Phaeohyphomycosis in a COVID-19 Patient Caused by *Neoscytalidium Dimidiatum*

Somayeh Dolatabadi

Hakim Sabzevari University

Sara Nasirharandi

Isfahan University of Medical Sciences

Morteza Pourahmad

Isfahan University of Medical Sciences

Kazem Ahmadikia

Tehran University of Medical Sciences

Mojgan Mokhtari

Isfahan University of Medical Sciences

Mohammad Javad Najafzadeh

Mashhad University of Medical Sciences

Rasoul Mohammadi (✉ Dr.rasoul_mohammadi@yahoo.com)

Isfahan University of Medical Sciences

Case Report

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Abstract

We report a case of 32-years-old male with a cerebral phaeohyphomycosis post COVID-19 infection from Iran. The causative agent was identified by histopathology, routine mycological methods and confirmed by DNA sequencing of the internal transcribed spacer (ITS) region of rDNA and proved to be *Neoscytalidium dimidiatum* which is an opportunistic dematiaceous fungus belonging to Dothideomycetes.

No apparent underlying disease was seen beside his COVID-19 complications and corticosteroid therapy. Symptoms indicative of his fungal infection appeared two weeks after his COVID-19 scenario. The brain MRI showed multi-focal CNS infection as abscess. Delayed identification of the fungus and antifungal treatment with amphotericin B may have affected the outcome, thus the patient died on day 25 post COVID-19 infection.

Considering, rare incidence of infections due to *N. dimidiatum*, reporting this case will add to our awareness and may lead us to a timely management.

Introduction

Neoscytalidium spp. (Dothideomycetes, Botryosphaerales) are generally known as phytopathogens. These endophytic, saprobic, and opportunistic species cause a wide range of symptoms, most commonly on woody plants [1]. They cause canker in a wide variety of trees in several regions of the world and are significant threat to commercial plantations. *Neoscytalidium dimidiatum* was dominantly isolated from *Quercus brantii* in Iran [2], leaf blight on *Sansevieria trifasciata* in Malaysia [3], tomato in Turkey [4], canker disease of pitaya in China [5], and fig trees in Iraq [6]. The *N. orchidacearum* sp. nov. was isolated from orchids in Thailand [7]. Traumatic implantation of contaminated plant material is the most common route of infections in humans [8].

Neoscytalidium spp. are opportunistic, dematiaceous fungi which may also lead to a variety of clinical conditions, mainly in patients with predisposing factors such as diabetes mellitus and corticosteroid therapy [9], solid organ transplant [10], systemic lupus erythematosus [11], and trauma [12] but few cases in apparently healthy people has been reported [13].

Neoscytalidium dimidiatum, being a phytopathogen in nature, is mostly the cause of chronic superficial infections in human skin and nail [14]. In a study done in USA, *N. dimidiatum* was dominant among other ascomycetous fungi, mostly affecting the superficial tissue [15] and was also dominant causative agent among 81 onychomycosis cases in another report [16].

Other forms of infections ranging from cutaneous to disseminated infection [17–18], such as rhinosinusitis [19–20], pneumonia [21], cerebral pheohyphomycosis [13], sinusitis [10], and endophthalmitis [12] due to *N. dimidiatum* have been reported. First case of fingernail onychomycosis due

to *N. novaehollandiae* from Iran [22] and a new species of *N. oculus* sp. nov from a corneal ulcer [23] from Latin America were reported.

Case Report

A 32-year-old male was diagnosed with COVID-19 infection on 26th July 2021 and hospitalized for 10 days at Shahreza's hospital, Iran. Single dose of tocilizumab (8 mg/kg/day), 6 doses of remdesivir (200 mg loading dose for the first day and 100 mg daily for another 4 days), and dexamethasone (16 mg/kg/day for 10 days) were prescribed. After two weeks, he was hospitalized again for another 10 days presenting persistent fever of unknown origin (FUO). He was treated with meropenem (1 g loading dose and 1 g every 8 hours making 3000 mg per day) and vancomycin (30 mg/kg/day) for 10 days. Considering his deteriorating condition, decreased left eye vision, and bilateral maxillary sinus opacity, he was suspected with mucormycosis. The patient didn't have nocturnal sweating or weight loss but suffered from nocturnal fever, productive cough, and shortness of breath.

Then he was sent to our center 'Alzahra hospital' in Isfahan on 29th Aug (day 0). At this point, his vital signs were: blood pressure = 115/75 mmHg, pulse rate = 110 beats per minute, respiratory rate = 20 breaths per minute, fever = 38°C, oxygen saturation (O₂ sats) = 92%, glasgow coma score (GCS) = 15, and pupils were reactive and normal. Meropenem (1g/8 hrs.) and vancomycin (30 mg/kg/day) were prescribed. Paranasal sinuses computed tomography (PNS-CT) scan showed no chronic sinusitis, no fungal infection or pathological bone change. Multi-detector computed tomography (MDCT) of lung showed no sign of pulmonary thromboembolism (PTE) or COVID-19 and MDCT of neck, abdomen, and pelvic was normal. Blood culture was negative.

On day 5, sulfamethoxazole: trimethoprim (TMP-SMX: (15 mg/kg/day TMP), (75 mg/kg/day SMX) IV/po divided in 2–4 doses] was added to his medications. Ophthalmology consultation reported no toxoplasma.

On day 8, first magnetic resonance imaging (MRI) of brain showed acute ischemic change due to post COVID-19 vasculitis versus demyelinating disease. The MRI of orbit, and resonance angiography (MRA) and magnetic resonance venography (MRV) of brain were normal. Fiberoptic laryngoscopy showed no evidence of mucormycosis. Several transthoracic echocardiogram (TTE) showed no endocarditis vegetation. Other immunological tests such as wright, coombs wright, 2ME test, Brucella Ag, PPD test, tumor and viral markers such as HBs Ag, HCV Ab, and HIV Ab were negative. Peripheral blood smear (PBS) showed no sign of leishmaniasis. Meropenem dosage was increased to 2 g/8 hrs.

On day 13, second brain MRI showed multi-focal CNS infection as abscess, ventriculitis, and cerebritis. As a result, both intravenous (5 mg/kg/day) and intrathecal liposomal amphotericin B (0.5 mg/day) were added to his medications. Cervical MRI was normal. Toxic panel was positive for morphine (Fig. 2).

His first lumbar puncture (LP) result was: white blood cells (WBC) = 160 cells/μL, protein = 321 mg/dL, red blood cells (RBC) = 50 cells//μL, neutrophil = 72%, and CSF sample from external ventricular drain (EVD)

was sent to the microbiology laboratory. Due to several focal status seizures, levetiracetam (3 g/8 hrs.) and phenytoin (10–15 mg/kg stat and 100 mg/8 hrs.) were added to his medications. Second LP on day 16 showed WBC = 100 cells/ μ L, protein = 80 mg/dL, glucose = 15 mg/dL, neutrophils = 85%, and CSF sample was sent to microbiology laboratory again.

Neurosurgery consultation recommended no surgery until the day 16 that patient's level of consciousness dropped and he went through intubation and external ventricular drain (EVD) installation surgery due to hydrocephalus. He became conscious for a while but then his level of consciousness dropped gradually and CSF sample was analyzed again.

On day 23, due to the patient's creatinine rise and GFR drop, the medications were adjusted. Patient's level of consciousness dropped gradually and reached 1T1. Patient died by cardiopulmonary arrest on day 25 at 23rd September 2021.

Fungal morphological examination: The CSF samplings, negative for bacterial culture, were sent to the mycology lab and grown on Sabouraud's dextrose agar (SDA, Merk, Germany) with chloramphenicol (50 mg/liter) and incubated at 30°C and 37°C. Hairy colonies, dark gray to blackish brown, with a deep ochraceous colony on reverse, were observed after seven days.

For histopathological examination, slides were stained with haematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) and observed with 40 $\times$ magnification. Septated hyphae could be traced. Slide cultures revealed chains of darkly pigmented arthroconidia, and brown, septate, and branching sub-hyaline to dark-colored hyphae (Fig. 1).

The typical morphological feature of these fungi is the production of asexual spores within fruiting bodies called conidiomata which could be seen in slide culture preparation (Fig. 1).

Fungal molecular identification: Fungal DNA was extracted by glass-beads preparation followed by phenol-chloroform purification as described previously [24]. Internal transcribed spacer 1 and 2 including the intervening 5.8S rDNA region (ITS) was amplified using ITS1 (forward primer: 5'-TCCGTAGGTGAACCTGCGC-3') and ITS4 (reverse primer: 5'-TCCTCCGCTTATTGATATGC-3'). Same primers were used for sequencing.

The PCR amplification was performed as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles (each 30s at 95°C, 30s at 54°C and 1 min at 72°C) prior to a final extension of 5 min at 72°C. PCR reaction was performed according to Bakhshizadeh et al. [19] and was purified with Sephadex G-50 fine (GE Healthcare Bio-Sciences, Uppsala, Sweden). The PCR fragments were sequenced with the ABI Prism® Big Dye™ Terminator v. 3.0 Ready Reaction Cycle sequencing Kit (Applied Biosystems) and analyzed on an ABI PRISM™ 3700 Genetic Analyzer (Applied Biosystems). The generated ITS sequence was compared with other reference sequences available at National Center for Biotechnology Information (NCBI) nucleotide database (GenBank; <https://www.ncbi.nlm.nih.gov/genbank/>). Similarity of 100% was

considered a reliable identification. The strain was identified as *N. dimidiatum*. The ITS sequence was deposited in GenBank database with accession number OL684981.

Discussion

Our case has received tocilizumab, remdesivir, and corticosteroids for his COVID-19 infection. It seems COVID-19 complication and steroid therapy could make patients vulnerable for secondary fungal and bacterial infections [25]. Other co-infections such as aspergillosis and candidiasis have been widely reported in association with COVID-19 [26–27] but few reports are yet available from other fungal infections.

Based on his symptoms, it seems that emergence of fungal infection has happened two weeks after his hospitalization for COVID-19. Brain MRI showed multi-focal CNS infection as abscess indicating cerebral phaeophycomycosis and morphological and molecular methods proved the causative agent to be *N. dimidiatum*, an opportunistic dematiaceous fungus.

Infections caused by this species generally occur in endemic tropical and subtropical areas, such as Africa, South America, Caribbean, India, and Asia, through direct or indirect exposure to contaminated soil and plant materials [13, 16–17, 28]. For instance, all cases reported by Garniet et al. [29] with renal transplantation as underlying condition were from tropical areas. In endemic regions, *Neoscytalidium* spp. was an important cause of onychomycosis, accounting for 9% of cases in Nigeria, 24% in Gabon, and up to 56% in the West Indies [14]. The occurrence of these infections has been linked with patients' travel in areas of endemicity [9, 30]. *Neoscytalidium dimidiatum* predominately cause chronic dermatomycosis and onychomycosis in humans, mainly involving the feet [16, 29] and happens in farm workers, such as onychomycosis cases in tea leaf pluckers from India [8].

Most clinical reports are limited to immunocompromised cases such as renal [17–18, 29] and lung transplantation [10]. Sigler et al. [9] reviewed 11 cases of subcutaneous and invasive infection in which underlying risk factors included diabetes mellitus, chronic obstructive lung disease, corticosteroid therapy, hypertension, trauma, and immunosuppression.

Cerebral phaeohyphomycosis is known to occur commonly in immunocompetent hosts, but such infections generally are caused by neurotropic fungi like *Cladophialophora bantiana* [31] and relatively few cases of deep infection caused by *N. dimidiatum* have been reported. Although it is a common phytopathogen, its poor invasive capacity, inability to grow in routine fungal culture media and the possibility of being overlooked as a laboratory contaminant may explain its low incidence in deep infections as we could see in our case where we faced a delayed recovery of the fungus in the lab.

Our case was affected by phaeohyphomycosis. First case of cerebral phaeohyphomycosis due to *N. dimidiatum* in Iran was reported from a 17-years-old boy with systemic lupus erythematosus (SLE) [11]. Cerebral phaeohyphomycosis is rare but it's the most serious form of this infection [32]. Cerebral phaeohyphomycosis infection due to *N. dimidiatum* was also reported in an immunocompetent host

from India [13]. Another report of an immunocompetent host was from Israel affected by dermatomycosis [33]. A cutaneous phaeohyphomycosis from Kuwait [34] has been reported.

Due to the rarity of invasive infections caused by this species, there is limited information and literature available regarding its treatment. Delay in identification of the causative pathogen is the main obstacle and rates of false-negative cultures can be as high as 30% [21]. This group of fungi is taxonomically problematic and their morphological identification based on characters like pigmentation is inaccurate [1]. The nomenclature of *N. dimidiatum* has been controversial as well due to the production of both arthroconidia and pycnidial synanamorphs and also hyaline as well as phaeoid colonies. As a result, there are many other names by which this organism has been classified such as *Scytalidium dimidiatum*, *Nattrassia mangiferae*, *Fusicoccum dimidiatum*, and *Neoscytalidium hyalinum* (species fungorum: <http://www.speciesfungorum.org/Names/SynSpecies.asp?RecordID=500869>). One should take care of these name changes and thus in this article, we identify our strain as *N. dimidiatum*, according to the latest classifications [1] based on molecular identification of ITS sequencing which proved to be reliable and thus many species have been reclassified in recent studies [1, 6]. In a study from Iran, based on sequencing of the same gene, majority of samples from respiratory tracts were identified as *N. dimidiatum* [35].

Neoscytalidium spp. infections have no standardized treatment and are frequently resistant in vivo despite in vitro susceptibility tests showing low minimal inhibitory concentrations [MICs] [29]. In superficial infections, oral and topical treatments are often ineffective with *Neoscytalidium* spp., possible due to melanin production [29]. Successful eradication of mycosis has been obtained with systemic use of azoles or with amphotericin B [28, 33]. However, in most cases, healing is protracted and combinations of drugs are often necessary. Combination of amphotericin B with terbinafine [21–22], and with itraconazole [9, 20] showed effectiveness while these species were almost resistant towards the new antifungal luliconazole [35–36]. In study done by Gil-González et al. [37], clinical isolates of *N. dimidiatum* showed low sensitivity to itraconazole but high to terbinafine. Voriconazole may provide options for treating patients with invasive disease who are unable to tolerate amphotericin B formulations due to poor renal function [10]. Our case has received AMB on day 13 post COVID-19 hospitalization in Alzahra hospital which was relatively late and could have affected the outcome. Fast and reliable identification methods not merely based on morphological characters, timely management of the cases, proper antifungal treatments, as well as controlled use of corticosteroids in COVID-19 patients may lead to less casualties and improved outcome.

Declarations

Ethical statement:

This research was approved by the Ethics Committee of Isfahan University of Medical Science (no. IR.MUI.MED.REC.1400.669), and written informed consent was obtained from the patient.

Consent to participate:

Not applicable

Consent to publish:

Not applicable

Author's contribution:

SD has contributed with writing the manuscript. SN, MP, MM helped with the clinical data and analysis. KA confirmed the fungal identification and MNJ consulted the case. RM designed and supervised the work and contributed to manuscript editing.

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Conflict of interest:

None

Availability of data and material:

not applicable

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Figures

Figure 1

colony growth on SDA after 7 days at 25°C, A: reverse, B: front, c: arthroconidia, d,e: uni to tricellular dark rectangular arthroconidia, f, g: hyaline hyphae, h: chain of arthroconidia.

Figure 2

Brain MRI with post contrast T1W and DWI imaging, indicative of lesion with central diffusion restricted in right parietal lobe suggestive for brain abscess.