

A Case of Superficial Fungal Infection caused by *Naganishia diffluens*

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Most superficial fungal infections of the skin are caused due to dermatophytosis or yeasts. However, it is rare for *Cryptococcus* species to cause infections. Among the fungal infections by *Cryptococcus* species, *Cryptococcus (C.) neoformans* and *C. gattii* are the most common pathogenic species. However, other rare infections have been reported. Subcutaneous cryptococcosis due to *Naganishia (N.) diffluens* (formerly *Cryptococcus diffluens*) was first reported in Turkey. We present the case of a 53-year-old man who had mild pruritic annular, erythematous, scaly patches with inner small, crusted papules and nodules on both axilla and trunk for 3 months. A potassium hydroxide test on his abdomen scales was positive. Fungal culture, light microscopic findings, and the sequencing of an internal transcribed spacer of rRNA gene confirmed the presence of *N. diffluens*. As a result we diagnosed the patient with a superficial fungal infection caused by *N. diffluens* and treated it successfully with oral itraconazole and topical isoconazole.

Key Words: Mycoses, *Naganishia diffluens*

INTRODUCTION

Superficial fungal infections are some of the most common dermatologic diseases seen worldwide. It is estimated that 13.8~20% of the population has had a dermatophyte infection, and approximately 12~13% has onychomycosis¹. *Trichophyton (T.) rubrum*, *T. tonsurans*, *T. mentagrophytes*, *Microsporum (M.) canis* are the most common causative agents of superficial fungal infection in the US¹.

Other species, such as *Cryptococcus* species, can also cause superficial fungal infection¹.

Cryptococcus species are basidiomycetous yeasts that are thought to be the cause of a variety of diseases²⁻⁴. *Crypto-*

coccus (C.) neoformans and *C. gattii* are the most common pathogenic species²⁻⁴. However, the incidence of infection due to non-neoformans cryptococcal species such as *Naganishia (N.) albida* and *Papillotrema laurentii* (formerly *C. laurentii*) has increased recently^{5,6}. Other very rare infections including *N. diffluens* (formerly *C. diffluens*) have also been reported (Table 1)⁷. We present a case of *N. diffluens*-induced superficial fungal infection.

CASE REPORT

A 53-year-old man presented to our clinic with a pruritic

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Table 1. 2 reported cutaneous infection cases of *Naganishia diffluens*⁷

No	Age/sex/year	Location	Host status	Examination	Treatment	Outcome
1	17/Male/2007	Turkey	Not indicated	Culture, biopsy, DNA sequencing	Itraconazole	Cured
2	53/Male/2021	Korea	Alcoholic liver cirrhosis, Hypertension	Culture, DNA sequencing	Itraconazole	Cured

**Fig. 1.** (A, B) Broad annular erythematous scaly patches and plaques on both axilla, back and abdomen (C) A large well-demarcated plaque with inner reticulated patch and nodules

skin rash on his trunk and axilla.

For three months, he had mildly pruritic annular, erythematous, scaly patches with inner small, crusted papules and nodules on both axilla and trunk (Fig. 1). The lesions enlarged after the application of topical corticosteroids, administered by a local medical center approximately 2 months ago. Additionally, he suffered from hypertension and alcoholic liver cirrhosis. The patient had recurrent ascites and a history of being hospitalized in a bed-ridden state regularly.

Furthermore, because of his low socio-economic status, his surroundings were unclean and humid.

A potassium hydroxide test performed on the scales on the lesions of multiple sites was all positive, and multiple round yeast cells were observed. Specimens were collected at least twice from multiple lesions and cultured at 28°C for two weeks on Sabouraud dextrose agar. All of the agars produced white and creamy colonies with smooth surfaces (Fig. 2A). Lactophenol cotton blue staining of the smears

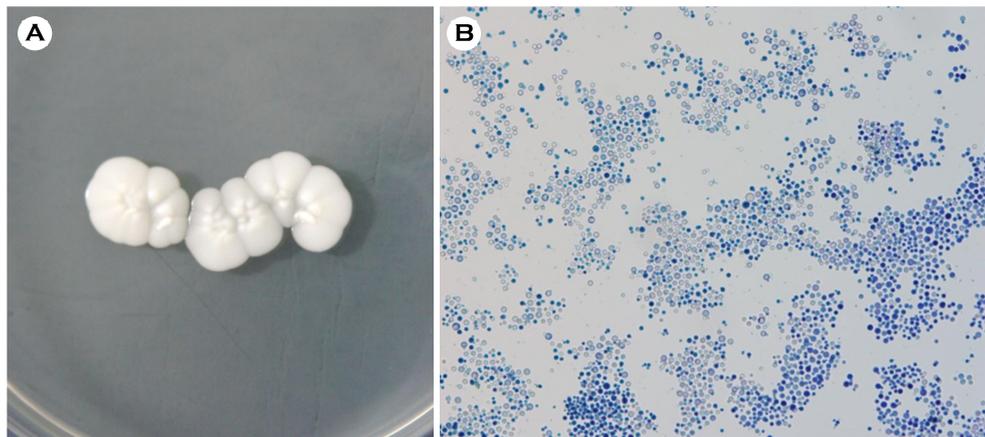


Fig. 2. (A) White and creamy colonies with smooth surfaces developed in Sabouraud dextrose agar at 28°C for 2 weeks (B) Regular in size and round yeast cells are seen. (Lactophenol cotton blue preparation ×400)

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Query      1  ATCATTAAATGATTAACGTCTGTCGAGCTTGCTCACAGACTTATCATATCCATAACACCT  60
CBS 160   24  ATCATTAAATGATTAACGTCTGTCGAGCTTGCTCACAGACTTATCATATCCATAACACCT  83
Query     61  GTGCACTTGTCCGATGGCTTAGTGAAGACCGCAAGGTTGGATCTATCCATCTACTTTACA  120
CBS 160   84  GTGCACTTGTCCGATGGCTTAGTGAAGACCGCAAGGTTGGATCTATCCATCTACTTTACA  143
Query    121  TAACAATTTAGTAACAAATGTAGTCTTATTATAACATAATAAAACTTTCAACAACGGATC  180
CBS 160  144  TAACAATTTAGTAACAAATGTAGTCTTATTATAACATAATAAAACTTTCAACAACGGATC  203
Query    181  TCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA  240
CBS 160  204  TCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA  263
Query    241  ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCCTCCCTGGTATTCGGGGAGCAT  300
CBS 160  264  ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCCTCCCTGGTATTCGGGGAGCAT  323
Query    301  GCCTGTTTGAGTGTGCATGAAAACCTCAACCTTAGATTGGTTAACACCTTTCTTTGGCTT  360
CBS 160  324  GCCTGTTTGAGTGTGCATGAAAACCTCAACCTTAGATTGGTTAACACCTTTCTTTGGCTT  383
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CBS 160  384  GGATTTGGACGTTTGCCGATGATAAGTCGGCTCGTCTTAAAAGTAATAGCTGGATCTGTC  443
Query    421  TCGCGACATGGTTTGACTTGGCGTAATAAGTATTTTCGCTAAGGACATCTTCGGATGGCCG  480
CBS 160  444  TCGCGACATGGTTTGACTTGGCGTAATAAGTATTTTCGCTAAGGACATCTTCGGATGGCCG  503
Query    481  CGTTGCAAGACTAAAGACCGCTTTCTAATCCATTGATCTTCGGATTAATATTCTTGACAT  540
CBS 160  504  CGTTGCAAGACTAAAGACCGCTTTCTAATCCATTGATCTTCGGATTAATATTCTTGACAT  563
Query    541  CTGGCCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA  598
CBS 160  564  CTGGCCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA  621
    
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Fig. 3. Alignment of ITS sequences of rRNA gene: Query is the sample from the patient, CBS 160 (GenBank accession number NR111051) is type strain of *Naganishia diffluens*. The sequences of ITS region of clinical sample were 100% match to that of *Naganishia diffluens* strain: CBS 160(NR111051.1, type strain), CBS 8985 (KY104323.1), CBS:8990 (KY104324.1).

showed yeast cells similar to *Cryptococcus* under the microscope (Fig. 2B). The presence of *N. diffluens* was finally confirmed through sequencing of the rRNA gene' internal transcribed spacer (ITS) (Fig. 3). Furthermore, no evidence of systemic infection was discovered in X-ray, CT, or laboratory findings. Thus, we diagnosed the patient with a superficial fungal infection caused by *N. diffluens*. For two weeks, the patient was treated with an oral antifungal agent (itraconazole, 100 mg) and a topical antifungal agent (isotretinoin), and the lesions significantly improved. There was no evidence of recurrence for 6 months.

DISCUSSION

Most rare *Cryptococcus* species belong to the *C. albidus* clade (*N. adeliensis*, *N. diffluens*, *N. liquefaciens*, *Filobasidium magnus*, and *N. uzbekitanensis*). They were misidentified as *N. albida* before molecular identification (formerly *C. albidus*). The ITS region and the 28S D1/D2 region of rRNA DNA sequence analysis reclassified them as *N. diffluens*⁸.

The genus *Naganishia* has asexually reproducing yeast species. *Naganishia* species are basidiomycetous yeasts and are characterized by the lack of basidiocarps. Typically, hyphae and pseudohyphae are absent, while budding cells and ballistoconidia are present⁹. *N. diffluens* are globose or elliptical, encapsulated yeast cells of varying sizes⁸. The histopathological features of *N. diffluens* are similar to those of cryptococcal infection, and a phenol oxidase test that is negative can aid in the differentiation from *C. neoformans*, the most common cause of cryptococcosis. Biopsy, culture, and molecular analysis are required for a definitive diagnosis. For accurate identification of *N. diffluens* isolates, DNA sequence analysis of the ITS region and the 28S D1/D2 region of rRNA are necessary⁷.

Typically, primary cutaneous infections caused by *Cryptococcus* species occur infrequently as a result of organisms being directly inoculated into the skin by a parasitized object. The disease's clinical manifestation is determined by the host's response, the size of the inoculum, and the organism's innate virulence. Cryptococcosis can present with a variety of skin and soft tissue manifestations including purpura, vesicles, nodules, abscesses, ulcers, acneiform eruptions, granulomas, pustules, draining sinuses, and cellulitis^{10,11}. The majority of skin and soft tissue manifestations occur in the context of a disseminated disease, which is seen in 10~15% of patients with systemic cryptococcosis¹¹. The symptoms of systemic cryptococcosis can range from a mild fever and cough to meningitis, osteomyelitis, pyelonephritis, prostatitis, hepatitis, and peritonitis¹⁰. Thus far, most of the reported cases have

been by *C. neoformans*. Subcutaneous cryptococcosis due to *N. diffluens* (formerly *C. diffluens*) was first reported in Turkey (Table 1)⁷. In that case, the patient has a two-month history of nontender nodules that began on the right wrist and progressed to the right arm. The patient was treated through oral itraconazole for 3 months after diagnosis through culture, biopsy, and DNA sequence analysis. In addition to *N. diffluens*, the first case of *N. albida* superficial infection was reported in Iran¹². In that case, the patient has been suffering from a hyperpigmented patch on his axilla for more than a month. The patient was treated through oral itraconazole for 10 days after diagnosis through culture and DNA sequence analysis. The case described here is the first report of a superficial infection caused by *N. diffluens* in South Korea. The patient's alcoholic liver cirrhosis and long-term bed-ridden condition as a result of cirrhosis complications are thought to be risk factors for the superficial fungal infection.

The first choice of treatment for cryptococcosis is the combination of amphotericin B and flucytosine^{13,14}. However, the treatment of *N. diffluens* superficial infection is not well defined. Oral itraconazole was used successfully in reported cases, including this one⁷, and *in vitro* susceptibilities of *N. diffluens* to several antifungal agents (amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and posaconazole) revealed that voriconazole and posaconazole have good activity against *N. diffluens*¹⁵.

Unfortunately, the biopsy was not performed in this case. However, it is of diagnostic significance in that the test (including KOH, culture, molecular analysis) conducted on the multiple sites of lesion produced the same results. The ability of *N. diffluens* to cause superficial infection has been discovered through this report and literature reviews. As a result, we present a rare case of *N. diffluens* caused superficial fungal infection.

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The authors declare that there is no acknowledgment.

CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

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PATIENT CONSENT STATEMENT

The patient provided written informed consent for the publication and the use of his images.

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