ORIGINAL ARTICLE

A study on the fungal rhinosinusitis: Causative agents, symptoms, and predisposing factors

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Background: In natural conditions, inhaled fungi are considered a part of the microflora of nasal cavities and sinuses. However, subsequent to the protracted use of corticosteroids and antibacterial agents, suppression of the immune system by chemotherapy, and poor ventilation, these fungi can become pathogens. Fungal colonization in the nose and paranasal sinuses is a prevalent medical issue in immunocompetent and immunosuppressed patients. In this study, we aimed to categorize fungal rhinosinusitis (FRS) among immunocompetent and immunosuppressed patients and identified the etiologic agents of disease by molecular methods. **Materials and Methods:** A total of 74 cases were evaluated for FRS. Functional endoscopic sinus surgery was performed for sampling. The clinical samples were examined by direct microscopy with potassium hydroxide 20% and subcultured on Sabouraud Dextrose Agar with chloramphenicol. Polymerase chain reaction sequencing was applied to identify causative agents. **Results:** Thirty-three patients (44.6%) had FRS. Principal predisposing factors were antibiotic consumption (n = 31, 93.9%), corticosteroid therapy (n = 22, 66.6%), and diabetes mellitus (n = 21, 63.6%). Eyesore (n = 22, 66.6%), proptosis (n = 16, 48.5%), and headache (n = 15, 45.4%) were the most common clinical manifestations among patients. *Rhizopus oryzae* (n = 15, 45.4%) and *Aspergillus flavus* (n = 10, 30.3%) were the most prevalent fungal species. **Conclusion:** Diagnosis and classification of FRS are crucial, and a lack of early precise diagnosis can lead to a delay in any surgical or medical management. Since there are a variety of treatments for FRS, accurate identification of etiologic agents should be performed based on phenotypic and molecular methods.

Key words: Clinical signs, etiologic agents, fungal rhinosinusitis, invasive, noninvasive, predisposing factors

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INTRODUCTION

Fungal colonization in the nose and paranasal sinuses is a prevalent medical issue in immunocompetent and immunosuppressed patients. Fungal sinusitis can be subdivided into noninvasive and invasive.[1] The invasive type is a destructive infection with intracranial and orbital implications and usually affects immunocompromised individuals; however, the noninvasive type is typically more chronic. Owing to an elevating number of diabetes mellitus, human immunodeficiency virus infections, bone marrow transplant recipients, and chemotherapy, the number of vulnerable immunosuppressed hosts is increasing.[2] *Aspergillus* species and the Mucorales are the main causative agents of fungal rhinosinusitis (FRS), nevertheless, other genera such as *Alternaria, Bipolaris, Schizophyllum, Ulocladium,* and *Curvularia* are also not infrequent.[3] Facial pain, nasal obstruction, headache, rhinorrhea, and epistaxis are common nonspecific clinical signs that are often seen in patients with FRS. This study was planned to determine the prevalence of FRS in the university hospitals in Isfahan, Iran, and

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to identify the etiologic agents using polymerase chain reaction (PCR)-sequencing.

MATERIALS AND METHODS

Clinical specimens

The protocol of the study was approved by the Ethics Committee of Isfahan University of Medical Science (no. IR.MUI.MED.REC.1399.384). From February 2020 to October 2021, a total of 74 cases were referred to the University hospitals (Al-Zahra and Kashani) of Isfahan University of Medical Sciences, Isfahan, Iran, due to microbial sinusitis. Thirty-five out of 74 cases were immunocompromised (47.3%). Nineteen patients were hospitalized in the intensive care unit (ICU) (25.7%), 16 patients (21.6%) in the Ear, Nose and Throat (ENT) ward, eight patients (10.8%) in the infectious disease ward, three patients (4%) in the surgical ward, two patients (2.7%) in the oncology ward, and the rest (35%) were outpatients.

Inclusion criteria

Patients with persistent fever despite antibiotic therapy, patients with facial swelling, patients with necrotic lesions in the mouth, patients with ptosis, and patients with nasal discharge and prolonged congestion were included in the study.

Exclusion criteria

Patients who did not agree to participate in the survey or sign the informed consent form were excluded from the study.

Functional endoscopic sinus surgery was carried out using the Messerklinger technique using KARL STORZ Endoscope (Germany). It is a diagnostic method demonstrating that the maxillary and frontal sinuses are subordinate cavities. All clinical specimens were transferred to the mycology reference laboratory for direct microscopic examination (DME) with potassium hydroxide 20%, culture on Sabouraud Dextrose Agar (Merck, Germany) with chloramphenicol, and molecular characterization. The periodic acid–Schiff was used to stain histological sections.

Molecular techniques for species identification

Polymerase chain reaction

Genomic DNA was extracted from fresh colonies by DNA Isolation Kit (MoBio Inc. Solana Beach, CA, USA), according to the manufacturer's instructions. For *Aspergillus* and *Penicillium* spp., the beta-tubulin protein-coding gene (BT2) and for other molds, internal transcribed spacer regions were used for species identification.[4-6] PCR reactions were carried out by Corbett Research thermal cycler (mod. CG1-96) (Sydney, Australia) in a final volume of 25 µL including 2.5 µL of 10 × reaction buffer (0.1 M Tris-HCl, pH 8.0, 0.5 M KCl, 15 mM MgCl2, 0.1% gelatin, and 1% Triton X-100), 1.5 mM MgCl2, 0.2 mM of each dNTP, 30 pmol of forward and reverse primers, 1.25 U of Taq polymerase (ITK diagnostics, Leiden, The Netherlands), and 2 µL of extracted genomic DNA. Amplification was started with one cycle of 5 min at 94°C for primary denaturation, followed by 32 cycles of 95°C for 40 s, 55°C for 60 s, and 72°C for 60 s, with a final extension of 72°C for 7 min. Five microliters of amplicons were run on 1.5% agarose gel and stained with SYBR Safe DNA gel stain (1:10,000 dilution in Tris/Borate/EDTA buffer), and in the end, visualized by gel documentation system (UVITEC, UK).

DNA sequencing

All PCR products were purified using QIAquick PCR Purification Kit (Hilden, Germany), and used for Sanger sequencing in a forward direction (Bioneer, South Korea). The products were analyzed with Chromas 2.4 (https://chromas.software.informer.com/2.4/) and then compared with fungal sequences existing in DNA databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool.

Statistical analysis

SPSS software version 23 (IBM, Chicago, Illinois, USA) was used for data analysis. The correlation between fungal species and type of sinusitis was adjusted using Chi-square, Fisher's exact test, and Mann–Whitney *U*-test. A $P < 0.05$ was considered statistically significant.

RESULTS

FRS was diagnosed in 33 patients (44.6%) according to clinical manifestation, DME [Figure 1], culture, and histopathological findings [Figure 2]. The age range of the patients was between 6 and 74 years with a median age of 47.1 years. The age ranges of 50–59 (27.3%) and 70–79 (6%) years had the highest and lowest frequency, respectively. The male-to-female ratio of the present study was 19/14. Thirty-one out of 33 patients (93.9%) used

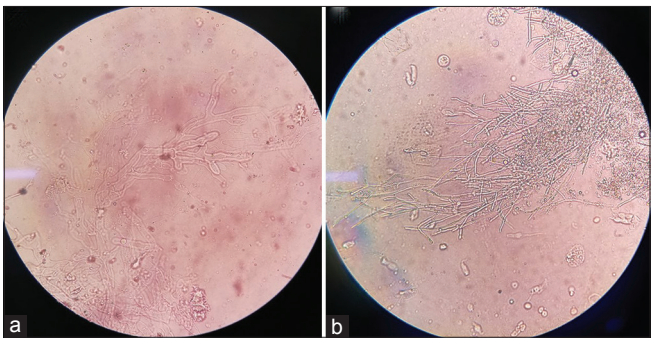

Figure 1: Direct microscopic examination with potassium hydroxide 20%, aseptate ribbon-like hyphae in *Rhizopus oryzae* (a) and septate hyphae with dichotomous branching in *Aspergillus flavus* (b)

antibiotics. Meropenem, cephalexin, and tazocin were the most antibacterial agents consumed by patients. Eight patients (24.2%) had chronic sinusitis with an average time of 3.5 years. Figure 3 shows predisposing factors among patients. Eyesore (n = 22, 66.6%), proptosis (n = 16, 48.5%), headache (n = 15, 45.4%), ptosis (n = 14, 42.4%), and blurred vision (n = 11, 33.3%) were the most common clinical manifestations among patients [Table 1]. Twenty patients revealed invasive FRS [Table 2]. *Rhizopus oryzae* was the most prevalent fungal species (n = 15, 45.4%), followed by *Aspergillus flavus* (n = 10, 30.3%), *Alternaria multiformis* (n = 2, 6.1%), *Neoscytalidium novaehollandiae* (n = 2, 6.1%), *Uncinocarpus reesii* (n = 1, 3%), *Curvularia hawaiiensis* (n = 1, 3%), *Aspergillus tubingensis* (n = 1, 3%), and *Penicillium polonicum* (n = 1, 3%). The mortality rate was 39.4% in the present study. The causative agent was *R. oryzae* in nine patients who died, *A. flavus* in three patients, and *A. tubingensis* in one patient. Maxillary sinuses were the most common involved sinuses among patients [Figure 4]. Fisher's exact test showed that the association between the type of FRS and fungal species was statistically significant in the invasive group (P = 0.037), but it was not statistically significant in the noninvasive FRS (P = 0.128). Statistically, there was no significant relationship between the etiologic agents and suppressed immune system (P = 0.113).

Table 1: Clinical signs of fungal rhinosinusitis among patients in the present investigation

Symptoms	*n* (%)
Eyesore	22 (66.6)
Proptosis	16 (48.5)
Headache	15 (45.4)
Ptosis	14 (42.4)
Blurred vision	11 (33.3)
Facial numbness	10 (30.3)
Nasal congestion	9 (27.3)
Blindness	7 (21.2)
Toothache	5 (15.1)
Facial paralysis	3 (9.1)
Hemoptysis	3 (9.1)
Fever	2 (6)
Nose bleeding	1 (3)

Table 2: Causative agents of fungal rhinosinusitis depending on the invasive or noninvasive type

Type of FRS	Etiologic agent	*n* (%)
Invasive	*Rhizopus oryzae*	14 (42.4)
	Aspergillus flavus	3 (9.1)
	Curvularia hawaiiensis	1 (3)
	Uncinocarpus reesii	1 (3)
	Aspergillus tubingensis	1 (3)
Noninvasive	*Aspergillus flavus*	7 (21.2)
	Alternaria multiformis	2 (6.1)
	Neoscytalidium novaehollandiae	2 (6.1)
	Penicillium polonicum	1 (3)
	Rhizopus oryzae	1 (3)

FRS=Fungal rhinosinusitis

DISCUSSION

Fungal spores are ubiquitous in the environment, and human vulnerability to fungi is inevitable. The spectrum of infections of the nose and paranasal sinuses is characterized by the presence or absence of fungi, and the immune system

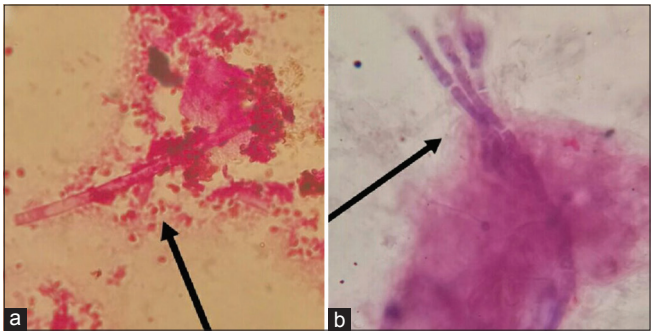

Figure 2: Black arrows show nonseptate hyaline hyphae of *Rhizopus oryzae* (a) and septate hyphae of *Aspergillus flavus* (b) in histopathological findings, periodic acid–Schiff staining, original × 40

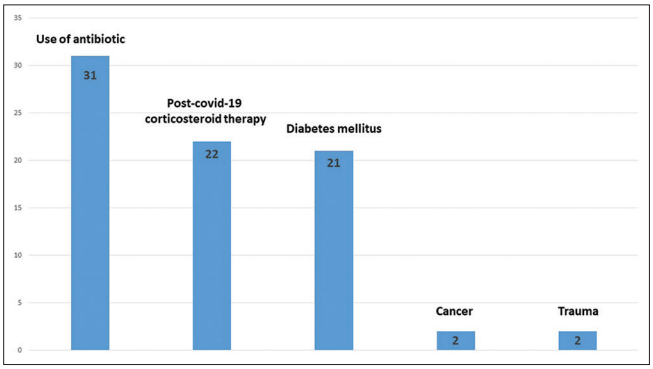

Figure 3: Predisposing factors among suspected cases of FRS. FRS: Fungal rhinosinusitis

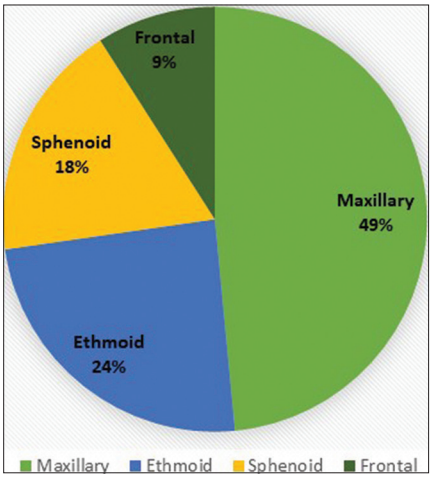

Figure 4: The type of sinuses involved with the fungal elements

status and may range from saprophytic colonization to invasion of the orbit and cerebrum with often serious effects.[1] Hora reported two wide categories of FRS containing invasive and noninvasive types, depending on being restricted to the superficial epithelium in noninvasive form, and the possible invasion of the fungal hyphae to the tissues through the mucosa, neurovascular structures, bones, and surrounding organs.[7] Noninvasive FRS is categorized into saprophytic fungal infestation, fungus ball, and allergic FRS (AFRS).[8,9] Fungal ball is designated as a thick fungal hypha causing partial mucosal inflammation.[10] In the present study, no cases of fungus balls were found in patients. AFRS is a type I hypersensitivity reaction to fungal components and the most prevalent type of fungal sinusitis.[2] The disease is mostly asymmetric and bilateral implicating several sinuses. Patients usually have a history of atopies such as asthma, an intact immune system, or facial dysmorphia. Hyaline molds (*Aspergillus* and *Fusarium*) and dematiaceous fungi (*Alternaria, Curvularia*, and *Bipolaris*) are considered etiologic agents.[11,12] According to Bent and Kuhn criteria,[13] we detected 13 cases of AFRS in the present investigation (27.3%). Causative agents of AFRS were *A. flavus* (66.6%), *A. multiformis* (22.2%), and *P. polonicum* (11.1%). AFRS commonly is found among younger individuals (21–33 years) with a competent immune system;[14] however, the age range of patients with AFRS in the present study was 39–68 years. In agreement with Glass and Amedee,[12] the most commonly affected sinuses in patients with AFRS were the ethmoids, and the disease was mostly asymmetrical. In our study, *R. oryzae* was found to be the most prevalent fungal species causing FRS. This contradicts the results of studies conducted in India and the United States, where *Aspergillus* spp. were the most common cause of the infection.[15-17] This difference can be related to geographical regions and climate. In the present investigation, all clinical specimens revealed positive mycological and histopathological findings; nevertheless, one of the major limitations of our survey was the lack of direct PCR on the samples for diagnosis of FRS. Noninvasive FRS (NFRS) mainly affects immunocompetent patients of all age groups;[18] however, 8 out of 13 patients (61.5%) with NFRS had risk factors including diabetes mellitus and cortone therapy. Invasive fungal sinusitis can be more frequently found in immunosuppressed patients and remarkably may lead to consequential morbidity and mortality. Suppression of the immune system in these patients can be a result of different sources containing diabetes mellitus, bone marrow transplantation, hematologic malignancies, and cytotoxic chemotherapy-induced neutropenia.[19] Infection is commonly appears in the nasal cavity and develops in the paranasal sinuses.[20] It can be caused by various fungal agents, such as Mucorales (commonly in patients with diabetes), *Aspergillus* species (mostly in neutropenic patients), and dematiaceous fungi.[19] In the present study, most cases of the invasive type were caused by *R. oryzae* (42.4%); however, *Aspergillus* species (12.1%) and *C. hawaiiensis* (as a dematiaceous fungus) (3%) were also isolated from patients. In agreement with our findings, Bakhshaee *et al*.[19] reported *R. oryzae* as the most prevalent etiologic agent of invasive rhinosinusitis, nevertheless, *A. flavus* was the leading agent of invasive fungal sinusitis in a study conducted by Alotaibi *et al*.[21] Coronavirus disease-2019 (COVID-19) is a primary respiratory infection which may lead to secondary bacterial or fungal infections owing to the use of corticosteroids and immune system impairment.[22] In the present survey, 20 out of 33 patients (60.6%) had coinfection with COVID-19 of which 18 cases (90%) had the invasive type of FRS; however, not known whether these patients were in the wards or in the ICU is another limitation of the present study.

CONCLUSION

FRS is a wide spectrum and challenging disease seen in immunocompetent or immunodeficient patients. With the excess knowledge about this spectrum of conditions, we can use proper medical management strategies to control the disease. In this regard, accurate criteria to classify invasive and noninvasive disease are essential to reduce the mortality rate of invasive FRS.

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Conflicts of interest

There are no conflicts of interest.

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