First case of fungal rhinosinusitis due to *Aspergillus nomius* in a child with aplastic anaemia

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Summary

Recently, infections caused by *Aspergillus* species have increased dramatically. Invasive aspergillosis (IA) is one of the most important causes of morbidity and mortality in immunocompromised patients, such as those with haematological malignancies who undergo chemotherapy, bone marrow and solid organ transplant recipients, and patients with other immunodeficiencies. The most common species causing invasive infections include *Aspergillus fumigatus*, followed by *Aspergillus flavus*. *Aspergillus nomius* is an anamorphic species belonging to *Aspergillus* section *Flavi*, which currently include 22 species that can be grouped into seven clades (*Aspergillus flavus, Aspergillus tamarii, Aspergillus nomius, Petromyces alliaceus, Aspergillus togoensis, Aspergillus leporis* and *Aspergillus avenaceus*) based on morphological characters, sequence data, and exrolite profiles. These species may also produce toxic and carcinogenic aflatoxins. However, *Aspergillus nomius* is an emerging pathogen as a cause of IA; we found only two reported cases of invasive infection caused by this fungus in literature up till now. We reported a case of fungal rhinosinusitis caused by *Aspergillus nomius* in a child with aplastic anaemia and to our knowledge, it is the first case as an agent of rhinosinusitis. The isolate was identified by sequencing based methods.

Introduction

In recent years, the importance of *Aspergillus* species has increased dramatically. Invasive aspergillosis (IA) is one of the most important causes of morbidity and mortality in immunocompromised patients, such as those with haematological malignancies who undergo chemotherapy, bone marrow and solid organ transplant recipients, and patients with other immunodeficiencies (1-4). The most common species causing invasive infections include *Aspergillus fumigatus*, followed by *Aspergillus flavus*. *Aspergillus nomius* (*A. nomius*) is a heterothallic and also sexually reproducing species belonging to *Aspergillus* section *Flavi*, which currently include 27 species that can be grouped into seven clades (*A. flavus, A. tamarii, A. nomius, Petromyces alliaceus, A. togoensis, A. leporis, and A. avenaceus*) based on morphological characters, sequence data, and extrolite profiles (5). Several of these species are known to produce highly toxic and carcinogenic aflatoxins (6). *A. nomius* is an emerging pathogen as a cause of IA; we found only two reported cases of invasive infection due to *A. nomius* in literature up till now. We reported a case of fungal rhinosinusitis caused by *A. nomius* in a child with aplastic anaemia; to our knowledge, it is the first case as an etiological agent of rhinosinusitis.

Case Report

An 11-year-old girl was hospitalized for 22 days with aplastic anaemia and febrile neutropenia. She was receiving corticosteroid and vancomycin, amikacin, imipenem empirically for persistent fever. Microbiological examination of blood, urine, stool and throat swab specimens was performed for three times during her hospitalization. A consolidation area of lung parenchyma was detected on routine chest X-ray and high-resolution computed tomography (HRCT) revealed a single nodule with ground glass sign and atelectasis suggestive of a fungal infection. Intravenous voriconazole was started in addition to empirical antibacterial treatment.

On day 28, pain, hyperaemia and oedema appeared in the left wing of her nose, progressing to a necrotic lesion. On day 29, nasal and sinus biopsies were obtained and sent to Clinical Microbiology...
Laboratory for microbiological evaluations. Patient persisted as febrile on day 34, when paranasal computed tomography examination consistent with a fungal infection was performed. Considering being mucormycosis, voriconazole treatment switched to liposomal amphotericin B. On the 46th day of hospitalization, posaconazole was added to antifungal treatment because neither radiological nor clinical regression was subsequently detected with amphotericin B therapy. After another 20 days, cranial Magnetic Resonance Imaging (MRI) and HRCT examination showed the fungal infection progression with left maxillary and infraorbital oedema, bone destruction and mucosal thickening. Imipenem was stopped and the therapy switched to piperacillin-tazobactam, amikacin and metronidazole on the 70th day. Furthermore, caspofungin was added to posaconazole on the 72th day. Diffuse fungal progression was detected in cranial, orbital and paranasal MRI on 79th day, and surgical debridement was recommended for maxillary sinus but this latter could not be performed due to the patient’s general condition. Nasal and maxillary lesions spread to her hard palate and upper mouth mucosa. Stenotrophomonas maltophilia was isolated from blood culture the 92th day. She died for septic emboli in spite of broad spectrum anti-bacterial and antifungal therapy the 97th day of the hospitalization.

Mycological studies

Nasal and maxillary specimens were used for microscopic examination, that revealed hyaline and septate hyphae, and for microbiological investigation.

The samples were plated on sheep blood agar, McConkey agar, chocolate agar and three Sabouraud dextrose agar (SDA) plates. The SDA plates were incubated at three different temperatures (26, 30 and 37 °C) for a period of 7 days. After 48 hours of incubation, filamentous colonies began to grow on all plates. Initially pale yellow-green, the colonies then became velvety to floccose consisting in light orange-brown vegetative mycelium. At maturity, colonies had green, yellow-green granule like loose mycelial mesh in central, yellow-orange colour in periphery and the colony reverse was light yellow-orange on SDA. Microscopically, conidial heads were uniseriate or biseriate, radiate; vesicles were spherical to subspherical. The conidiophores were variable in length, hyaline and echinulate; conidia were spherical to subspherical, echinulate, and variable in size (Figures 1 and 2). Based on macroscopic and microscopic features, the fungal isolate was identified as Aspergillus flavus (7).

All bacterial cultures were negative.

Precise identification of this fungus was made by PCR amplification of D1-D2 region of 28S rRNA gene, and sequencing of the resulting amplicons. The amplification of genomic DNA was carried out by semi-nested PCR. We used broad-range primer pairs ITS1 (5’ TCC GTA GGT GAA CCT GCG G) in the first reaction followed by D1 (5’ GTA TAT CAA TAA GCG GAG GA) in the second reaction; D2R (5’ TTG GTC CGT GGT TCA AGA CG) was used as the reverse primer in both reactions. Also, a segment of the β-tubulin gene was amplified using primers bT-F (5’ CAACTCCTGAC- CGCTTCTCC 3’) and bT-R (5’ GACATGACAGCAGA-GACCGAG3’) and a segment of the calmodulin gene was amplified using primers emd-F (5’ TGTAAGTAGTTATCGTCG 3’) and emd-R (5’ ATCACTCTCATCAACTTCGT 3’). DNA sequence was determined using a BigDye Terminator ver 5.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI 3130 DNA sequencer. Sequence analysis was carried out by BLASTN similarity search at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). A sequence of 534 bp was detected, exhibiting 100% identity with Aspergillus nomius isolates present in the publicly available GenBank sequence database of NCBI. After identification, viable cultures were deposit-
humans (18-20). To our knowledge, our patient is the first fungal rhinosinusitis case caused by *A. nomius* in Turkey. Initially this isolate was identified as *A. flavus* based on morphologic characteristics because of the high similarity between these sister species. The final identification was carried out by molecular methods. It is possible that other *A. nomius* isolates have been misidentified as *A. flavus* in our laboratory until now. The differentiation of *A. nomius* from other members of *Aspergillus* section *Flavi* can be made by only molecular techniques, including calmoduline, beta tubuline and ITS sequence analysis as it was done in this study (13,21). It should be noted that molecular methods are not applicable in most routine clinical microbiology laboratory.

The present case shows that clinicians should be aware of a possible *Aspergillus* species when the manifestations of sinusitis appeared in immunocompromised patients, and invasive fungal rhinosinusitis must be taken into consideration. Although *A. fumigatus* is the most common agent of invasive aspergillosis, rare species such as *A. nomius* may also cause an invasive infection. However, correct identification of uncommon fungal pathogens is difficult for most of clinical microbiology laboratories. Accurate identification of these rare pathogens is important for both epidemiological and management of treatment.

**References**

1. Del Gaudio JM, Clemson LA. An early detection protocol for invasive fungal sinusitis in neutropenic patients successfully reduces extent of diseases at presentation and long term morbidity. Laryngoscope 2009;119:180-3.
2. Sulu AE, Ogretmenoglu O, Sulu N, et al. Acute invasive fungal rhinosinusitis: our experience with 19 patients. Eur Arch Otorhinolaryngol 2009;266:77-82.
3. Takahashi H, Hinohira Y, Hato N, et al. Clinical features and outcomes of four patients with invasive fungal sinusitis. Auris Nasus Larynx 2011;38:289-94.
4. Montone KT, Virginia A Livolsi VA, et al. Fungal rhinosinusitis: A retrospective microbiologic and pathologic review of 400 patients at a single university medical center. Int J Otolaryngol 2012;684835.
5. Horn BW, Moore GG, Carbone I. Sexual reproduction in aflatoxin-producing Aspergillus nomius. Mycologia 2011;103:174-83.
6. Peterson SW, Ito Y, Horn BW, et al. Aspergillus bombycis, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. Mycologia 2001;93:689-703.
7. De Hoog GS, Guarro J, Gene J, et al. Atlas of the Clinical Fungi, a pilot CDROM version of the 3rd edition, 2009.
8. Clinical and Laboratory Standards Institute. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard, 2nd ed.CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
9. Samson RA, Visagie CM, Houbraken J, et al. Phylogeny, identification and nomenclature of the genus Aspergillus. Stud Mycol 2014;78:141-73.
10. Balajee SA, Lindsay MD, Iqbal N, et al. Nonsporulating clinical isolate identified as Petromyces alliaceus (anamorph Aspergillus alliaceus) by morphological and sequence-based methods. J Clin Microbiol 2007;45:2701-3.
11. Ozhak-Baysan B, Alastruey-Izquierdo A, Saba R, et al. Aspergillus alliaceus and Aspergillus flavus co-infection in an acute myeloid leukemia patient. Med Mycol 2010;48:995-9.
12. Balajee SA, Houbraken J, Verweij PE, et al. Aspergillus species identification in the clinical setting. Stud Mycol 2007;59:39-46.
13. Kurtzman CP, Horn BW, Hesseltine CW. Aspergillus nomius, a new aflatoxin-producing species related to Aspergillus flavus and Aspergillus tamarii. Antonie van Leeuwenhoek 1987;53:147-58.
14. Ehrlich KC, Kobeseman K, Montalbano BG, et al. Aflatoxin-producing Aspergillus species from Thailand. Int J Food Microbiol 2007;114:153-9.
15. Moody SF, Tyler BM. Restriction enzyme analysis of mitochondrial DNA of the Aspergillus flavus group: A. flavus, A. parasiticus, and A. nomius. Appl Environ Microbiol 1990;56:2441-52.
16. Razzaghi-Abyaneh M, Shams-Ghahtarokhi M, Allameh A, et al. A survey on distribution of Aspergillus section Flavi in corn field soils in Iran: population patterns based on aflatoxins, cyclopiazonic acid and sclerotia production. Mycopathologia 2006;161:183-92.

17. Olsen M, Johnsson P, Moller T, et al. Aspergillus nomius, an important aflatoxin producer in Brazil nuts? World Mycotoxin J 2008;1:123-6.

18. Manikandan P, Varga J, Kocsobe S, et al. Mycotic keratitis due to Aspergillus nomius. J Clin Microbiol 2009;47:3382-85.

19. Zotti M, Machetti M, Persi A, et al. Onychomycosis: first case due to Aspergillus nomius. Acta Derm Venereol 2011;91:5912.

20. Caira M, Posteraro B, Sanguinetti M, et al. First case of breakthrough pneumonia due to Aspergillus nomius in a patient with acute myeloid leukemia. Med Mycol 2012;50:746-50.

21. Hinrikson HP, Hurst SF, Lott TJ, et al. Assessment of ribosomal large-subunit D1-D2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as targets for molecular identification of medically important Aspergillus species. J Clin Microbiol 2005;43:2092-103.