First report of rhinosinusitis caused by Neoscytalidium dimidiatum in Iran

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Received 10 July 2013
Accepted 14 May 2014

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The study describes isolation of Neoscytalidium dimidiatum from a case of eosinophilic fungal rhinosinusitis (EFRS). The isolate was identified by routine mycological methods and confirmed by DNA sequencing of the internal transcribed spacer (ITS) region of rDNA. To our knowledge, this is the first report of its kind from Iran.

Introduction

Sinusitis, or more accurately rhinosinusitis (RS), is a common disorder that affects approximately 20 % of the adult population (International Rhinosinusitis Advisory Board, 1997). The aetiology of RS is not completely understood. However, a variety of factors such as bacteria, viruses, fungi, allergens and environmental irritants have all been incriminated in the development of RS, and fungi are considered to be important agents in the disease process. Fungal RS (FRS) has been known as a medical entity for the last several hundred years, but has only gained in importance in recent years. Aspergillus species, zygomycetes and a number of dematiaceous fungi are commonly associated with FRS (Challa et al., 2010). FRS encompasses a variety of disease processes that differ in their presentation, histological appearance, mucosal involvement and fungal agents involved. Precise categorization is important for planning management and assessing the patient’s prognosis.

Broadly, there are two varieties of FRS: non-invasive and invasive. Differentiating these two varieties is important because the treatment and prognosis differ for each (Chakrabarti et al., 2009). Reports of human infection by Neoscytalidium dimidiatum are rare. This may be a result of difficulties with identifying the organism, or differences in its geographical distribution. To our knowledge, this is the first report from Iran in which N. dimidiatum was isolated from a patient with EFRS. The pathogen was identified by routine mycological methods and confirmed by molecular methods.

Case report

A 20-year-old man from Turkmenistan presented to Razavi Hospital, Mashhad, Iran, complaining of fever, frequent pain in the forehead and orbit, nasal discharge, stuffy nose, exertional dyspnoea and cough. His past medical history consisted of recurrent pains in the cheeks, under the eyes. In addition, he had a brief history of asthma and had received prednisolone and a respiratory spray (topical steroids).

The patient’s physical examination was unremarkable and systemic examination of the respiratory, cardiovascular and central nervous systems and an abdominal examination were all normal. A nasal examination did not show any significant deformities or abnormalities. Sonographic findings of liver and spleen were normal. Computed tomography scan results showed mucosal hypertrophy with choanal atresia, increased density in the soft tissue of the left nasal cavity with airway obstruction and bilateral obstruction of the maxillary sinus with mucosal reaction. There were no other abnormalities except a deviated septum of the nose.
Laboratory investigations included a complete blood count, tests for hepatitis B virus surface antigen (HBsAg) and human immunodeficiency virus, and C-reactive protein and liver function tests. All parameters were within normal limits except HBsAg, which was positive, and glutamic oxaloacetic transaminase and glutamate pyruvate transaminase were raised.

Cystoscopic maxillary sinus debridement was performed, and secretions and tissue specimens were submitted to the microbiology laboratory for further work-up.

**Mycological studies**

The clinical specimens were processed for microscopy, culture and histopathology. Direct microscopic examination of tissue and secretions in 20% potassium hydroxide showed septate, branched, subhyaline to dark-coloured hyphae (Fig. 1).

The clinical specimens were inoculated on Sabouraud dextrose agar (SDA) with chloramphenicol, brain heart infusion agar and blood agar. The plates were then incubated at 27 °C and 35 °C. After 48 h of incubation, a rapidly expanding hyaline fungal colony appeared. On further incubation, the rate of growth became rapid and the colour of the colony changed to bluish-green to dark olivaceous. Initially, the reverse of the colony was cream to deep ochraceous-yellow, but gradually darkened to ashen black. Slide cultures on SDA showed chains of one- to two-celled, darkly pigmented arthroconidia, 3.5–5 × 6.5–12 μm, produced by the holothallic fragmentation of undifferentiated hyphae (Fig. 2). The clinical specimen and isolate were sent to the Department of Medical Mycology, Mashhad University of Medical Sciences, Mashhad, where the isolate was identified as *N. dimidiatum*.

In an effort to provide a definitive identification, the slant cultures were sent to the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, Utrecht, The Netherlands, for DNA sequencing.

**Histopathological examination**

The slides were stained with haematoxylin and eosin (H&E) and periodic acid–Schiff (PAS). Examination of H&E- and PAS-stained sections showed eosinophilic distributed diffusely and in clusters, along with occasional Charcot–Leyden crystals. However, fungal elements were not seen in the multiple sections studied. Based on the clinical history, signs and symptoms and laboratory findings, the patient was diagnosed as having EFRS (Chakrabarti et al., 2009).

**Molecular studies**

The fungus was grown on a malt extract agar plate, and was then transferred to a 2 ml Eppendorf tube containing 400 μl TEX-buffer (Tris 1.2% w/v, Na-EDTA 0.38% w/v, pH 9.0) with glass beads (Sigma) and homogenized by MO-BIO vortexing for 5–10 min. DNA was extracted and molecular identification was performed as described previously (Najafzadeh et al., 2011a, b).

The entire sequence of the rDNA ITS domain was compared with the GenBank database. The nearest neighbour to our isolate within the ITS BLAST in GenBank was *N. dimidiatum*, with 99% similarity. The ITS sequence was deposited in GenBank with accession number KF571862. The isolate was deposited in the reference collection of the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre with accession number CBS 135275.

**Discussion**

The coelomycete *N. dimidiatum* is a filamentous fungus. Infections with *N. dimidiatum* have been observed predominantly in patients from tropical and subtropical
regions, such as South America, south-east Asia, India, the Caribbean and West Africa (Gupta & Elewski, 1996). The taxonomy of *N. dimidiatum* and *Scytalidium hyalinum* is very confusing, with descriptions having evolved over the years, leading to several nomenclature changes since the original definition of the genus and species (Machouart et al., 2013). In the early 1930s, Nattrass (1933) described the conidial state of *Scytalidium dimidiatum* for the first time, under the name *Hendersonula toruloidea*. More than 50 years later, Sutton & Dyko (1989) proposed a name change from *H. toruloidea* to *Nattrassia mangiferae*, with the mycelial synanamorph named *S. dimidiatum*. More recently, Crous et al. (2006) proposed a taxonomic revision of the *Botryosphaeriaceae* family based on molecular analysis and concluded that genus *Scytalidium* is a polyphyletic group, and that *Neoscytalidium* can accommodate *S. dimidiatum* and *N. mangiferae* as *N. dimidiatum* (Crous et al., 2006).

*N. dimidiatum* is known to cause a variety of clinical conditions, including superficial (Jabbarvand et al., 2004) and subcutaneous (Rockett et al., 1996; Sigler, et al., 1997) infections, RS (Dunn et al., 2003), endophthalmitis (Gumum et al., 2002) and disseminated infections (Geramishoar et al., 2004; Tan et al., 2008) in humans. However, reported infections by *N. dimidiatum* in immunocompetent patients show that other risk factors may also be important in the development of infection. Whether such local and systemic factors result in any predisposition for infection has not yet been clearly established.

**Acknowledgements**

We thank the staff of the Medical Mycology Laboratory in Ghaem Hospital at Mashhad University of Medical Sciences.

**References**


