

Exophiala oligosperma involved in a refractory chronic rhinosinusitis

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Abstract. – Fungal rhinosinusitis refers to a wide variety of conditions caused by fungal infections of the paranasal sinuses. Allergic fungal rhinosinusitis and sinus fungus balls are mostly noted in healthy individuals. *Aspergillus* species are supposed to be the most common etiologic agents of the disorder, but melanized fungi also occur, and these potentially are able to lead to fatal dissemination into brain parenchyma. We report on a case of fungus ball in a 20-year-old female with refractory chronic rhinosinusitis (RCRS) and bronchial asthma due to the black yeast *Exophiala oligosperma* which was confirmed by mycological and molecular (sequences of ITS rDNA) investigations. *Exophiala oligosperma* has previously not been reported to cause fungus balls or invasive fungal rhinosinusitis. Patient underwent functional endoscopic sinusitis surgery and the hypertrophic mucosa was removed completely. Without antifungal therapy, successful cure was achieved after spray therapy with corticosteroids for 1 month, without any relapse after a 6 month-follow up.

Key Words:

Fungal rhinosinusitis, Fungus ball, *Exophiala oligosperma*, *In vitro* activity, ITS rDNA.

Introduction

Fungal rhinosinusitis (FRS) refers to a wide range of conditions associated with fungal growth in the paranasal sinuses of otherwise

healthy individuals. The disorder has been reported with high frequency worldwide¹. Invasive infections, based on histopathology, can be acute and fulminant, or chronic and granulomatous, whereas intracavitary fungus balls (incorrectly referred to as mycetomas) and allergic fungal rhinosinusitis (AFRS) are classified as non-invasive types². The immunological status of the host determines the degree of infection of the paranasal sinuses, which means that patients with invasive fungal rhinosinusitis are usually immunocompromised and may, therefore, potentially lead to complications. AFRS starts off with an allergic hypersensitivity response to the presence of extra-mucosal foreign bodies. Occasionally fungal material in the maxillary sinus cavity slowly aggregates into fungus balls, which consist of hyphal masses mixed with allergic mucin, intact and degenerating eosinophils, Charcot-Leyden crystals and cellular debris². Fungus balls are clinically significant because of nasal obstruction, facial pain, pressure and discharge. However, total specific IgE concentrations are lower than with AFRS². Most patients with fungus balls are healthy individuals. To date, a wide variety of saprobic fungi has been seen on the mucosa and crusts in the sinonasal cavity of immunocompetent patients following sinus surgery. *Aspergillus* is supposed to be the most common etiologic agent of the disorder, but culturing of samples from sinus fungal balls is positive in only 23-50% of the cases and thus other agents may have been missed³. In histopathology, *As-*

pergillus, *Scedosporium* and *Schizophyllum* are characterized by colourless hyphal elements which can not be differentiated from one another while infections usually remain indolent. In contrast, melanized fungi, characterized by olivaceous brown or greenish black hyphal elements, may lead to fatal dissemination into the brain parenchyma, known as secondary cerebritis⁴. Particularly species of *Bipolaris*, *Exserohilum* and relatives belonging to the fungal order *Pleosporales* are known as potential agents⁵. Other melanised fungi may be involved in fatal, cerebral or disseminated, often disfiguring infections in otherwise healthy individuals primarily. Symptoms included headache, seizures cerebral irritation neurological deficits, fever. Among these are *Cladophialophora bantiana*, *Fonsecaea monophora*, *Exophiala dermatitidis* and *Rhinoctadiella mackenziei*^{4,6}. These species have a probable pulmonary portal of entry and exhibit the first clinical symptoms in the brain; the disorder is, therefore, referred to as primary cerebritis. They are classified in the fungal order *Chaetothyriales* in the family of *Herpotrichiellaceae*⁷, which have rarely been reported to be involved in sinusitis⁸. In this study we present a case of melanised fungus ball due to *Exophiala oligosperma* in a 20 year-old female.

Case Report

A 20 year-old HIV negative Iranian female with bronchial asthma and a 4-year history of rhinosinusitis with nasal septal deviation was admitted for evaluation early 2009 to the Department of Ear, Nose and Throat of the Boo-Ali Hospital, Mazandaran University of Medical Sciences, Sari, Iran. Clinical history was suggestive of allergic rhinitis with complaints facial pain, purulent nasal discharge, epistaxis, nasal obstruction, nasal stiffness, fever, cough, sneezing, anosmia, halitosis and dental pain. There was no underlying immunodeficiency or diabetes mellitus. Computerized tomography (CT) of the paranasal sinuses revealed nasal septal deviation, mucosal thickening, complete opacification of the involved sinuses, unilateral obstruction of ostiomeatal complex and no evidence of bone erosion. Patient underwent functional endoscopic sinus surgery (FESS) and the hypertrophic mucosa or paranasal sinuses secretions were removed completely and collected into a suction trap. Then fragments of the tissue were sent for further investigation.

Mycological Investigation

The entire biopsied tissue was adjusted for mycological and histopathological investigations. Macroscopically, mucopurulent friable cheesy material that was easily peeled off the mucosa within the sinus was seen. Direct examination with KOH (20%) supplemented by periodic acid-Schiff (PAS) stain revealed tangled mats of hyphae (Figure 1). Histological examination showed no evidence of invasive fungal infection. Direct cytological examination was also performed with hematoxylin and eosin (H&E) staining revealed a mild nonspecific chronic inflammatory response with predominance lymphocyte, few plasma cell and scant and scattered mast cell and neutrophils and no eosinophils in nasal mucus specimen. The specimens were cultured by standard techniques on Sabouraud's glucose agar (SGA) and SGA supplemented with chloramphenicol (0.5 µg/ml) and incubated at 27-30°C for two weeks. Growth of a dematiaceous fungus was observed which morphologically revealed the presence of numerous brownish, septate, branched hyphae with annelloconidia identified as an *Exophiala* species. Stock cultures were maintained on slants of 2% malt extract agar (MEA, Difco) and oatmeal agar (OA) at 24°C⁹, and a voucher strain was deposited in the CBS-KNAW culture collection as CBS 124085.

Microscopy was done with slide culture techniques using potato dextrose agar (PDA, Difco) or OA because these media readily induce sporulation and suppress growth of aerial hyphae¹⁰. Slides were made after 2 weeks in lactic

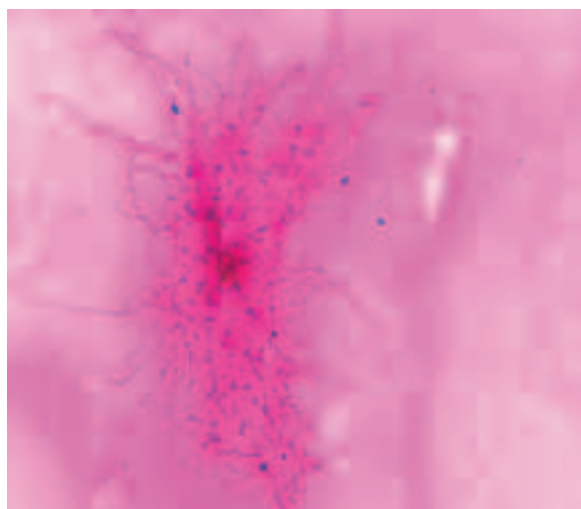


Figure 1. Tangled mats of mycelium as observed in KOH (10%) wet mount supplemented by PAS stain.

acid or lactophenol cotton blue and light micrographs were taken using a Nikon Eclipse 80i microscope with a Nikon digital sight DS-Fi1 camera. Colonies were moderately expanding and restricted, velvety, olivaceous grey, and dry, mostly with an insignificant yeast phase on MEA with olivaceous black reverse (Figure 2A-B). Budding cells were abundant, pale olivaceous, broadly ellipsoidal, $3 \times 2.5 \mu\text{m}$, often appears encapsulated in India ink preparation, later inflating and developing into broadly ellipsoidal germinating cells often bearing a single, short, irregular annellated zone. Hyphae were pale olivaceous to brown, somewhat inflated, $1.5\text{--}3.2 \mu\text{m}$ wide, and irregularly septate. Conidiogenous cells mostly arose at acute angles as part of a slightly differentiated conidial apparatus, also arising at right angles from creeping hyphae. Conidial branches were of the same colour as the hyphae or only slightly darker and one- to

three-celled; the ultimate cells were rocket-shaped or cylindrical, tapering towards the end with a flaring, irregular annellated zone. Conidia adhering in small groups, subhyaline, $3\text{--}5 \times 2.2\text{--}3.2 \mu\text{m}$. Spherical, subhyaline chlamydospores up to $13 \mu\text{m}$ in diameter were present (Figure 2C-G). Cardinal growth temperatures of strain CBS 124085: growth between $9\text{--}37^\circ\text{C}$, optimum at 27°C , no growth observed at 40°C .

For molecular analysis mycelia were grown on 2 % MEA plates for 2 weeks at 24°C . DNA was extracted using an Ultra Clean Microbial DNA Isolation Kit (Mobio, Carlsbad, CA, USA) according to the manufacturer's instructions. ITS rDNA was amplified using primers V9G (5'-TTA CGT CCC TGC CCT TTG TA-3') and LS266 (5'-GCAT TCC CAAACA ACT CGA CTC-3') and sequenced with the internal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3').

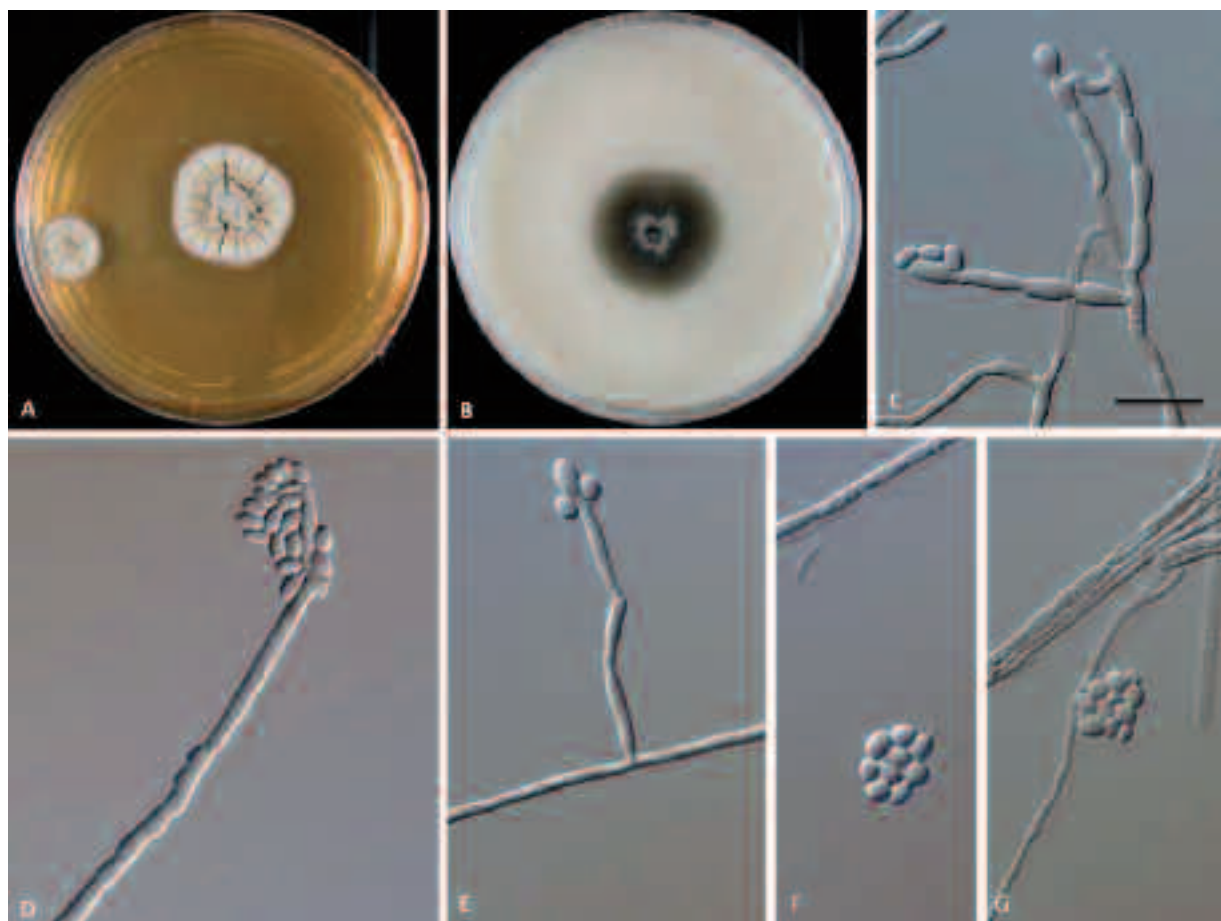


Figure 2. *Exophiala oligosperma* (CBS 124085). (A-B) Colony on malt extract agar (MEA, Difco) and Oat meal agar (OA) at 30°C after 2 weeks in darkness produced dark, moist, olivaceous-black yeast-like colony at room temperature. (C-G) Conidia clustered at the apices of the tapered annellides and long, thick-walled, septate conidiophores. Scale bar = $10 \mu\text{m}$.

PCR amplification and sequencing were according to Badali *et al.* 2009¹¹. Sequences were compared with GenBank and through local blast with a molecular database maintained at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, for research purposes. The obtained isolate was subjected to DNA sequencing of internal transcribed spacer region (ITS rDNA) and identified as *Exophiala oligosperma* by showing less than 1% difference with the ex-type strain of that species (CBS 725.88 = AY163551) which had originally been isolated from a 45-year-old woman with a tumour of the sphenoidal cavity.

The case was proven by CT, positive direct examination with KOH supplemented by PAS stain and sinus cultures were positive for *Exophiala oligosperma* (Figure 1). The agent was identified and confirmed by sequencing of the internal transcribed spacer region of rDNA. The total serum IgE was not elevated. Therefore, these observations led to the diagnosis of a sinus fungal ball due to *Exophiala oligosperma*. Successful cure was achieved after surgery and spray therapy with corticosteroids for 1 month, without any relapse after 6 month follow up without antifungal therapy.

In vitro Antifungal Susceptibility

The strain isolated was tested to determine its *in vitro* antifungal susceptibilities to eight antifungal agents. MICs (minimum inhibitory concentration) and MECs (minimum effective concentration, echinocandins only) were evaluated by broth microdilution which was performed by the reference method according to the Clinical and Laboratory Standard Institute (CLSI) document M38-A2¹². *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality control¹². Methods for conidial suspensions were those of Badali *et al.*¹¹. MICs for the current strain (CBS 124085) was as follows: amphotericin B (2 µg/ml), fluconazole (16 µg/ml), itraconazole (0.063 µg/ml), voriconazole (1 µg/ml), posaconazole (0.063 µg/ml), and isavuconazole (1 µg/ml). The two echinocandin agents gave MECs of 8 µg/ml for caspofungin and of 1 µg/ml for anidulafungin.

Discussion

Most cases of fungal sinusitis are benign or non-invasive, except when they occur in im-

munocompromised individuals. Allergic fungal rhinosinusitis is quite common in healthy individuals and starts off with an allergic hypersensitivity response to the presence of extra-mucosal foreign bodies. Several reports are available that have shown invasive fungal infections are exceptional and the disorder may lead to higher morbidity and mortality. Sinus fungus balls due to *Aspergillus* species or *Fusarium* species that become invasive by spreading beyond the sinus region and eventually lead to fulminant disseminated disease after solid organ transplantation have been described¹³. Moreover, case reports have been published in which the fungus ball was thought to be secondary to a zygomycotic infection. Therefore, rhinosinusitis may take quite different clinical courses.

Species of *Bipolaris*, *Curvularia*, *Drechslera* and *Alternaria* belonging to the fungal order *Pleosporales* are the most common agents of allergic fungal sinusitis¹, and in general are prone to cause secondary cerebritis, even in otherwise healthy individuals. Shivaprakash *et al.* 2009¹⁴, reported the first case of AFRS due to *Neosartorya hiratsukae* in a 40 year-old healthy Indian male complaining of nasal blockage, headache without any diplopia. The CT showed no signs of mucoperiosteal change, but thick mucus in the left sphenoid and posterior ethmoid was found without bone destruction. In contrast, Guarro *et al.*, 2002¹⁵ reported invasive cerebral aspergilliosis by *N. hiratsukae* in a 75 year-old Brazilian female.

Exophiala oligosperma is a member of *Chaetothyriales*, known for causing primary encephalitis. After a thorough search of the published literature, we have found only a single proven case¹⁶ of secondary cerebritis in the *Chaetothyriales*, out of a total of 126 cases, in immunocompetent individuals; in contrast, in the *Pleosporales* this ratio was 5:17.

Exophiala is a genus of black yeast and relatives in the order of *Chaetothyriales* characterized by annellidic conidiogenesis, which are remarkably frequently encountered in human infections, ranging from allergic sinusitis to fatal cerebral phaeohyphomycosis in otherwise healthy individuals with fatal outcome despite antifungal therapy⁴. Among the most virulent species are *Exophiala dermatitidis*, *E. spinifera*, *E. oligosperma* and *E. lecanii-corni*. Although infection by these species is rare, its incidence is increasing, particularly in immunocompromised patients. Pulmonary colonization by *E. dermatitidis* is regularly observed in patients with cystic fibrosis¹⁷.

Most fungus balls are diagnosed clinically and histopathological examination is often not performed. However, it is recommended that melanized etiologic agents of chronic sinusitis are identified down to the species level at all times. Fungal growth with septate hyphae can be detected in the mucosa. Early identification of agents of non-invasive fungal sinusitis especially in the order of *Pleosporales* may help to prevent invasion. Sinus fungus balls due to *E. oligosperma* have thus far not been published.

Identification of the black yeast was confirmed by combined molecular and morphological techniques. Detection of allergic mucin in the sinus content due to fungi is crucially important and should be considered in all patients for early diagnosis and may prevent multiple surgical interventions.

Acknowledgements

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