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**Original Article**

# Fungal Rhino Sinusitisin in Tehran, Iran

***Mehdi NAZERI*** [1]***,*Seyed Jamal HASHEMI*** [1]***, Mohammad ARDEHALI*** [2]***, Sasan REZAEI*** [1]***, SeyedMojtaba SEYEDMOUSAVI*** [3]***, Mahdi ZAREEI*** [1]***, Emaddodin HOSSEINJANI*** [4]

1. *Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Amir-alam Hospital, Tehran University of Medical Sciences, Tehran, Iran*
3. *Dept. of Medical Microbiology, Radboudumc, Nijmegen, The Netherlands*
4. *Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

***Corresponding Author:** Email: sjhashemi@tums.ac.ir

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**Abstract**

**Background:** Fungal rhino sinusitis (FRS) is an important infection of para nasal sinuses, which encompasses two main categories; invasive and noninvasive forms according to histopathological findings. *Aspergillus* spp are the most common species isolated from noninvasive form, while Mucorales are more frequently isolates from acute infections.

**Methods:** Four hundred fifty patients suspected to fungal rhino sinusitis were investigated in a cross-sectional prospective study from June 2009 to Sep 2013. All patients under went endoscopic sinus surgery of the middle meatus. Tissue biopsies were investigated for culture, histopathology and molecular examination.

**Results:** Totally, 87 patients were diagnosed with fungal rhinosinusitis. *A. flavus* was the most common etiological agent of chronic invasive form (CIFRS), allergic fungal rhino sinusitis (AFRS) and fungus ball (FB), while *Rhizopus oryze* (26.7%) was the most common cause of infection in acute invasive fungal rhino sinusitis (AIFR). However, a few rare species such as *Shyzophyllum commune* and *Fusarium proliferatum* were also isolated.

**Conclusion:** Diabetes is the most important predisposing factor for patients with acute invasive form of sinusitis and the most involved sinuses were unilateral multiple sinuses and maxillary sinus.

**Keywords:**Fungal rhinosinusitis, AFRS, AIFR, Fungus ball

## Introduction

Fungal rhino sinusitis (FRS) is a group of infections affecting in paranasal including invasive and noninvasive forms. The invasive infections encompasses acute invasive or fulminant (AIFRS), Chronic granulomatos invasive (CGFRS) and chronic invasive (CIFRS). The noninvasive diseases include saprophytic fungal infestation (SFI), fungus ball (FB) and Allergic fungal rhino sinusitis (AFRS) (1, 2). These forms have been classified according to histopathology finding such as fungal invasion to vessels, bone erosion and infection period (1, 3). Acute invasive commonly happen in patients with immune suppressed status in a period less than 4 weeks (4).However, in all of invasive forms hyphal invasion to vessels and bone erosion are observed (2,5). In other hand noninvasive are an extra mucosal infection and most of the patient are immune competent (6,7).

*Aspergillus* sp. is the most common species reported as a major cause of fungal sinusitis(5,8-11) with the exception of acute invasive form that the most frequent fungal isolated belong to the Zygomycete order (8). However, etiologic agents of these infections may vary according to type of sinusitis and geographical epidemiology (4).

Up until now, the majorities of studies performed in Iran were limited to a few case reports or limited to particular form of fungal rhino sinusitis in a special patient (12, 13). In addition, the identified fungal agents were most fungal species identified by conventional methods (13,14).
The link between clinical features and etiological agent is an important factor to apply proper administration of antifungal agent (15). The application of complete identification of fungal agent seems quite necessary. This may apply a combination of culture, morphology, and molecular base assays.
In the present study we used both microscopic morphology and sequence-based identification assays using ITS and B-tubulin rDNA gene in order to identify causing agent own to species level. We also aimed to link the fungal sinusitis sub types to the etiological agent and the associated predisposing factors in patients with suspected rhino sinusitis.

## Materials and Methods

In a prospective cross-sectional study, 450 patients with suspected fungal rhino sinusitis referred to Amir-Alam Hospital, Tehran were examined. The patients had headache, rhinorrhea, obstruction and nasal discharge; inflammation or polyps in endoscopic examinations with sinus involvement in CT scan findings after obtaining informed consent were included in this study. All patients underwent endoscopic sinus surgery of the middle meatus. Tissue biopsies were taken to evaluate histopathological and mycological characteristics of these infections. Some portions of tissue samples were inoculated on two multi points of Sabouraud Dextrose Agar plate (belief, Italy) supplemented with chloramphenicol (0.5mg/ml) and incubated for 10 days at 30 and 37°C (16). All samples with positive culture identified by macroscopic features and morphological criteria were confirmed by PCR. From the 10 days colony on 4% sabouraud dextrose agar fungal DNA was extracted by using a conical grinder according to the previously described method (17).

Three pairs of forward and reverse primers were designed based on the sequence of translation elongation factor-1, ITS rDNA and tubulin genes for cases of *Fusarium* sp., *Aspergillus* sp. and *mucoral* sp. Demateaceous and indistinguishable species) were identified respectively by macroscopic features and morphological criteria (18-20).PCR was carried out according to PCR programs, selected for each primer (16, 19, 20). The PCR products were subjected to DNA sequencing with the same primers.
The obtained sequences were analyzed in Gen Bank database and revealed high identities recorded and then the results were compared with macroscopic features and microscopic structures.

## Results

In total, 87 patients were diagnosed with fungal rhino sinusitis over the period of this study. Fungal elements were identified in both direct examination and pathological finding.Thirty-three (30.7%) out of 87 patients had negative culture. Overall the mean patient age was (10-86) with a female to male ratio of 1.16/1 and based on clinical histological criteria patients were classified (Table 1). Overall, 81% of patients lived in urban areas and most prevalent of disease were house keeper women and worker men.
Depending on the type of sinusitis there were differences between fungal species. *A. flavus* was the most common etiological agent in CIFRS, AFRS and FB while *Rhizopusoryze* (26.7%) was the most common cause of infection in AIFR although some rare species such *Syzophyllum commune* and *F. proliferatumalso* report (Table2).
All patients with acute invasive form of sinusitis had underlying disease such as diabetes (71.4%) and malignancy (28.6%) whereas diabetes and malignancy in patients with non- invasive fungal sinusitis was 12.2% and 6.1% respectively. Headache (46.2%), nasal discharge (34.6%) and facial pain (33.3%) were most symptoms but these were different according to sinusitis form. The most involved sinuses were unilateral multiple sinuses and maxillary sinus.

**Table 1:** Distribution of patients with fungal rhino sinusitis based on age, Tehran, 2012-13

| Sinusitis type | NO | Mean | Range | F:M |
|---|---|---|---|---|
| AIFRS | 28 | 41 | (10-86) | 1.15/1 |
| FB | 33 | 44 | (20-69) | 1.5/1 |
| AFS | 16 | 36 | (11-67) | 1:1/3 |
| CIFRS | 1 | 55 | 55 | - |
| Total | 78 | 41 | (10-86) | 1.16/1 |

**Table 2:** Distribution of isolated fungal species based on rhino sinusitis type, Tehran, 2012-2013

| Fungal species | Rhino sinusitis types | | | | Total |
|---|---|---|---|---|---|
| | AIFRS No-PER (%) | FB No-PER(%) | AFRS No-PER (%) | CIFRS No-PER (%) | |
| *Asergillus flavus* | 2-4.4 | 14-31.1 | 9-20 | 0 | 25-55.5 |
| *Asergillus fumigatus* | 0 | 1-2.2 | 2-4.4 | 0 | 3-6.6 |
| *Rhizopus oryza* | 12-26.7 | 0 | 0 | 0 | 12-26.7 |
| *Fusarium proliferatum* | 0 | 1-2.2 | 0 | 0 | 1-2.2 |
| *Shizophyllum commune* | 0 | 1-2.2 | 0 | 0 | 1-2.2 |
| *Nattrassia mangiferae* | 0 | 0 | 1-2.2 | 0 | 1-2.2 |
| *Candida albicans* | 0 | 0 | 2-4.4 | 0 | 2-4.4 |
| Total | 14-31.1 | 17-37.7 | 14-31 | | 45- 100 |

## Discussion

Fungal rhino sinusitis has been considered as an uncommon disorder and its frequency are increasing in recent years. (21).This study is the first large study from Iran, and other studies had been conducted of limited (12-14, 22, 23). Thus many of fungal isolates particularly Zygomycets are identified in genus level that show different in vitro susceptibility to antifungal agents (24). Isolation and identification of fungal agent are tremendously important for proper management of infections caused by the less common fungi (25, 26). Therefore, combination of PCR sequencing with conventional methods able us to identify fungal isolates precisely specially in some rare species such as *S. commune* which despite the morphological study, due to the lack of diagnostic structure the only successful method for identification was PCR sequencing.

In this study majority of patient had non-invasive disease. This is similar to previous studies but different in sub type disorder frequency (5, 8, 27).

In contrast with our study, Challa et al. in South India reported a low frequency of non-invasive fungal sinusitis (25%) vs. invasive form (75%) (9). FB was the most common form that was similar to previous studies (5, 7). The only one case (1.3%) of CIFRS reported in a diabetic man was similar to Granville et al. , Monotone et al. and Das et al. study who reported 2.1% , 1.2% and 1% of patients with CIFRS in their patient groups respectively (4, 28) although the incidence CIFRS was higher (10%) in the Micheal et al. study (8).

Incidence AFRS was lower than other studies (28 ,29) but Panda et al. and Chakrabarti, et al. reported much lower incidence of this type of sinusitis (5,11). This difference could be due to variation in diagnostic criteria for disease defining because the existence of eosinophilic mucin (EM) with fungal elements in histopathological examination or the existence EM without fungal elements in histopathological examination (30). So it should not be ignored which could be due to contamination especially in some study samples collection by nasal irrigation fluid

through nasal passage and nasal brush or swab from nasal septums (31-34).

*Aspergillus* species are the most common fungal agents of the paranasal sinuses while according to geographical conditions there is a difference between *Aspergillus* species (5, 7-9, 27). *A. fumigatus* has the highest frequency in some reports (4,7) but in our study *A. flavus* had the highest frequency similar to other studies (5,8,27,35).

*Rhizopus* sp., *A. fumigatus* and *A. flavurs* are as the main causes of the acute invasive sinusitis in around the world (5, 8, 9, 27). In our study *Rhizopus oryza* was the most common fungal isolated in acute invasive sinusitis and all patients with this form showed an immune deficiency such as malignancy or uncontrolled diabetes.

Most variation in fungal species reported in AFRS. Although the *Aspergillus* sp. and dematiaceous species are most isolated but fungal species vary in disparate geographic area in different studies (4). However in our study dematiaceous had a very small role in AFRS and like some other study *A. flavus* was most common fungal species isolated in patients with AFRS (5, 9, 35).

Although the over growth of the *Aspergillus* sp. compared to the dematiaceous species in routine culture media should not be ignored (4), but hyaline septate hyphae, which branch at a 45 ° angle observed in direct examination was able to rollout ınfection due to dematiaceous fungal species (36).

33.3% of all samples submitted to laboratory with normal saline container had negative culture that was similar to some other studies (2, 36, 37). This could be due to the damaged hyphea during processing or when limited specimen is available and fungal agent lost their vitality. So histopathological examination provide only a presumptive diagnosis (38).

## Conclusion

This study showed the potential assistance by using the PCR sequencing method in combination with a conventional assay for precise identification of fungal species in fungal rhino sinusitis. However, it seems in cases where fungal elements envisage and where either culture result is negative or culture is not done, fungal DNA detection from tissue specimens for improvement of detection is necessary. In this situation we have more complete information about fungal rhino sinusitis epidemiology in Iran that help to better management and control of this infection by ENT specialists.

## Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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