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CASE REPORT/CAS CLINIQUE

Implantation phaeohyphomycosis caused by a non-sporulating *Chaetomium* species

Phaeohyphomycose d'inoculation causée par une espèce non sporulante de Chaetomium

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KEYWORDS
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Summary We report the case of a 66-year-old Iranian woman with a phaeohyphomycotic cyst (approximately 3 × 2.5 cm in size) on the right lateral side of the neck. She had dysphagia and hoarseness, without any pain. She complained about discharge of black liquid on the skin and irritation. Histological examination of biopsy fragments from the lesions showed septate, branched brown hyphae. The fungus was cultured, but sporulation remained absent from 4- week-old cultures on Sabouraud dextrose agar (SDA), malt extract agar (MEA), potato dextrose agar (PDA), and water agar with sterile filter paper. Identification with the genus *Chaetomium*

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was achieved by sequencing the internal transcribed spacer (ITS) and the small subunit (SSU) domains of the rDNA gene and comparison with sequences held at GenBank and at the Centraalbureau voor Schimmelcultures (CBS). Sequencing of the SSU rRNA gene reveals this strain as belonging to the genus *Chaetomium*. The sequence of ITS did not fully match with any sequence of available ex-type strains of *Chaetomium*, *Thielavia*, *Madurella* and *Papulaspora* and hence might belong to an undescribed species. However, without diagnostic morphological features the taxon cannot be introduced as a novel member of the genus *Chaetomium*. After local excision of the cyst and antifungal therapy with ketoconazole (200 mg twice a day), the lesion regressed and healed completely.

MOTS CLÉS
Phaeohyphomycose cutanée ;
Chaetomium ;
Sordariales

Résumé Nous rapportons le cas d'une femme iranienne de 66 ans avec un kyste phaeohyphomycotique (environ 3 × 2,5 cm de diamètre) sur la face latérale droite du cou. Elle se plaignait d'un écoulement de liquide noir sur la peau et d'irritation. L'examen histologique des fragments de biopsie de la lésion a montré des hyphes bruns, cloisonnés et ramifiés. Le champignon a été cultivé, mais la sporulation est restée absente après 4 semaines de culture sur *Sabouraud dextrose agar* (SDA), agar à l'extrait de malt (MEA), agar pommes de terre dextrose (PDA), et de l'agar à l'eau avec du papier filtre stérile. L'identification avec le genre *Chaetomium* a été réalisée par séquençage de l'espaceur transcrit interne (ITS) et de la petite sous-unité (SSU) domaines de l'ADNr et la comparaison avec des séquences détenues dans la GenBank et au voor Schimmelcultures Centraalbureau (CBS). Le séquençage du gène ARNr SSU révèle cette souche comme appartenant au genre *Chaetomium*. La séquence de son ITS ne correspondait pas entièrement avec toutes les séquences de souches disponibles ex-type de *Chaetomium*, *Thielavia*, *Madurella* et *Papulaspora* et par conséquent pourrait appartenir à une espèce non décrite. Cependant, sans caractéristiques morphologiques diagnostiques le taxon ne peut pas être présenté comme un nouveau membre du genre *Chaetomium*. Après exérèse locale du kyste et le traitement antifongique avec le kétoconazole (200 mg deux fois par jour), la lésion a régressé et a guéri complètement.

Introduction

Chaetomium, a genus in the ascomycete family *Chaetomiaceae* (order *Sordariales*), is widespread in soil, on dung, and in indoor environments. Taxonomy of the genus is still in a state of flux, since no modern, molecular revision of the genus is as yet available. *Chaetomium* species are characterized by elaborated, ornamented fruit bodies producing numerous, thin-walled asci which contain unicellular, dark brown ascospores. In many species, anamorph sporulation is absent or insignificant. *Chaetomium* species are regularly encountered as causative agents of infections in humans. Some species are thermophilic and have been reported from brain infections [4,15,23,24,28]. Using molecular phylogeny data, de Hoog et al. [5,6,9] and Van de Sande et al. [25] proved that the *Madurella* agents of eumycetoma cluster in *Chaetomium*, underlining the potential clinical significance of the genus. Despite antifungal treatment, the mortality rate among patients with systemic *Chaetomium* infections is high [22]. The most prevalent clinical species is *Chaetomium globosum*, but several other species such as *C. atrobrunneum*, *C. funicol* and *C. murorum* have been reported [8,22]. Most infections can be listed under the umbrella term ''phaeohyphomycosis'' using the presence of melanized hyphal fragments in tissue as a prime criterion, although clinically the infections may be widely divergent [14]. As mentioned above, another frequent clinical syndrome in the *Chaetomium* relationship is mycetoma, characterized by compact, black grains in tissue. In addition, the case of chromoblastomycosis outside the black yeast-like fungi was caused by a *Chaetomium* species [21].

The present paper describes a subcutaneous phaeohyphomycosis caused by *Chaetomium*-like species in an immunocompetent patient.

Case report

A 66-year-old immunocompetent female residing in a rural countryside of Birjand, Iran, presented with a cyst (approximately 3 × 2.5 cm in size) on the right side of the neck (Fig. 1). She had a history of thyroidectomy 2 months before. She had fever, dysphagia and hoarseness, but no pain. She complained about discharge of black liquid on the skin and irritation.

The results of clinical laboratory tests were: Blood culture negative, Urine culture negative, platelets = 337,000 per mm^3, glucose = 96 mg/dL, white blood cells = 5500 cells per microliter, hemoglobin (HB) = 9.6 g/dL, hematocrit (Hct) 40%, urea = 30 mg/dL, Creatinin = 1.2 mmol/L. Histological examination of biopsy fragments after hematoxylin-eosine staining showed the presence of dematiaceous catenulate fungal cells and septate mycelium surrounded by polymorphonuclear leukocytes (Fig. 2). The patient was initially treated with local excision of the lesion followed by administration of oral fluconazole (150 mg twice a day), but patient complained of a dry mouth and body edema and did not comply with treatment. After 3 months, oral ketoconazole (200 mg twice a day) was prescribed for twelve

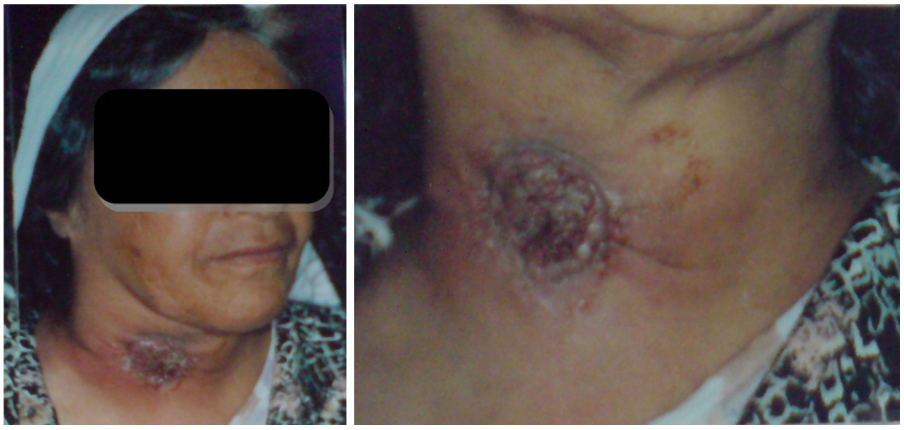

Figure 1 A cyst on the right lateral of neck of a 66-year-old Iranian woman.
Kyste sur le côté droit du cou chez une femme iranienne de 66 ans.

weeks and the lesion resolved (Fig. 3). The patient was considered clinically cured with ketoconazole and after 2 years follow up, no relapse of the fungal infection was observed.

Mycology

A filamentous fungus was isolated twice from biopsy specimens. Colonies on malt extract agar (MEA) grew rapidly with a white to grey aerial mycelium and a black colony reverse (Fig. 4). After 4 weeks of growth on MEA, Sabouraud's glucose agar (SGA), potato dextrose agar (PDA) and water agar with sterile filter paper at 25 °C and 35 °C, no sporulation was obtained, the culture entirely being composed of olivaceous brown hyphae. The strain was enlisted in the reference collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands, with accession number CBS123940. Cardinal temperatures of CBS123940 on MEA plates showed optimal development at 30 – 33 °C, with a growth maximum at 40 °C. Because the fungal isolate failed to produce any identifiable diagnostic structure, the isolate was subjected to routine methods of molecular identification involving the ribosomal internal transcribed spacer (ITS) and small subunit (SSU) domains.

DNA extraction was performed using glass beads (Sigma G9143) according to the protocol described previously [18]. ITS amplicons were generated with primers V9G and LS266 and were sequenced with primers ITS1 and ITS4 [6,17] and small subunit (SSU) rDNA amplicons were generated with primers NS1 and NS24 and were sequenced with primers BF83, Oli1, Oli9, BF951, BF963, BF1438, Oli3 and BF1419 [7].

PCR amplification and sequencing were conducted as described previously [19]. Briefly, PCR was performed in a 25 mL volume reaction mixture with 7 mL GoTaq master mix (Promega, Leiden, The Netherlands) containing dNTPs, $MgCl_2$, reaction buffer, 1 mL of each primer (10 pmol) and

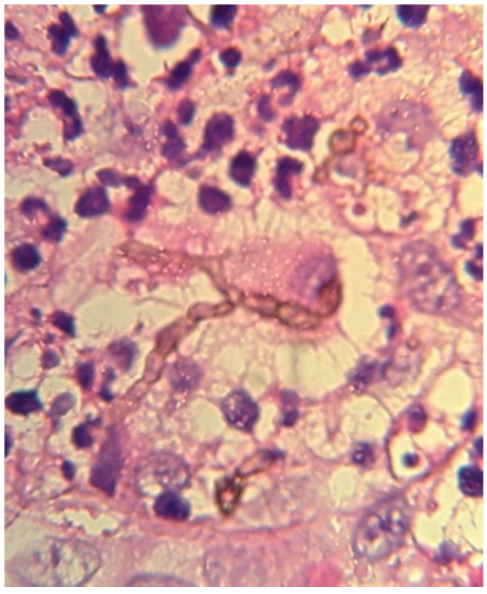

Figure 2 Dematiaceous catenulate fungal cells and septate mycelium in histological examination of biopsy fragments in H&E stains from the lesions surrounded by polymorphonuclear leukocytes.
Cellules fongiques dématiées en chainette et mycélium cloisonné à l'examen histologique des fragments de biopsie (H & E coloration) de lésions entourées par des leucocytes polynucléaires.

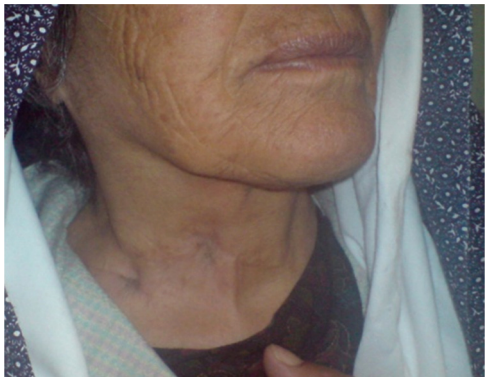

Figure 3 Successful treatment after local excision and using oral ketoconazole.
Guérison après exérèse locale et l'utilisation de kétoconazole par voie orale.

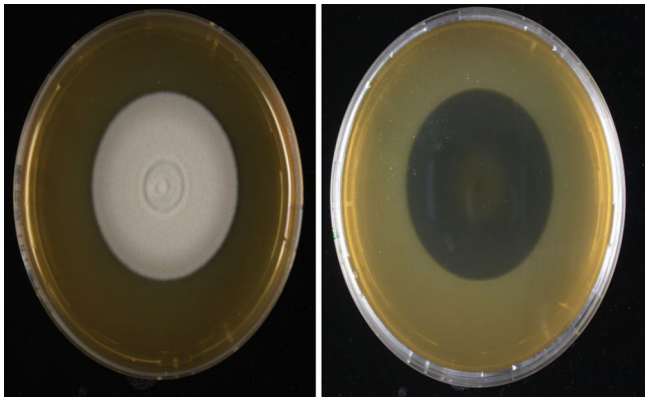

Figure 4 Pictures of *Chaetomium* species (CBS 123940) on MEA.
Photos de Chaetomium sp. (CBS 123940) sur MEA.

1 mL DNA. Amplification was performed in an ABI PRISM 2720 (Applied Biosystems, Foster City, U.S.A.) thermocycler as follows: 95 °C for 4 min, followed by 35 cycles consisting of 95 °C for 45 s, 52 °C for 30 s and 72 °C for 2 min, with a delay at 72 °C for 7 min. Amplicons were subjected to direct sequencing, with PCR conditions as follows: 95 °C for 1 min followed by 30 cycles consisting of 95 °C for 10 sec, 50 °C for 5 s and 60 °C for 2 min. Sequencing was done using ABI Prism BigDye sequencing kit (Applied Biosystems) and sequences were analyzed on an ABI Prism 3730XL sequencer. Sequence data obtained were adjusted using Lasergene SeqMan software (DNAStar, Madison, WI, USA).

The lengths of ITS and SSU regions of the patient's isolate were 561 bp and 1590 bp respectively. The sequences were deposited in GenBank with accession number KC283189 for ITS and KC790427 for SSU. The ITS and SSU sequences were compared and aligned with those of sequences maintained at Genbank and of all available *Chaetomium* type strains made available by CBS. The nearest neighbors based on SSU sequence were *Chaetomium globosum*, *C. elatum* and *Madurella mycetomatis* at 99% similarity and 100% coverage. These data suggested that the strain belonged to the genus Chaetomium; In contrast with ITS, no 100% match was found with any of the sequences deposited in GenBank, The nearest neighbor was *Chaetomium globosum* at 86% similarity. In the CBS database it clustered in *Chaetomium* but no match was found either. The fungus was concluded to be an as yet undescribed, non-sporulating member of the genus *Chaetomium*.

Discussion

Chaeomium species are widespread worldwide in soil and plant debris, and in indoor environments. Phylogenetically, the genus *Chaetomium* is nested within the order *Sordariales*, with the genera *Madurella*, *Thielavia*, and *Papulaspora* as its nearest neighbour [5,6,9,25]. More than 100 described species [27] have been described, but only very few species ribosomal sequences are available in the public domain. Thus far, only a limited number of clinical cases have been reported under the name *Chaetomium*. Most of these concern traumatic causes such as cutaneous infections, onychomycosis, keratitis and peritonitis, but also brain abscesses have been reported [2,3,11—13]. With systemic infections in immunocompromised or alcoholic patients, mortality is high [16].

In our case, she had a history of thyroidectomy 2 months before and the lesion was in the thyroidectomy scar and probably the patient got the infection during surgery. Fungal infections have become a major source of morbidity and mortality in the modern surgical intensive care unit [10]. Pauzner et al. [20] reported a case of phaeohyphomycosis due to *Bipolaris spicifera* in an immunocompetent patient following cardiac surgery.

In the present study, we document a case of phaeohyphomycosis due to a *Chaetomium* species, identified as such with sequences of SSU and ITS genes.

The ITS sequence of the present case (CBS123910) did not fully match with any sequence available of *Chaetomium*, *Thielavia*, *Madurella* and *Papulaspora* available in GenBank, and in the CBS database and hence might represent a hitherto undescribed species. However, without diagnostic morphological features of the teleomorph the taxon cannot be introduced as a novel member of the genus *Chaetomium*. *Chaetomium* species normally reproduce by the formation of elaborate ascomata, but often no anamorphs are produced. Hence, in the absence of fruit bodies, cultures may remain sterile. If clinical strains are degenerate and have lost their ability to sporulate, they cannot be identified by morphology, and hence in the past many cases due to *Chaetomium* species might have passed unnoticed. Mootha et al. [26] reported a keratitis due to a non-sporulating *Chaetomium*-like species, while de Hoog et al. [5,9] and Van de Sande et al. [25] demonstrated that the well-known opportunist genus *Madurella*, comprising several agents of human mycetoma, is phylogenetically close to *Chaetomium*. These data suggest the genus *Chaetomium* may be clinically more important than it was before.

Our patient was initially treated with local excision of the cyst, followed by oral administration of fluconazole (150 mg twice a day), but she complained of a dry mouth and body oedema and did not comply with treatment. After 3 months, when the identity of the fungus was established by sequencing, ketoconazole (200 mg twice a day) was prescribed, and the lesion regressed and was considered to be successfully treated. The resistance to fluconazole and sensitivity to ketoconazole corresponds with profiles of *Chaetomium* species reported previously [12,13,24].

Aranegui et al. [1] report a case of subcutaneous phaeohyphomycosis by *Exophiala jeanselmei* that was treated successfully with wide surgical excision and posaconazole.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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