

Ochroconis humicola Coexisting with Esthesioneuroblastoma: An Incidental Coloniser or Allergen?

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Abstract *Ochroconis humicola*, a fish pathogen, is rarely reported to cause disease in human. We report its first isolation from nasal tissue of a human immunodeficiency virus-positive young female patient. Histopathologically, the nasal mass was diagnosed as esthesioneuroblastoma. She presented with right-sided nasal obstruction and bleeding for two and half months. Computed tomography scan showed the nasal mass filling the whole right nasal cavity, maxillary, ethmoid and sphenoid sinuses. The direct microscopy of the nasal tissue and mucin demonstrated the presence of septate hyphae. On culture, *O. humicola* was isolated from the same tissue and the fungus was identified by morphologic, physiologic and molecular data including sequencing of ITS and 28S rDNA regions. No antifungal was prescribed, and the whole mass was resected out by endoscopic surgery. The patient was treated further by radical radiotherapy. After 1 year of

follow-up, patient is stable with no recurrence of tumour. The role of this fungus was not clear, as it may be bystander or producing allergic fungal rhinosinusitis.

Keywords *Ochroconis humicola* · Esthesioneuroblastoma · Allergen · HIV patient · AFRS

Introduction

Phaeoid fungi are a group of fungi causing phaeohyphomycosis with cutaneous, subcutaneous and systemic involvement. These fungi are present ubiquitous and can lead to human infections especially in immunocompromised population. *Ochroconis* is an important member of this group of fungi. Human infections due to *O. gallopava* and *O. tshawytschae* have been reported in immunocompromised individuals, while *Ochroconis humicola* has been reported to cause infections in fish [1–5]. *O. humicola* is sparsely prevalent in the environment and has a less chance to colonise on human skin [6]. The only report of its association with human disease has been documented by Goldschmied-Reouven et al. from an ulcerative lesion [7]. We report here the first case of isolation of *O. humicola* from nasal tissue of human immunodeficiency virus (HIV)-positive patient diagnosed with malignancy of nasal cavity.

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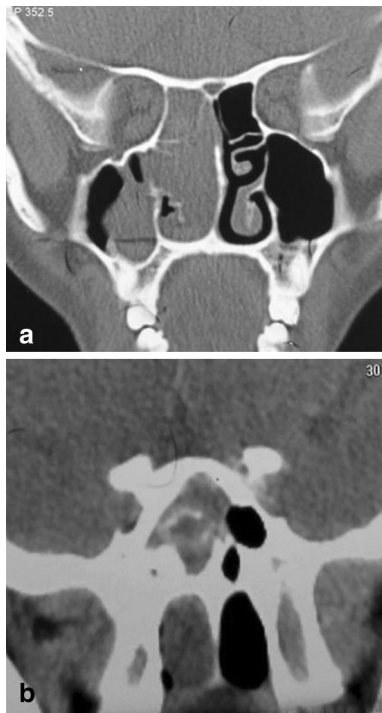


Fig. 1 **a** Coronal CT, anterior part of nasal cavity and paranasal sinuses showing a mass lesion occupying right nasal cavity, ethmoids and maxillary sinus. **b** Coronal CT, posterior part of nasal cavity and paranasal sinuses showing a heterogenous density in right sphenoid sinus

Case Report

A 27-year-old female presented to Otolaryngology outpatient department with complaints of right-sided nasal obstruction and intermittent bleeding from nose for the past two and half months. She had no associated ear or throat complaints. She was seropositive for HIV for the last 3 years and was on regular highly active anti-retroviral therapy (HAART) for 2 years. Anterior rhinoscopy revealed pinkish mass filling the right nasal cavity. CT scan showed a heterogenous mass lesion filling the right nasal cavity, maxillary and ethmoid sinuses (Fig. 1). In addition, inspissated secretions were noticed in right sphenoid sinus.

With a probable diagnosis of right paranasal malignancy, patient was taken up for endoscopic excision under general anaesthesia. Intraoperatively, friable reddish mass was noticed, which occupied whole of the right nasal cavity and nasopharynx with moderate bleeding. Brownish-coloured debris and

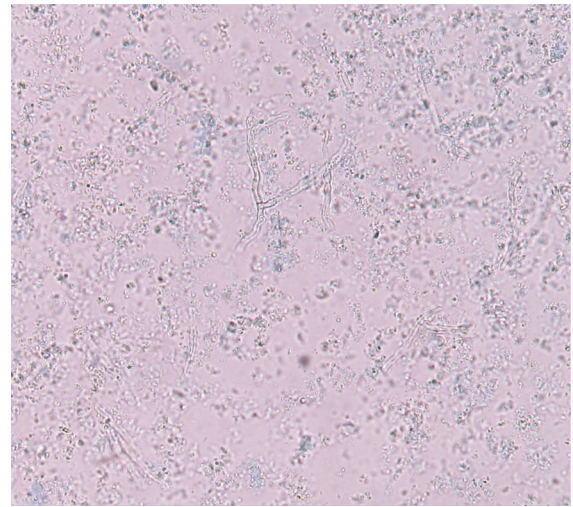


Fig. 2 Septate hyphae in potassium hydroxide mount of tissue including mucin

tissue along with allergic mucin were suctioned from right nasal cavity/sinuses and sent for microbiological and histopathological examination. As the histopathology of the biopsy showed features of poorly differentiated ethmoidal carcinoma (esthesioneuroblastoma), patient underwent complete removal of the mass and radical radiotherapy. She was discharged with prescription of xylometazoline nasal drops. Steroids were not prescribed due to her immunocompromised status, though allergic fungal rhinosinusitis was suspected. Check endoscopy performed after 8 months of initial treatment did not reveal any residual mass or any new growth. Patient is on regular follow-up, and after a year, she is still stable without any symptoms related to nasal tumour.

Mycological Study

The tissue and mucin material collected from nose were examined in microbiology laboratory. Septate hyphae were demonstrated in the mucinous material of the sample on potassium hydroxide wet mount (Fig. 2). The tissue with mucinous material was inoculated on Sabouraud dextrose agar (SDA) containing chloramphenicol and gentamicin and incubated at 25 and 37 °C. The sample was also inoculated on brain heart infusion (BHI) agar with actidione (cycloheximide) and incubated at 25 °C. The growth of a dark brown to black dry flat colony on SDA at 25 °C suggested phaeoid fungus which also grew on



Fig. 3 Dark brown flat dry colony of *O. humicola*

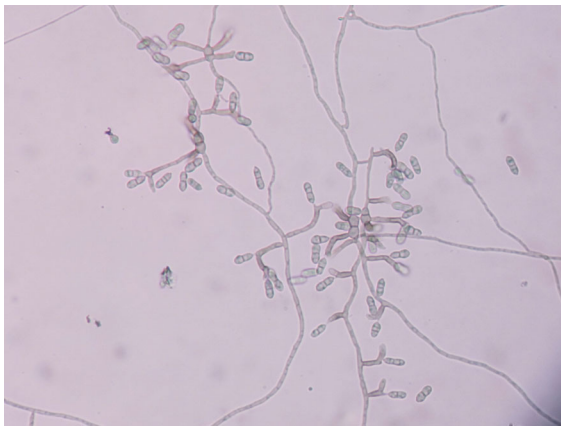


Fig. 4 Lactophenol cotton blue mount of slide culture after 2 weeks

BHI showing its resistance to actidione (Fig. 3). Slide culture prepared on potato dextrose agar (PDA) medium after 2 weeks of incubation at 25 °C revealed brown septate hyphae with thick walls and two-celled, pale brown, smooth-walled, cylindrical to clavate conidia measuring 10–15 µm × 3–4 µm in size and constricted at the septum, suggesting it to be a member of *Ochroconis* genus (Fig. 4). Physiologic tests performed to identify species of *Ochroconis* include temperature tolerance at 30, 40 and 45 °C on SDA

medium, gelatin liquefaction and urease test. Fungus grew at 30 °C, but was unable to grow at 37, 40 and 45 °C. Gelatin was not liquefied, and urease test was positive. Based on the morphology and physiologic results, the isolate was presumptively identified as *O. humicola*. It was further subjected to molecular identification.

Antifungal Susceptibility

Antifungal susceptibility testing was performed as per Clinical Laboratories Standard Institute document for broth microdilution technique (M 38 A2). The minimum inhibitory concentration (MIC) (or minimum effective concentration for echinocandins) of isolate was as follows: Amphotericin B-1 µg/ml; Itraconazole-0.5 µg/ml; Voriconazole-0.5 µg/ml; Caspofungin- 0.5 µg/ml; Anidulafungin-0.5 µg/ml.

Molecular Study

The DNA was extracted by method described previously [8]. Briefly, growth of *O. humicola* in potato dextrose broth incubated at 30 °C for 4 days on a rotary shaker (Innova, Ependroff, Hamburg, Germany) was filtered and the mycelial mass was thoroughly washed with sterile normal saline. It was then treated with liquid nitrogen, and the crushed powder was put into a 1.5 ml microcentrifuge tube having 600 µl of lysis buffer (100 mM Tris–HCl, pH 8.0, 50 mM EDTA, 3 % SDS). Proteinase K (Sigma St. Louis, MO) at a final concentration of 20 g/ml was added followed by incubation at 56 °C for 1 h. The phenol–chloroform extraction method was used to extract DNA. DNA was precipitated with an equal volume of isopropanol in the presence of 3 M sodium acetate. Washing of pellet was carried out using 70 % alcohol, and finally, it was dissolved in 100 µl of TE (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) and stored at –20 °C till further use.

DNA was subjected to PCR using internal transcribed region 1 (ITS 1) (TCCGTAGGTGAACCTGCGG), ITS4 (TCCTCCGCTTATTGATATGC) and 28S rDNA primers. Sequencing was done by Big Dye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster city, CA, USA) and analysed by ABI 3130 Genetic Analyser (Applied Biosystems). The sequence obtained was compared with GenBank DNA database. The sequence obtained

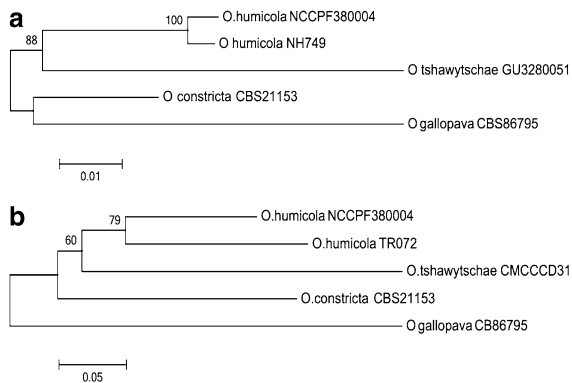


Fig. 5 Phylogram of the currently known *Ochroconis* species of human importance generated using the maximum composite likelihood method based on **a** 28S region and **b** ITS region of rDNA gene sequence data. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

by ITS primers showed 98 % identity with standard strain of *Ochroconis* (CBS 102086; GenBank accession HQ667538), while the sequence obtained by 28S rDNA primers showed 99 % identity with *O. humicola* strain (NH749; GenBank accession: AB701672). The isolate was deposited at the National Culture Collection of Pathogenic Fungi (NCCPF), PGIMER, Chandigarh, India with accession NCCPF 380004. The nucleotide sequence of 28S rDNA region is deposited in GenBank with accession KF705043. Phylogenetic analysis was conducted using CLUSTALX 2 and MEGA 5. (Fig. 5).

Discussion

Dematiaceous fungi usually exist as environmental saprobes and are known to cause infections in both immunocompetent and immunosuppressed individuals. Phaeohyphomycosis is the broad term, which encompasses different spectra of infections caused by these phaeoid fungi ranging from superficial to deep-seated disease. The present case is a rare event as it describes the isolation of *O. humicola* from the nasal tissue of HIV patient. *O. humicola* has been isolated from litter collections in humid tropical forests. Though its isolation has been reported from skin and nail specimens, the association of this agent with the disease is still doubtful [6, 7]. The present case is a HIV-positive patient with esthesioneuroblastoma, which is an uncommon malignant neuroectodermal tumour originating from neuro-epithelial cells of the olfactory membrane [9]. The excised tissue along with the mucin sent to Mycology laboratory grew *O. humicola*. The presence of mucin in the sample leads to the possible diagnosis of allergic fungal rhinosinusitis (AFRS), though further investigation like total immunoglobulin E (IgE) or skin test was not conducted in the patient. The detection of septate hyphae in the mucinous tissue and the isolation of this fungus may indicate its etiologic role for causing AFRS. However, the fungus may also be mere coloniser of nasal tissue of the patient. The presence of *O. humicola* in the neuroectodermal tumour indicates its possible affinity for the neural tissue such as other pathogenic species under this genus, *O. gallopava*. However, unlike *O. gallopava*, *O. humicola* failed to grow at 37 °C. That

Table 1 Characteristics of various species of *Ochroconis* genus

Characteristics	<i>O. humicola</i>	<i>O. gallopava</i>	<i>O. tshawytschae</i>	<i>O. constricta</i>
Growth at 37 °C	Absent	Present	Present	Present
Growth at 40 °C	Absent	Present	Absent	Present
Growth at 45 °C	Absent	Absent	Absent	Present
Growth in cycloheximide (0.05 %)	Present	Present	Present	Absent
Urease	Positive	Not known	Not known	Not known
Gelatin liquefaction	Negative	Not known	Not known	Positive
Conidial morphology	2 celled, cylindrical, smooth, 7–15 × 2.5–4.0 µm	2 celled, clavate, smooth-verrucose, apical cell wider than basal cell 11–18 × 2.5–4.5 µm	2–4 celled, cylindrical, verrucose, 12–25 × 3.5–5.5 µm	2 celled ellipsoidal, verrucose, 5–12 × 2.5–4.0 µm
Conidiophore	Up to 100 µm long and 2–3 µm wide	Cylindrical to acicular	Up to 30 µm long and 2–3.5 µm wide	Short non-septate

was the possible reason also for the presence of fungus in nasal tissue without invasion. Nasal cavity maintains lower temperature than that of the host body temperature.

The nomenclature of genus *Ochroconis* was suggested by de Hoog and von Arx in 1983 to include all morphologically similar fungi, i.e. *Scolecobasidium constricta*, *Scolecobasidium tshawytschae* and *Dactylaria gallopava*, but this classification was not entirely accepted. The genus *Scolecobasidium* was designated by Abbott in 1927 [10]. Salkin and Dixon in 1986 compared the morphologic and physiologic properties and proposed that *D. gallopava* be regarded as *D. gallopava* var. *gallopava* and the remaining isolates be given a separate variety of *D. humicola* var. *humicola*. The genus *Ochroconis* thus contains *O. gallopava*, *O. tshawytschae*, and *O. humicola*. These fungi are present in environment and have been associated with infections in cats, birds, fish and amphibians. *O. gallopava* is a well-known human pathogen causing CNS infection, pulmonary infections and subcutaneous abscesses [1, 2, 11–14]. On the other hand, *O. tshawytschae*, a rare fish pathogen, has been reported to cause subcutaneous infection in an immunocompetent host [4].

Identification of *O. humicola* was based on combination of microscopy, physiologic characteristics and molecular data. The various differentiating features of *O. humicola*, *O. gallopava*, *O. tshawytschae* and *O. constricta* are given in Table 1 [4, 7, 15]. *O. humicola* was identified by the presence of brown hyphae with two-celled, pale brown, smooth-walled, cylindrical conidia, constricted at the septum, inability to liquefy gelatin, inability to grow at 37, 40 and 45 °C, ability to grow at 25 and 30 °C and urease positivity. Nucleotide sequence data of ITS region of rDNA were unable to identify *Ochroconis* to species level as it provided only 98 % identity. However, 28S region identified it to the species level with 99 % similarity. This may be due to the scarcity of the sequence data of *O. humicola*.

The present case, thus, probably represents either nasal colonisation or allergy by *O. humicola* in HIV-positive patient with nasal malignancy.

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