

Original Article

Frequency and Identification of Fungal Strains in Patients with Chronic Rhinosinusitis

Ali Eslamifar¹, Mehdi Razzaghi-Abyaneh², Mohsen Vazir-Nezami³, Habibollah Moghadasi³, Amitis Ramezani¹, Masoomeh Shams-Ghahfarokhi⁴, Ali Khameneh⁵, Mohammad Ali Shokrgozar⁶, Ali Akbar Amir-Zargar⁷, Zahra Deljoodokht¹, Arezoo Aghakhani¹

1. Dept. of Clinical Research, Pasteur Institute of Iran, Tehran, Iran.
2. Dept. of Mycology, Pasteur Institute of Iran, Tehran, Iran.
3. Dept. of ENT, Loghman Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Dept. of Mycology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
5. Dept. of Microbiology, Lahijan Azad University, Lahijan, Iran.
6. Dept. of National Cell Bank, Pasteur Institute of Iran, Tehran, Iran.
7. Dept. of Immunology, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Background and Objective: Chronic rhinosinusitis (CRS) is one of the most common chronic diseases. In the past decades, there has been an increase in fungal infections of sinuses and fungal rhinosinusitis (FRS) has been diagnosed more frequently. Knowing the fungal flora and its prevalence in CRS patients will allow a better understanding of this disease, permitting a correct diagnosis, treatment and formulating its prognosis. This study was performed to assess the fungal flora and its prevalence in CRS patients.

Materials and Methods: In this prospective study fungal cultures were obtained from nasal and paranasal sinuses mucus of 100 patients suffering from CRS and 40 controls who did not show any evidence of CRS. In addition, in CRS patients, paranasal sinuses mucus and tissue were histologically investigated for evidence of eosinophilic granulocytes and fungal elements besides fungal culture.

Results: Fungal cultures of nasal mucus were positive in 62.5% and 60% of cases and controls respectively. Positive fungal cultures of paranasal sinuses mucus were seen in 49% of cases and 5% of controls. *Aspergillus* was the most frequent, followed by *Penicilium* and *Cladosporium* in both nasal and paranasal sinuses mucus culture. Eosinophilic mucin was found in 90% of CRS patients.

Conclusion: Our data showed that fungal infection is frequent in patients with chronic rhinosinusitis. Based on our results, *Aspergillus* was the most frequent isolated fungus in CRS patients.

Key Words: Chronic, Rhinitis, Sinusitis, Fungi

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Address communications to: Dr. Arezoo Aghakhani, Department of Clinical Research, Pasteur Institute of Iran, Tehran, Iran.

Email: aaghakhani@pasteur.ac.ir

Introduction

Chronic rhinosinusitis (CRS) is defined as an inflammatory disease of the nasal and paranasal mucosa persisting and symptomatic for longer than 3 months, with polypoid mucosal thickening and nasal polyps as an ultimate end stage of that chronic inflammation (1;2). Presently, the etiology and pathogenesis of the disease are still unknown (3). The group of CRS disorders annually accounts as many as 22 million office visits and more than 500,000 emergency department visits in the USA according to some estimates (4). During the past 10 years, the prevalence of CRS has increased more than 50%. This dramatic increase in the incidence of this disease has occurred despite improved surgical techniques and the development of more powerful, broader-spectrum antibiotics (5). Although insights into the pathophysiology of CRS have largely been expanded over the last two decades, the exact etiology and mechanism of persistence is still unrevealed. CRS is a multifactorial disease and with some evidence, impaired ostial patency, mucociliary impairment, allergy, bacterial or fungal infection (or triggering), immunocompromised state, and environmental and genetic factors have been suggested to be associated or as risk factors (6). The eosinophil leukocytes are suspected to play a significant role in pathogenesis of CRS (6-8). The Mayo Clinic work is the first to provide data for the role of air-borne fungi in chronic rhinosinusitis and to show that several immune system branches appear to collaborate in response to the fungi, resulting in an abnormally enhanced response that causes troublesome inflammation and congestion. The research team's data showed that specific cells in 90 percent of chronic rhinosinusitis patients produce an enhanced immune-system response to one fungus in particular, *Alternaria*. Another kind of common fungus, *Cladosporium* also provoked an abnormally enhanced immune response (4). Previous studies revealed that one or more species of fungi could be cultured from the nasal mucus of CRS patients and healthy controls (5).

In the past decades, there has been an increase in fungal infections and fungal rhinosinusitis (FRS) has been diagnosed more frequently. Knowing the fungal flora, its prevalence and symptomatic presentation in CRS patients will allow a better understanding of this disease, permitting a correct diagnosis, treatment and formulating its prognosis (9). This study was performed to assess the fungal flora and its prevalence

in CRS patients.

Patients and Methods

In this prospective study, 100 patients with CRS and 40 controls that did not show any evidence of CRS were included. We considered the patients with signs and symptoms of inflammation of paranasal sinuses that persisted for more than 12 weeks, associated with the documented affections by imaging techniques after at least 4 weeks of appropriate clinical treatment. All patients and controls gave their written consent to participate in the study. The study was approved by the ethics committee of Pasteur Institute of Iran.

Fungal cultures were obtained from nasal and paranasal sinuses mucus of patients and controls. In addition, in patients with CRS, paranasal sinuses mucus and tissue were histologically investigated for evidence of eosinophilic granulocytes and fungal elements besides fungal culture.

Paranasal sinuses secretions were collected unilaterally under endoscopic view with aspirator sterilized in autoclave at 2 mm diameter coupled in a collection recipient specimen trap. The principle of maximum mucus preservation was adhered during the acquisition of specimens. This enabled the assigned pathologist to find eosinophilic mucin and fungal elements within the mucus. The specimens were then fixed in formalin and embedded in paraffin. Multiple serial sections of different specimens from each patient were stained with H&E and with Gomori methenamine silver (GMS). The pathologists were alerted to pay special attention to the mucin focusing on fungal elements and eosinophils.

Mycological analysis was performed through the culture of the mucus in 3 fungal culture mediums [Sabouraud Dextrose Agar, Brain Heart Infusion (BHI) Agar and Selective Agar (Merck, Darmstadt, Germany)]. Incubation was at 25 and 35°C and cultures were observed up to 20 days before release as negative for fungi. The identification of fungi and yeasts were made from macroscopic features, microscopic structures, use of biochemical properties and special tests such as Germ tube test.

Chi-square and t test were applied using SPSS 11.5 package program for statistical analysis. Data are presented as means \pm standard deviations or when indicated as absolute number and percentage. A P-value of <0.05 was considered significant.

Results

A total of 100 CRS patients with a mean age 33.9 ± 15 years and 40 controls with a mean age of 29 ± 15 years were included in our study. In this respect, 58% of CRS group were males and 42% were females.

Positive fungal cultures of nasal mucus were obtained in 62.5% and 60% of cases and controls respectively. Positive fungal cultures of paranasal sinuses mucus were seen in 49% of cases and 5% of controls. Mycological culture exam showed *Aspergillus* as the most frequent species, followed by *Penicillium* and *Cladosporium* in both nasal and paranasal sinuses mucus. There was not any significant difference between CRS patients and

controls regarding nasal mucus fungal culture but we found significant difference between CRS patients and controls regarding paranasal sinuses mucus culture ($p < 0.001$).

Out of the 100 CRS cases, fungal elements were found in 41%; eosinophilic mucin containing clusters (or sheets) of necrotic eosinophils was found in 90% and Charcot-Leyden crystals, a by-product of necrotic eosinophils present in eosinophil-associated diseases were present in 30%. Figure 1 shows GMS staining of fungal elements in paranasal sinus tissue of a CRS patient (Figure 1). Number of fungi isolated from patients with CRS and controls were summarized in Table 1 (Table 1).

Table 1: Number of fungi isolated from patients with chronic rhinosinusitis and controls

	Cases (number of isolates)	Controls (number of isolates)
<i>Aspergillus</i>		
<i>A. clavatus</i>	2	0
<i>A. flavus</i>	27	11
<i>A. fumigatus</i>	6	3
<i>A. nidulans</i>	1	0
<i>A. niger</i>	32	10
<i>A. terreus</i>	12	1
<i>A. tamari</i>	0	1
<i>A. species*</i>	27	4
<i>Penicillium species*</i>	52	19
<i>Cladosporium species*</i>	15	5
<i>Candida</i>		
<i>C. albicans</i>	7	0
<i>Alternaria species*</i>	6	1
<i>Rodotrolla species*</i>	6	0
<i>Scopulariopsis species*</i>	5	1
<i>Fusarium species*</i>	3	0
<i>Rhizopus species*</i>	2	1
<i>Acremonium species*</i>	2	0
<i>Aureobasidium species*</i>	1	0
<i>Cryptococcus neoformans</i>	1	0
<i>Trichoderma species*</i>	1	0

*identified to the species level

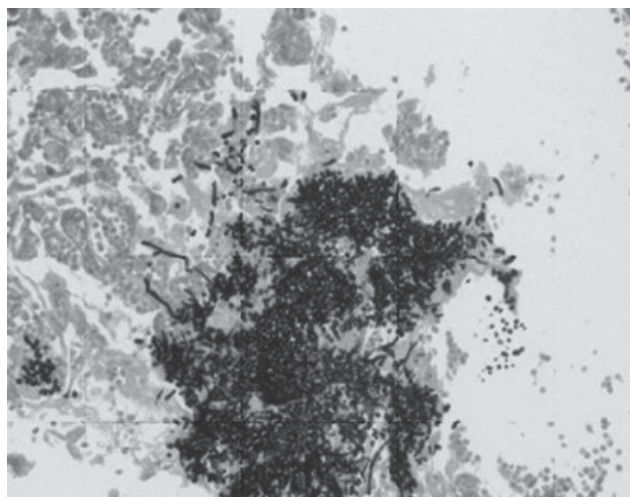


Figure 1: GMS staining showing fungal elements in paranasal sinus tissue of a CRS patient

Discussion

Chronic rhinosinusitis (CRS) is mostly referred to as a multifactorial disease, which has been variously defined by far (4). Published data have proposed a common etiological pathway for all clinical variations of CRS, describing a systemic immunological response to fungal organisms located in sinonasal mucus as a causative mechanism (8-11). The role played by fungi in most chronic rhinosinusitis patients as the target antigen of initiation of such chronic inflammation is still debated. With the presence of chronic eosinophilic inflammation in chronic rhinosinusitis, an exaggerated reaction to various inhaled antigens is anticipated (12).

The potential relationship between fungal organisms and CRS was first proposed in 1981 by Millar et al (13) who found extramucosal fungi in 5 patients with CRS. In 1983, Katzenstein et al (14) concurred with this finding, noting the histopathologic triad of fungal hyphae, "allergic" (eosinophilic) mucin, and Charcot-Leyden crystals in the mucus of 9 patients with CRS.

Jiang et al demonstrated that fungi were grown in 50.9% of ethmoid sinus specimens in patients with chronic rhinosinusitis (15). Erbek et al reported that fungi grew in the cultures from 80% of CRS patients and 85% of healthy volunteers (16). In another study, positive cultures for fungi were obtained in 63.0% of CRS patients and 62.2% of normal volunteers. *Cladosporium*, *Aspergillus*, *Alternaria*, and *Penicillium* were frequently isolated from CRS patients and normal volunteers (17). Ragab et al reported that in the normal control group nasal lavages showed 100%

positive fungal cultures. In the CRS patient group, the BALs showed positive fungal cultures in 28%, nasal vestibule cultures were positive in 8% and positive middle meatal cultures were obtained in 44% of the patients (12). The other investigation from Austria showed that fungal cultures were positive in 91.3% of patients with chronic rhinosinusitis. In all positive cultures, 33 different genera grew, with 3.2 species per patient, on average. Fungal cultures from a control group of healthy volunteers yielded positive results in 91.3% of cases. Histologically, fungal elements were found in 75.5% and eosinophilic mucin in 94.6% of patients (3). Another study reported that the fungal recovery rate was 45.9% in patients with sinus disease and control subjects (18). Dosa et al demonstrated that yeasts and moulds could be detected from 83% of CRS patients. *Candida albicans*, *Candida spp.*, *Aspergillus spp.*, *Cladosporium spp.*, and *Penicillium spp.* were isolated most frequently. Fungi were isolated from 44% of healthy subjects (19). In a study in Iran fungal cultures were positive in 23.7% of rhinosinusitis patients. *Aspergillus flavus*, *Aspergillus fumigatus* and candida were isolated most frequently from these patients (20).

Our study showed that fungal cultures of nasal mucus were positive in 62.5% and 60% of cases and controls respectively. Positive fungal cultures of paranasal sinuses mucus were seen in 49% of cases and 5% of controls. These variable rates of fungi in CRS patients in different studies may be explained by epidemiological and geographic variations and study conditions such as the number of the patients and the method using for detection of fungi. In our survey, there was not any significant difference between CRS patients and controls regarding nasal mucus fungal culture but we found significant difference between CRS patients and controls regarding paranasal sinuses mucus culture. Fungi can be cultured from nasal mucus as soon as contact with the environmental air exists. Fungal cultures can be obtained from almost everyone's nose. Therefore fungal spores must be considered a normal content of nasal mucus. Fungal spores are inhaled with every breath, some stick to the mucus, are transported to the nasopharynx and swallowed. This does not cause any clinical symptoms and is therefore not a pathological finding at all (21;21). So positive fungal cultures of paranasal sinuses mucus is a better predictor for rate of fungi in CRS. Our results showed that *Aspergillus* was the most frequent species, followed by *Penicillium* and *Cladosporium*. These species were also isolated most

frequently in other studies (17;19).

Conclusion

Our data showed that the fungal infection is frequent in patients with chronic rhinosinusitis. Based on our results, *Aspergillus* was the most frequent isolated fungus in CRS patients.

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