



Fungal characterization using polymerase chain reaction in patients with fungal sinusitis

Sir,

Fungal infection is a cause of sinusitis and nowadays is

increasingly implicated in the etio-pathology of rhino-sinusitis. *Aspergillus* spp., dematiaceous fungi, and zygomycetes have been reported as etiological agents. Diabetes mellitus, hematological malignancy, primary immunodeficiency state, human immunodeficiency virus (HIV) infection, chronic renal failure, autoimmune diseases, and use of immunosuppressive drugs are the well-known predisposing factors. However, it has been reported in immunocompetent individuals with indolent course.^[1] Five distinct histologic categories of fungal sinusitis (FS) have been described: allergic, noninvasive fungal colonization (mycetoma or "fungus ball"), chronic granulomatous, chronic invasive, and acute fulminant.^[1] Allergic mucin with Charcot-Leyden crystals are common histologic features of allergic fungal sinusitis (AFS). Patients with chronic rhino-sinusitis commonly present with nasal polyps, inhalant atopy, and elevated total serum immunoglobulin E (IgE). This condition is believed to be an allergic reaction to aerosolized environmental fungi. In chronic noninvasive FS, the fungal balls (mycetoma) are easily seen by H and E staining, without any mucosal invasion. Chronic invasive FS revealed non-necrotizing granulomas with infiltration of eosinophils. In acute invasive FS, vascular invasion by fungal hyphae and thrombosis are present. It has an aggressive course with a high mortality rate.^[1,2]

The aim of this study was to determine the etiology of FS in patients from Shiraz, a city in the south of Iran. In a review of pathology files of Khalili hospital affiliated to Shiraz University of Medical Sciences, from June 2008 to July 2009, five cases of FS were diagnosed based on histopathologic findings. The patients were evaluated in the ENT out-patient department. The medical history and clinical examination of them were suggestive of chronic rhinosinusitis. All of them had radiologically proven sinusitis. Rigid nasal endoscopy with swab and biopsy collection from middle meatus was done.

The pathological report of three patients revealed allergic mucin, Charcot-Leyden crystals, and degenerated fungal hyphae, with no evidence of fungal invasion [Figure 1]. The fourth case suffered from asthma and atopy. The histopathologic evaluation of sinus mucosa showed fungus ball without any relation to respiratory mucosa [Figure 2]. The final case was a known case of uncontrolled diabetes mellitus, which on histopathological examination showed necrosis with invasion of blood hyphae in nasal mucosa and blood vessels. The fungal culture was positive in two patients; however, the type of fungus was not determined.

In order to define the exact fungal pathogen, DNA was extracted from formalin-fixed, paraffin-embedded tissues, using a commercial kit (DNP, CinageneIran), and nested polymerase chain reaction (PCR) to detect DNA specific for *Aspergillus* species was carried out.^[3] The PCR was positive in all the five patients.

AFS is the most common form of FS. This finding is in line with other studies^[1] and differs from the findings of Chakrabarti *et al.*^[4] and Panda *et al.*^[5]

The *Aspergillus* spp. is the only isolated fungus in our study. The most common isolated fungus from South India was *Aspergillus* spp.^[1,5] Dematiaceous fungi such as *Bipolaris* spp. and *Curvularia* spp. were found to predominate AFS in North America.^[6]

In Sudan, Saudi Arabia, Northern India and along the Atlantic coast of the USA, *Aspergillus* spp. are the most common etiological agents,^[7,8] whereas the dematiaceous fungi are the commonest in the southwest of the USA.^[6]

The climatic and geographic conditions seem to be responsible for this difference. The weather in our area is hot and dry which is similar to the weather in India and Saudi Arabia.^[7] In contrast, in the United States, the humidity facilitates the growth of dematiaceous fungi. *Aspergillus* spp. was isolated from the fungus ball. This is in concordance with other studies which report that *Aspergillus* is the most commonly isolated in mycetoma.^[1] We have only one case of acute invasive FS, from which *Aspergillus* spp. was isolated. Our finding is in concordance with a report

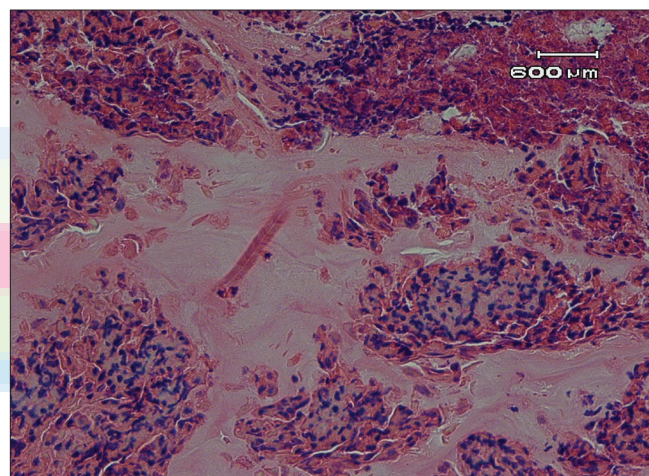


Figure 1: Allergic mucin with Charcot-Leyden crystals and degenerated hyphae (H and E ×200)

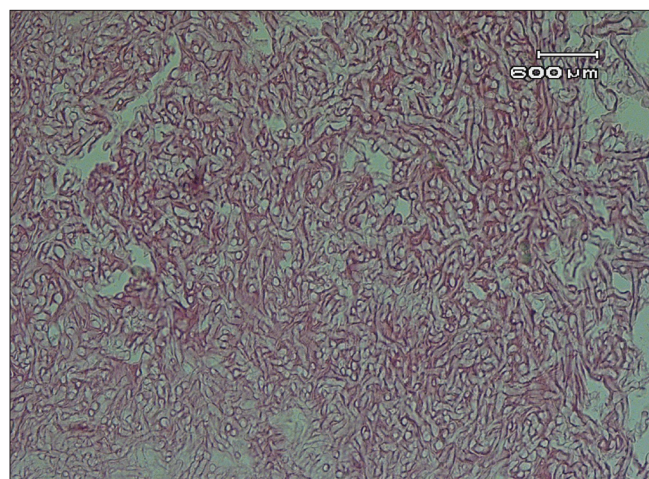


Figure 2: Mycetoma or fungus ball, aggregation of fungal hyphae (H and E ×200)

from India that *Aspergillus flavus* was the most common isolate.^[7] *Rhizopus* spp. that belongs to zygomycete and *Aspergillus* spp. were the first and second reported causes of invasive form in South India.^[1] In another study from North India, *Rhizopus arrhizus* and *Candida albicans* were found to be the causative agents.^[4]

In conclusion, this is the first report of FS from South Iran. The etiological agents involved in different forms of FS seem to be similar from India and Saudi Arabia, but differ from Western countries.

The sample size was small and fungal culture was not carried out for all of them. Therefore, further studies with larger patient numbers from different geographic parts of Iran are needed to investigate the cause of FS in our country.

**M. J. Ashraf, N. Azarpira, P. Badiiee¹,
B. Khademi², M. Shishegar²**

Department of Pathology, ¹Alborzi Clinical Microbiology Research Center, ²Department of Otolaryngology, Shiraz University of Medical Sciences, Shiraz, Iran

Address for correspondence:

Dr. Negar Azarpira, Transplant Research Center, Nemazi Hospital,
P.O.Box: 71935-1119, Shiraz University of Medical Sciences.
E-mail: negarazarpira@yahoo.com

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