pathology clinic.

Statistical analysis

All statistical analyses were performed with the SPSS version 15.0 statistical package (SPSS Inc., Chicago IL, USA). Values of $p<0.05$ were considered to indicate statistical significance.

RESULTS

Among all the 87 patients with CRS, 62 (71.3%) had NP and 25 (28.7%) did not have NP. Two patients (non-Hodgkin lymphoma and acute myeloid leukemia) with immunocompromised status were found and sent to our hematology clinic. Nine patients (10.3%) were asthmatic and received appropriate treatment. Five patients (5.7%) had aspirin sensitivity and four patients (4.5%) had asthma and aspirin sensitivity combined. Two patients had an immunocompromised status and one patient had dermographism so that a skin test was performed on 84 patients. Type 1 hypersensitivity was found against *A. fumigatus* in 4 (4.8%), grasses in 4 (4.8%), house-dust mite in 4 (4.8%), and trees in 4 (4.8%).

The serum total IgE was higher than 100 IU/mL in 32 patients (36.8%) and 4 patients had IgE for *A. fumigatus*. There was no significant correlation between total IgE levels and allergic symptoms in all patients: nasal obstruction ($p=0.285$), anterior/posterior discolored discharge ($p=0.806$), reduction/loss of smell ($p=0.732$), facial pain/pressure ($p=0.532$), and itching ($p=0.274$). However, a significant correlation was found between patients with NP and high IgE level ($p=0.012$). Statistical analyses of symptoms showed that there was no significant correlation in subtypes of CRS patients (Table 1).

None of the sinus CT scans showed evidence of AFRS. Twenty-nine (33.3%) perioperative paranasal sinus aspirates had material compatible with allergic mucin by macroscopic assessment. In these materials, fungal elements were not found histologically. These patients were classified as EMRS.

Positive cultures for fungi were obtained from four (4.6%) of the 87 patients. On histopathologic analysis, tissue invasive fungal infections were found in all of them. Two of these patients with hematologic diseases were referred for treatment to the hematology clinic. Diagnoses of these patients were acute necrotizing fungal sinusitis because of tissue necrosis with invasive fungal infection, clinical aggressiveness and immune deficiency status seen upon examination. Diagnoses for the other two patients were chronic invasive fungal sinusitis. In our study, none of the patients

Table 1. Comparison of clinical symptoms in subtypes of chronic rhinosinusitis

	EMRS	ECRS	Other CRS	Significance*
	%	%	%	*p*
Nasal obstruction	27.6	31.0	19.5	0.345
Anterior/posterior discolored discharge	18.4	18.4	14.9	0.870
Reduction/loss of smell	23.0	21.8	13.8	0.293
Facial pain/pressure	20.7	24.1	12.6	0.270
Itching	10.3	14.9	6.9	0.456

EMRS: Eosinophilic mucin rhinosinusitis; ECRS: Eosinophilic chronic rhinosinusitis; CRS: Chronic rhinosinusitis; * χ^2 test.

Table 2. Patients characteristics and laboratory data

	EMRS (n=29)			ECRS (n=33)			Other CRS (n=25)		
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD
Age			41.59±15.8			33.12±14			42.76±18
Sex									
Male	22			19			15		
Female	7			14			10		
Polyp	29			33			0		
Asthma	5	5.7		2	2.3		2	2.3	
Aspirin sensitivity	3	3.4		0	0		2	2.3	
IgE >100 (IU/mL)*	22	25.3†		3	3.4		7	8	
Fungal-specific IgE	0	0		0	0		4	4.6‡	
Positive skin test*	10	11.5†		0	0		6	6.9	
Culture positive	0	0		0	0		4	4.6‡	
Total IgE**			217.6†			57.3			–

SD: Standard deviation; IgE: Immunoglobulin E; † Statistically significance; * χ^2 test; ‡ Aspergillus fumigatus; ** Independent-samples t-test.

were confirmed with the diagnostic criteria for AFRS. All patients with NP were reported to show marked eosinophil infiltration in the nasal polyps. The patients with NP were classified as eosinophilic chronic rhinosinusitis (ECRS). A total of 87 patients were categorized into three groups: EMRS, ECRS, and other CRS (Table 2).

DISCUSSION

The role of fungal pathogens in CRS has been increasingly propagated during recent years. In 1981 the first report describing five patients with aspergillosis of the paranasal sinuses was made by Millar et al.[13] They found a similarity of this entity to ABPA clinically and histopathologically. Katzenstein et al.[14] reported that seven cases (6%) of 113 consecutive sinus specimens showed findings identical to those seen in ABPA, terming the condition allergic Aspergillus sinusitis in 1983. The obtained tissue was described as "allergic mucin" and its histology characterized as extramucosal eosinophil-rich mucin containing sparse numbers of fungal hyphae that resembled Aspergillus, Charcot-Leyden crystals, eosinophils and lymphocytes. After 1989, knowing that a different type of fungus in isolated material could explain the condition, AFRS was coined as a subgroup of fungal rhinosinusitis.[15]

The current diagnostic criteria evolved by Bent and Kuhn,[16] deShazo and Swain,[9] and Schubert and Goetz[2] include: characteristic allergic mucin seen histopathologically and/or grossly; positive fungal stain or fungal culture but not in mucosa; type 1 hypersensitivity to fungi by history, skin tests, or *in-vitro* testing; and characteristic CT scan findings. The extramucosal allergic mucin is described as thickened exudate "peanut-buttery" that is dark brown to green in color. Histologically, it is composed of masses of eosinophils with associated mucus where Charcot-Leyden crystals can often be seen.[2] In the present study, histologically, 29 cases with eosinophilic mucin were present without the presence of fungi.

The methods of collection, staining, and the culturing technique for fungi may affect proper categorization of cases. Ponikau et al.[17] diagnosed AFRS in 94 of 101 (93%) consecutive patients. At the same time, they also determined the presence of fungus in nasal lavage from healthy volunteers. They supposed that insufficiency of the methods used to identify the fungi was responsible for the low prevalence of AFRS. Guo et al.[18] in their study, using another detection method (a modification of GMS staining) improved the visualization of fungi in specimens. In addition, polymerase chain reaction is widely being used for fungal detection and is considered by some authors to be superior to fungal cultures.[19]

Researchers continue to discover the role of humoral immunity in AFRS. In some reports, fungal-specific IgE could not be shown so that

atopy has occasionally been removed from the criteria of AFRS.[9,17] Some investigators did not find specific IgE or positive skin tests in most patients who had noninvasive fungal sinusitis with allergic mucin.[20] In contrast, in many of the reports describing AFRS, the patients are atopic to causative fungi so that IgE sensitivity is accepted as a criteria of AFRS.[3,4,21] Hutcheson et al.[22] found that all patients were atopic and the mean serum total IgE in AFRS patients was significantly higher than in CRS patients. In our study, IgE levels were higher in 32 patients and fungus-specific IgE was only seen in four patients. Of 84 patients, positive skin tests were: 4 fungal, 4 trees, 4 grasses, and 4 mites. Analysis of EMRS and ECRS regarding the presence of atopy and higher IgE levels revealed a significant difference between the two groups (p=0.02, p=0.00 respectively) (Table 2).

Sinus CT always shows evidence for CRS, sometimes unilateral but often throughout multiple sinuses. A high density or hyperattenuation region within the abnormal sinus on CT is seen with AFRS.[16,23] This feature may also be seen with other forms of fungal CRS, particularly fungal ball, and is also the characteristic CT presentation for dense eosinophilic mucin without the presence of fungi.[12] None of our patients presented with radiographic evidence of skull-base erosion, hyperattenuating densities or erosion through the ethmoid lamina papyracea into the ipsilateral orbit.

Interestingly, some of our patients with a clinical picture of AFRS did not have evidence of fungus in their eosinophilic mucin. Histologically, four patients showed necrotic sinonasal mucosa with the presence of invasive fungal forms. These cases were caused by *A. fumigatus*. Fungal rhinosinusitis is considered a potentially progressive process.[24] It is possible that the noninvasive form may convert to the invasive form. However, we did not consider the noninvasive form turning into the invasive form in our patients during the brief preoperative follow-up. Based on our analysis, 33 patients with NP (37.9%) were classified as having ECRS and the other 29 patients with NP (33.3%) were classified as having EMRS. In the central region of Turkey, Hıdır et al.[25] showed the prevalence of AFRS to be 13%. We were unable to diagnose AFRS in the patients undergoing sinus surgery in the northwest of Turkey.

Conclusion

In our study, none of the patients were confirmed with the diagnostic criteria for AFRS. As mentioned above, although throughout the world AFRS is an increasingly recognized form of CRS with NP, the true incidence remains unclear. The variability of prevalence may be due to diagnostic error, insufficient sampling as well as geographical diversity. Eosinophilic mucin is not uniformly distributed throughout the sinus content. Insufficient sampling may cause problems with true diagnosis. In addition, in some cases hyphae could not be clearly distinguished from artifact. The pathologist must be alerted to pay special attention to specimens with a focus on fungal hyphae and spores. We concluded that our results may be due to geographical diversity. Questions regarding the proper diagnosis and pathogenesis of the disease have to be resolved for treatment. Future research on AFRS will further refine our understanding of its pathogenesis and correct diagnosis.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. de Shazo RD, Chapin K, Swain RE. Fungal sinusitis. N Engl J Med 1997;337:254-9.
2. Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. I. Demographics and diagnosis. J Allergy Clin Immunol 1998;102:387-94.
3. Manning SC, Holman M. Further evidence for allergic pathophysiology in allergic fungal sinusitis. Laryngoscope 1998;108:1485-96.
4. Stewart AE, Hunsaker DH. Fungus-specific IgG and IgE in allergic fungal rhinosinusitis. Otolaryngol Head Neck Surg 2002;127:324-32.
5. Bakhshaee M, Fereidouni M, Mohajer MN, Majidi MR, Azad FJ, Moghiman T. The prevalence of allergic fungal rhinosinusitis in sinonasal polyposis. Eur Arch Otorhinolaryngol 2013;270:3095-8.
6. Collins MM, Nair SB, Wormald PJ. Prevalence of noninvasive fungal sinusitis in South Australia. Am J Rhinol 2003;17:127-32.
7. Jain S, Das S, Gupta N, Malik JN. 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. Med Mycol 2013;51:164-9.
8. Ferguson BJ, Barnes L, Bernstein JM, Brown D, Clark CE 3rd, Cook PR, et al. Geographic variation in allergic fungal rhinosinusitis. Otolaryngol Clin North Am 2000;33:441-9.
9. deShazo RD, Swain RE. Diagnostic criteria for allergic fungal sinusitis. J Allergy Clin Immunol 1995;96:24-35.
10. Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinicopathological entity. Laryngoscope 2000;110:799-813.
11. Cody DT 2nd, Neel HB 3rd, Ferreiro JA, Roberts GD. Allergic fungal sinusitis: the Mayo Clinic experience. Laryngoscope 1994;104:1074-9.
12. Ramadan HH, Quraishi HA. Allergic mucin sinusitis without fungus. Am J Rhinol 1997;11:145-7.
13. Millar JW, Johnson A, Lamb D. Allergic aspergillosis of the maxillary sinuses. Thorax 1981;36:710. [Abstract]
14. Katzenstein AL, Sale SR, Greenberger PA. Allergic Aspergillus sinusitis: a newly recognized form of sinusitis. J Allergy Clin Immunol 1983;72:89-93.
15. Gourley DS, Whisman BA, Jorgensen NL, Martin ME, Reid MJ. Allergic Bipolaris sinusitis: clinical and immunopathologic characteristics. J Allergy Clin Immunol 1990;85:583-91.
16. Bent JP 3rd, Kuhn FA. Diagnosis of allergic fungal sinusitis. Otolaryngol Head Neck Surg 1994;111:580-8.
17. Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin Proc 1999;74:877-84.
18. Guo C, Ghadersohi S, Kephart GM, Laine RA, Sherris DA, Kita H, et al. Improving the detection of fungi in eosinophilic mucin: seeing what we could not see before. Otolaryngol Head Neck Surg 2012;147:943-9.
19. El-Morsy SM, Khafagy YW, El-Naggar MM. Allergic fungal rhinosinusitis: detection of fungal DNA in sinus aspirate using polymerase chain reaction. J Laryngol Otol 2010;124:152-60.
20. Collins M, Nair S, Smith W, Kette F, Gillis D, Wormald PJ. Role of local immunoglobulin E production in the pathophysiology of noninvasivefungal sinusitis. Laryngoscope 2004;114:1242-6.
21. deShazo RD. The fungal conundrum. Ann Allergy Asthma Immunol 2006;96:256-7.
22. Hutcheson PS, Schubert MS, Slavin RG. Distinctions between allergic fungal rhinosinusitis and chronic rhinosinusitis. Am J Rhinol Allergy 2010;24:405-8.
23. Mukherji SK, Figueroa RE, Ginsberg LE, Zeifer BA, Marple BF, Alley JG, et al. Allergic fungal sinusitis: CT findings. Radiology 1998;207:417-22.
24. Thakar A, Sarkar C, Dhiwakar M, Bahadur S, Dahiya S. Allergic fungal sinusitis: expanding the clinicopathologic spectrum. Otolaryngol Head Neck Surg 2004;130:209-16.
25. Hidir Y, Tosun F, Saracli MA, Gunal A, Gulec M, Yetiser S. Rate of allergic fungal etiology of chronic rhinosinusitis in Turkish population. Eur Arch Otorhinolaryngol 2008;265:415-9.