

Rate of allergic fungal etiology of chronic rhinosinusitis in Turkish population

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Abstract The aim of this study is to investigate intranasal fungal incidence in Turkish patients with chronic rhinosinusitis (CRS). One hundred patients with CRS and 40 healthy subjects as a control group were included in the prospective study. Nasal lavage samples were investigated for the presence of fungus, using new culture techniques and histopathologic examination. Epidermal and intradermal hypersensitivity tests were used to find out fungal atopy. Levels of serum eosinophils, total IgE and presence of fungus-specific IgE were investigated in the blood samples. Quantitative parameters were analyzed using Pearson χ^2 test. Allergic fungal sinusitis (AFS) was diagnosed in 13(13%) patients. There was no fungal growth in the control group. Eosinophilic mucin was detected in 94% of patients. Clinical and laboratory parameters of CRS were not significantly different in patient groups with or without intranasal fungi. As a result, fungal incidence was not as high as expected in Turkish patients with CRS. Furthermore, no significant correlation could be found between

presence of intranasal fungus and type-1 hypersensitivity in patients with CRS.

Keywords Rhinosinusitis · Fungi · Allergy

Introduction

Although many intrinsic and extrinsic factors have been blamed till now, etiopathogenesis of chronic rhinosinusitis (CRS) has not been explained completely [2]. Allergic aspergillus sinusitis was described as a new form of CRS at the beginning of the 1980s [13, 17]. In recent decades fungal etiology of CRS gained wide interest and, fungi were detected in 96% of nasal lavage samples, using new culture techniques [19]. Basic diagnostic criteria of Allergic fungal sinusitis (AFS) include radiologically confirmed CRS, presence of allergic mucin and detection of fungus without tissue invasion by culture or histopathologic examination [7, 19]. Fungal atopy and hyperattenuated areas on computerized tomography (CT) are accepted as additional diagnostic criteria by some authors [4, 12]. Fungi are widespread in nature and detection of intranasal fungi in patients with CRS is no surprise. Furthermore, similar ratio of fungal positivity was detected in the nasal passage of healthy controls in many of the studies [1, 19]. Rate of fungal colonization in the nasal passage may be affected by different features of climate such as humidity. Various host factors may also cause different response to fungi in the nasal passage. Therefore a wide range of AFS incidence was reported in different studies [1, 5, 11, 22].

This study aims to find out fungal incidence in Turkish patients with CRS and to investigate the role of type-I hypersensitivity in these patients.

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Materials and methods

This study was conducted at our tertiary referral hospital between October 2003 and June 2005. Patients with CRS, accompanied with or without nasal polyposis were included in the study. The procedure was informed to all cases and they signed the consent form. All subjects were questioned in terms of symptoms of CRS, asthma, aspirin hypersensitivity, previous history of paranasal sinus surgery, previous medical therapy and additional systemic diseases. Patients who have symptoms more than 12 weeks underwent further investigations with nasal endoscopy and CT of the paranasal sinuses on coronal plane to confirm the diagnosis of CRS.

Patients with diabetes mellitus, immunodeficiency disorders, renal transplantation, immunosuppressive therapy, malignant diseases and acute upper airway diseases were excluded. Three criteria were considered for the diagnosis of AFS: radiologically confirmed CRS, presence of allergic mucin and detection of fungus without tissue invasion by culture or histopathologic examination. Ethical committee of our institute approved the study protocol.

Sample collection

Nasal lavage materials were taken from patients and control cases. Sterile oxymetazolin (0.05%) spray was instilled into each nostril for decongestion before the procedure. The subjects were told to take deep breath and to keep it to prevent nasopharyngeal contamination and leakage. Then, 10 ml sterile isotonic saline solution (0.09%) was instilled into the each nostril and was collected into sterile plastic container by forced exhalation. Samples were sent to microbiology laboratory without waiting.

Microbiologic analysis

Microbiological investigation of nasal lavage samples was performed as described by Ponikau et al [19]. Briefly, the samples were suspended with an equal amount of dithiothreitol, vortexed for 30 s, allowed to stand at room temperature for 15 min, and then were centrifuged at 3,000g for 10 min. After inoculating a sample, taken from the vortexed sediment into Sabouraud's dextrose agar slants, supplemented with antibiotic (chloramphenicol, 50 mg/l), other part of the sediment was fixed with 20% KOH and examined using light microscope (40× magnification) to look for fungal elements. The cultures were incubated at 30°C for a month and examined for growth of fungi for every 3 days. Identification of fungi was made by conventional methods.

Immunologic analysis

Quantitative total IgE and fungal specific IgE measurements (for *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata*, *Helminthosporium halodes*) were performed in the serum samples taken from patients. Unicap 100 (Pharmacia Diagnostics, 2001, Germany) was used for measurements. IgE levels were measured by fluorometry and quantified by drawing calibration curves. The value of >0.35 kUA/L was accepted positive for specific IgE.

Histopathologic analysis

Nasal mucin and tissue samples were fixed in 10% formaldehyde. Prepared blocks were examined under a light microscope. Fungal elements, charchot–leyden crystals and eosinophils were sought by two different pathologists after they were painted with hemotoxyline–eosin, periodic acid Schiff and Gomori methenamine silver.

Allergy skin tests

Skin prick tests were carried out using a standard panel of 56 common aeroallergens including mites, molds, grass mix, tree mix pollen, weed mix, cockroach and animal dander (Greer Lab, NC, USA). Reactions were recorded 15 min after the test. Wheal diameter >3 mm with surrounding erythema was considered as positive, if the positive control histamine was positive and negative control was negative. The wheal without erythema was accepted as irritant reaction and excluded.

Lateral side of shoulder was used for intradermal prick test. About 0.2 ml of standard solution of intradermal test (*Candida albicans*, *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium notatum*, *Aspergillus fumigatus*-Allergopharma) was injected inside the dermis. Histamine was used as a positive control and saline was used as a negative control. Injection site was examined 15 min later- and in- duration, more than 4 mm was accepted positive.

Nasal smear was performed in all patients using nasal cotton swab. Nasal eosinophilia was evaluated using light microscope with 100× magnification after Wright staining. Eosinophil counting was made in every field for the following criteria: 1–5 eosinophils = low, 6–15 eosinophils = medium and more than 16 eosinophils = high level. Peripheral eosinophils counting were performed in the serum samples taken from the patients. Eosinophils numbers > 450 mm⁻³ was accepted as serum eosinophilia.

Statistical analysis

T-test was used to compare mean values. Quantitative parameters were analyzed by Pearson χ^2 test. (SPSS 11.5 program, SPSS Inc.Chicago, IL, USA). The value of $p < 0.05$ was considered statistically significant.

Results

One hundred patients (56 male and 44 female) with CRS, accompanied with or without nasal polyposis were included in the study. Ages of patients ranged from 12 to 80 years (mean 43, 65 ± 16 , 76 years). Ages of forty volunteers (28 male and 12 female) ranged from 15 to 67 years (mean 28, 05 ± 12 , 56 years).

Fungal growth in the culture was observed in the samples of 13(13%) patients in the patients group [*Aspergillus fumigatus* (n: 2), *Aspergillus niger* (n: 2), *Aspergillus flavus* (n: 1), *Alternaria* spp. (n: 3), *Penicillium* spp (n: 2), *Penicillium notatum* (n: 1), *Candida albicans* (n: 2), *Candida parapsilosis* (n: 1)]. Mixed fungal growth was observed in one patient (two fungi). Cultures in all cases of the control group were negative.

Rate of eosinophilic mucin was higher in both the patient groups with (100%) or without (93.1%) fungal growth in the culture but there was no significant difference between two groups ($p = 0.329$). Fungal elements were not detected histopathologically. All patients had radiologic findings, changing from mucosal thickening to total opacity of the sinuses, but hyperattenuated areas were not seen on CT. All of the culture positive patients ($n = 13$) met the

AFS criteria. Patients' clinical data according to fungal growth are shown in Table 1. Frequencies of symptoms and rate of bronchial asthma, aspirin sensitivity, nasal polyposis and number of previous sinus surgeries did not differ significantly for two groups of patients with or without fungal growth ($p > 0.05$).

Comparisons of laboratory findings between two groups of patients with and without fungal growth are summarized in Table 2. Rate of serum eosinophilia was significantly higher (53.8%) in patients with intranasal fungal positivity ($p = 0.019$). In contrast, rate of high level of total IgE was significantly higher (49.4%) in patients group without fungal growth in the culture ($p = 0.005$). Other laboratory findings, including fungal specific IgE positivity ($p = 0.119$), number of eosinophils in the nasal smear ($p = 0.182$), prick test positivity for fungi and intradermal test positivity for fungi ($p = 0.421$) did not differ significantly for two groups of patients with or without fungal growth in the culture.

Discussion

Millar and Katzenstein reported similar clinical and histopathologic features in patients with CRS and allergic bronchopulmonary aspergillosis for the first time, nearly two decades ago [13, 17]. In recent years allergy to intranasal fungi was accepted as one of the common causative factor of CRS by many authors [4, 9, 16, 21]. While the rate of AFS was reported 5–10% initially, it was increased up to 91% in recent studies [1]. However, controversy is still continuing on the topic. Fungi are ubiquitous in the nature and it may be a normal content of nasal mucus of patients

Table 1 Patients' clinical data according to fungal growth

Frequency of symptoms and related findings	Culture positive (n = 13)	Culture negative (n = 87)	χ^2
Nasal/postnasal drainage	13 (100)	84 (96.6)	0.497
Nasal obstruction	12 (92.3)	84 (96.6)	0.466
Hipposmia/anosmia	11 (84.6)	77 (88.5)	0.687
Sneezing	9 (69.2)	78 (89.7)	0.041 ^a
Facial pain/pressure	8 (61.5)	64 (73.6)	0.368
Headache	8 (61.5)	64 (73.6)	0.368
Nasal itching	8 (61.5)	62 (71.3)	0.475
Fatigue	10 (76.9)	53 (60.9)	0.265
Coughing	6 (46.2)	48 (55.2)	0.543
Dental pain	5 (38.5)	17 (19.5)	0.125
Fever	0 (0)	3 (3.4)	0.497
Asthma	4 (30.8)	25 (28.7)	0.880
Aspirin hypersensitivity	2 (15.4)	19 (21.8)	0.594
Nasal polyp	11 (84.6)	75 (86.2)	0.877
Previous sinus surgery	6 (46.2)	36 (41.4)	0.745

Culture positive and culture negative values are given as n (%)

^a Statistically significant

Table 2 Patients' laboratory findings according to fungal growth

Parameters	Culture positive (n = 13)	Culture negative (n = 87)	χ^2
Prick test positivity for fungi	0 (0)	2 (2.3)	–
Intradermal test positivity for fungi	9 (69.2)	50 (57.5)	0.142
High level of eosinophils in the nasal smear	10 (76.9)	50 (57.5)	0.182
Serum eosinophilia	7 (53.8)	20 (23)	0.019 ^a
Eosinophilic mucin	13 (100)	81 (93.1)	0.329
High level of total IgE	1 (7.7)	43 (49.4)	0.005 ^a
Fungal specific IgE positivity	0 (0)	14 (16.1)	0.119

Culture positive and culture negative values are given as n (%)

^a Statistically significant

with CRS. Ponikau et al. found 100% of intranasal fungal positivity in healthy volunteers, using new sample collection techniques [19]. In a recent study rate of intranasal fungi was 70% in controls and 46.6% in patients [10]. Lackner et al. detected positive fungal cultures in 20% of neonate, immediately after the birth (possible contamination from birth canal), then 7% on postpartum first day, 15% on postpartum 4th day, 72% on postpartum 2nd month and 94% on postpartum 4th month [14]. In a study from India, fungi were detected 80% of 251 CRS patients [20].

Kostamo et al. found that all of the nasal cultures were negative for fungi in January in healthy group, while 15.8% of them had intranasal fungal positivity in September [11]. The present study rate of AFS is 13%, which is lower as compared with the incidence of AFS, reported from Europe and from the North America. Different features of climate may affect incidence of intranasal fungi. Humidity is very low in Central Anatolia where the study was conducted and it may decrease the rate of intranasal fungal colonization. Furthermore, intranasal fungal colonization alone may not give rise to CRS for everyone. There should be some individual factors that contribute for the development of CRS in addition to fungi. In a study of Goh et al. [9], rate of intranasal fungi detected in culture was 16.7% from the samples of nasal lavages and 36.7% from the surgical specimens. Therefore, sample collecting method is another factor that may affect the rate of fungal growth in the cultures.

Eosinophilic infiltration is a common finding of AFS. It is suggested that the proteins secreted from eosinophils destroy fungi [15]. Benefit of steroid therapy in patients with AFS also supports the role of eosinophil in these patients. Braun et al. reported that they detected eosinophilic clusters in 94% of patients who underwent surgery for CRS [1]. Watanabe et al. showed fungal hyphae surrounded by eosinophils in one of five patients with AFS [24]. However, we don't know what causes the activation of eosinophils.

In a retrospective study consisting of patients who underwent endoscopic sinus surgery for CRS, the group with serum eosinophilia had a higher rate of postoperative

recurrent sinusitis, recurrent nasal polyps, more revision surgery for CRS, more need for postoperative antifungal agents and multiple course of antibiotic treatment [26]. Hafidh et al. [10] found, higher rate of serum eosinophilia in patients with CRS than in controls (30 versus 0%). However, Pant et al. [18] reported that most of the clinical characteristics of AFS patients were not significantly different from the other subgroups with eosinophilic chronic rhinosinusitis regardless of the detection of intranasal fungi or fungal allergy. At the present study, rate of serum eosinophilia was higher in patients with growth of fungi in culture. Rate of eosinophils was also higher in nasal smear in all patients with or without fungal growth in culture but the difference between two groups was not statistically significant ($p = 0.182$). All these findings indicate that eosinophilia is an important feature of the AFS but it is not associated with only intranasal fungi and may also elevate in other forms of CRS.

High level of fungus specific IgE and type-1 hypersensitivity may represent allergic reaction to fungi [3, 8]. Collins et al. [6] reported that presence of fungal specific IgE in sinus mucin was associated with systemic fungal allergy. Serum levels of specific IgE for *Aspergillus* were found to be elevated in 70% of cases with AFS in a study of Dhiwakar et al. [8]. Positive skin tests reactivity to fungal allergen in patients with AFS in other studies also supported the role of type-1 hypersensitivity in pathogenesis of AFS [9]. However findings of this study failed to demonstrate a clear correlation between parameters of type-1 hypersensitivity and intranasal fungi in patients with CRS.

Impact of intranasal fungal colonization on clinical course is another controversy. It is not clear whether severity of symptoms and persistence of disease in patients groups with or without fungal positivity is different or not. Scheuller et al. [23] reported that fungal positivity in the middle meatus did not correlate with quality of life outcomes. In a recent study, Weschta et al. [25] administered topical amphotericin B into the nostrils over a period of 8 weeks in patients with CRS and found no significant effect on symptoms. Frequencies of symptoms were not

significantly different in two groups of patients with or without intranasal fungal positivity in our study. Incidence of the triad of aspirin sensitivity, bronchial asthma and nasal polyposis did not differ in two groups of patients in the present study.

In conclusion, fungal incidence in patients with CRS in Turkish community was not as high as reported in the literature. Furthermore, findings of this study failed to demonstrate a clear correlation between the presence of intranasal fungi and type-I hypersensitivity in patients with CRS.

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