



Necrotizing fasciitis caused by *Apophysomyces variabilis* in a burn patient



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ABSTRACT

The genus *Apophysomyces* belonging to the order *Mucorales* is increasingly being reported as a cause of mucormycosis in immunocompetent patients. We report a case of necrotizing fasciitis caused by *Apophysomyces variabilis* in a 52-year-old immunocompetent male who sustained thermal burn in his right leg following a road-traffic accident. There was rapidly progressive necrosis of skin, soft tissues and underlying muscles which required extensive surgical debridement. Microscopic examination of excised tissues revealed broad aseptate fungal hyphae. Fungal culture on Sabouraud dextrose agar (SDA) showed growth of a mucoraceous mould which was identified as *A. variabilis* based on characteristic microscopic morphology and internal transcribed spacer sequencing of the ribosomal DNA. The isolate was found to sporulate on SDA, a finding that was unique as *Apophysomyces* spp. does not usually sporulate on primary isolation medium used in mycology laboratories. The disease progressed as there was an initial assumption of bacterial infection and the fungus was isolated late in the course of the disease because of which no antifungal drug was added to the regime. The patient left against medical advice and eventually underwent below-knee amputation at another city hospital a week later. Infection due to *A. variabilis* should be considered as a differential diagnosis of rapidly progressive necrosis of skin and soft tissues in immunocompetent individuals as early diagnosis and management will prevent the disease progression and a possible amputation.

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Introduction

Necrotizing fasciitis is a rapidly progressive inflammatory infection of the fascia with secondary necrosis of the skin and subcutaneous tissues which may occur following trauma, post-operative incision, intramuscular injections or burn [1]. It can be caused by both bacterial and fungal pathogens, the latter belonging to the order *Mucorales* are increasingly being reported from such infections. Species from the genera *Rhizopus*, *Lichtheimia*, and *Mucor* account for 80% of the mucormycosis cases worldwide, whereas *Cunninghamella*, *Syncephalastrum* *Apophysomyces*, and *Saksenaia* species are relatively rare causes [2]. However, in India, *Apophysomyces* spp. complex ranks second after *Rhizopus* spp. among the *Mucorales* causing mucormycosis and accounts for nearly 60% of the reported cases worldwide [3]. *Apophysomyces* complex comprises of multiple cryptic species of which *A. variabilis* is responsible for majority of the human infections in India [4]. The agent is abundantly present in Indian soils with low nitrogen

content, which act as a source of the infection [5]. Unlike other members of *Mucorales* that affect immunocompromised hosts and those with uncontrolled diabetes, *A. variabilis* predominantly affects immunocompetent individuals without underlying comorbidities [6]. Here, we describe a case of necrotizing fasciitis caused by *A. variabilis* in an immunocompetent male who sustained first degree thermal burn in his right leg following a road-traffic accident. This case report highlights the importance of a high level of clinical suspicion along with active co-ordination between clinicians and microbiologists for early diagnosis and management of such infections.

Case presentation

A 52-year-old male presented to All India Institute of Medical Sciences (AIIMS), Jodhpur, Rajasthan, India, on 7th August 2018 with complaints of fever, pain and swelling with extensive necrotizing lesion involving the right leg below the knee joint. He sustained first degree thermal burn at the same site from silencer of his motorcycle following a road-traffic accident one month back. He did not seek any medical care for first 10 days after the accident. Thereafter, he developed cellulitis at the site and was

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admitted to a government hospital where he received treatment for 22 days. He underwent debridement of the wound but his condition deteriorated following which he was referred to AIIMS for further management. On physical examination (day 0), the patient was febrile and had edema and severe tenderness with an area of necrosis extending from below the right popliteal region to two inches above the right ankle joint. A clinical diagnosis of necrotizing fasciitis following infected first degree thermal burn was made (Fig. 1A). His complete blood count showed leukocytosis (total leukocyte count = 21,400/ μ l) and neutrophilia (neutrophil count = 73%). He was not a known diabetic and his renal and liver function tests showed no significant abnormalities. The patient was admitted to plastic surgery ward and an emergency debridement was conducted. Broad spectrum antibiotic coverage consisting of piperacillin-tazobactam (4 g/0.5 g) intravenously every 8 h and ceftriaxone 1 gm intravenously every 12 h were initiated. There was no improvement in clinical condition of the patient and debridement of the wound was repeated on day +5. Wound swab was collected and submitted for microbiological examination. Gram staining of the specimen showed no organisms and aerobic bacterial cultures were sterile after 48 h of incubation. Culture of blood and tissue specimen sent on day +8 showed no growth after recommended duration of incubation. Debridement of the wound was done again on day +21 and the tissue specimen was sent for microbiological examination. Gross examination of the tissue showed white cottony filamentous growth over the surface (Fig. 1B). Direct (saline and 20% potassium hydroxide) mount and calcofluor white staining of the tissue showed broad, aseptate fungal hyphae with right angle branching resembling those of a mucoraceous mould (Fig. 2A & B). The specimen was inoculated onto routine bacteriological media and SDA with and without cycloheximide and incubated at 25 °C and 37 °C. Bacterial cultures were sterile after 48 h of incubation. The SDA tubes at both the temperatures showed profusely growing, white cottony mould with scarce aerial mycelia without any pigment on obverse and reverse after 72 h of incubation (Fig. 3). Lactophenol cotton blue (LPCB) mount was prepared from the growth after 3 days, 7 days and 14 days and observed under 400x magnification. Hyaline, branching, broad aseptate fungal hyphae without sporulation were observed in the three days old colony. Evidence of sporulation was noted after 7 days. LPCB mount prepared from the 14 days old colony showed hyaline branching broad aseptate hyphae with distinct foot cells. Sporangioophores were seen to arise singly, unbranched, of variable length upto 400 μ m long and slightly tapering towards the apex. The spogangia were pyriform in shape, multispored, hyaline at first, becoming light greyish brown when mature with hemispherical collumellae and distinctive funnel-shaped apophyses. There was prominent pigmented subapical thickening below the apophyses (Fig. 4A). Sporangiospores were

smooth-walled, variable in shape, trapezoid, ellipsoidal, subtriangular or claviform, and subhyaline to light brown in mass (Fig. 4B). All these features were consistent with *Apophysomyces variabilis*. The isolate was submitted to National Culture Collection for Pathogenic Fungi (NCCPF), Chandigarh, India for molecular identification. The DNA from the isolate was extracted using phenol: chloroform: isoamyl alcohol method and quantified using Nanodrop 2000/2000C (Thermo Fisher Scientific, Waltham, MA USA). The internal transcribed spacer (ITS) gene was amplified by PCR and sequenced using ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAAGG-3) primers. Both phenotypic characterization and molecular sequencing identified the isolate as *Apophysomyces variabilis*.

Specific antifungal therapy could not be instituted as the patient left against medical advice on day +28. There was no improvement in clinical condition of the patient and he eventually underwent below-knee amputation of the right leg on 11th September 2018 at Shree Ram Hospital, Jodhpur, Rajasthan.

Discussion

This is a case of necrotizing fasciitis caused by *Apophysomyces variabilis* in an immunocompetent individual. To our knowledge, this is the first such case report from Western India. The fungal isolate sporulated on SDA after 7 days of incubation, a finding that is not usually observed on primary isolation medium.

The type species of this genus, *Apophysomyces elegans*, was first isolated by Misra et al. (1979) from two soil samples in a mango orchard in northern India [7]. Since then, the taxonomy of the genus has evolved considerably and presently *Apophysomyces* spp. complex has been differentiated into five species, namely *A. elegans*, *A. variabilis*, *A. ossiformis*, *A. trapeziformis*, and *A. mexicanus*, based on morphological characters, biochemical properties and molecular characterization [8,9]. Although human infections due to *Apophysomyces* are uncommon, clinical cases are increasingly being reported from tropical and subtropical regions like India, Australia, United States, Sri Lanka, Thailand, and Central and South America [8]. Skin and soft tissue infections (52.7%) comprise the most common clinical presentation, followed by rhino-orbito-cerebral infection (25.7%), disseminated infection (10.8%) and isolated renal involvement (6.8%) [2]. Cutaneous involvement presents in the form of pain, erythema and induration with central necrosis. More advanced lesions may develop into necrotizing fasciitis with mortality around 80% [10]. *A. variabilis* is a thermotolerant fungus abundantly present in Indian soils with low nitrogen content. In a recent environmental study from India, it was observed that *A. variabilis* comprised 4.5% of all *Mucorales* isolated from the soil [5]. Traumatic inoculation of the pathogen from soil is the most common mode of acquisition of infection.

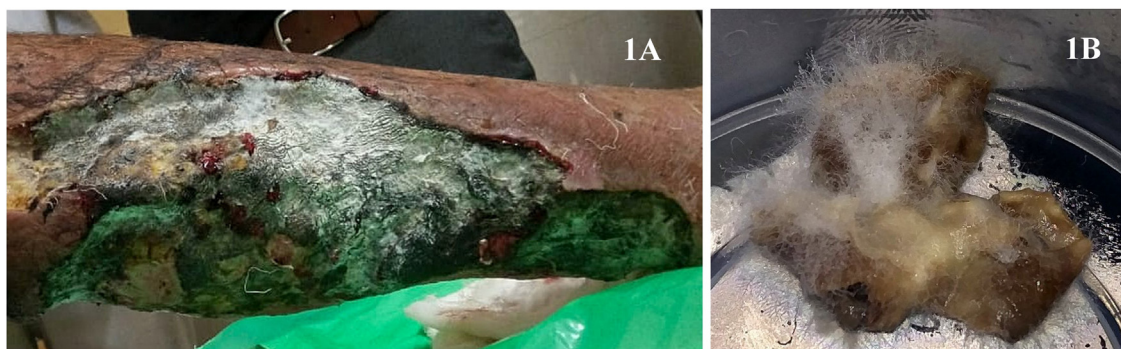


Fig. 1. Necrotizing fasciitis at flexor aspect of right leg. **1A:** Extensive necrosis of skin, subcutaneous tissue, fascia and muscles. **1B:** Gross appearance of excised tissue (day +21) showing white cottony filamentous growth over the surface.

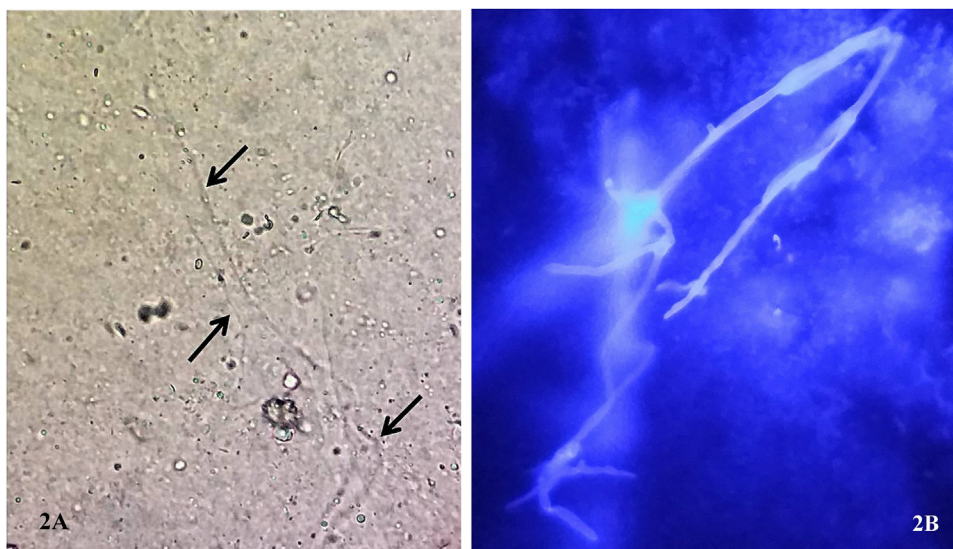


Fig. 2. Direct microscopy of excised tissue specimen. **2A:** Arrows showing coenocytic broad aseptate fungal hyphae in 20% potassium hydroxide mount (x 400). **2B:** Calcofluor white staining showing broad aseptate fungal hyphae suggestive of mucoraceous mould (x 400).

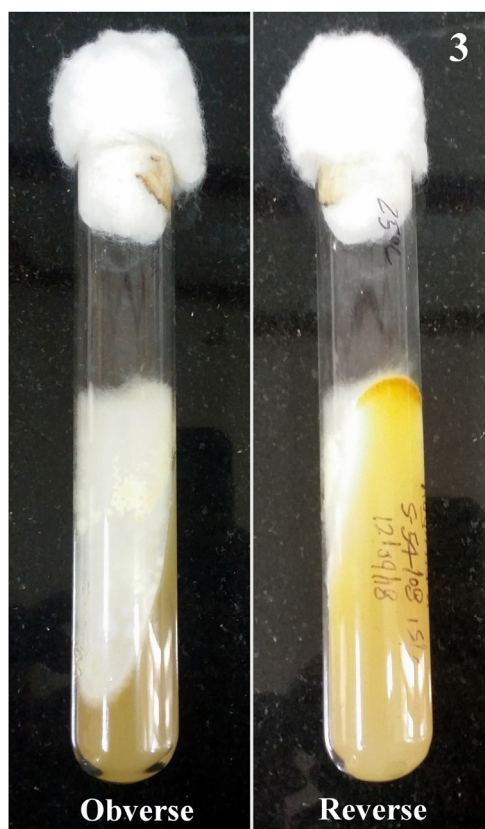


Fig. 3. Colony morphology of *Apophysomyces variabilis* on SDA showing white cottony growth with aerial mycelia filling-up the tube and without any pigment on obverse and reverse.

Other modes of entry include burns, surgery, arterial catheters, injection and biopsy, tattoos, and insect bites [11]. Pamidimukkala et al. (2019) conducted a retrospective study on mucormycosis due to *Apophysomyces* spp. complex over a period of 25 years and reported that *Apophysomyces* spp. was the causative agent in 60.5% of the patients with trauma as the underlying risk factor. The clinical presentation in 95.8% of the cases was primarily

necrotizing fasciitis that developed within 7–10 days following trauma [12]. Rapid progression of *Apophysomyces* infection can be explained by angioinvasion and growth of fungus within the vascular lumen leading to thrombus formation and consequent ischemic necrosis [11]. In our case, first degree thermal burn at the flexor aspect of right leg may be the portal of entry of this infective agent. The wound progressed to cellulitis which then extended along the fascial planes to cause necrotizing fasciitis. Rodriguez et al. [13] also reported similar findings in an immunocompetent male who sustained friction burns following a car accident. The infection may also be acquired in healthcare settings (healthcare-associated mucormycosis). In one study, healthcare-related *Apophysomyces* infection accounted for 29.2% of the mucormycosis cases [12]. Such infections have been attributed to contaminated wound dressings, bandages, medical devices, procedures like intramuscular injection or due to operating room contamination [14,15]. Whenever a case of HCM occurs, a prospective search for additional cases in hospitalized patients should be conducted along with investigations to detect and eliminate the potential source. Environmental and engineering controls along with other infection control strategies should be implemented to prevent further infections [16]. Unlike other members of the order *Mucorales* that tend to affect immunocompromised hosts, human infections by *Apophysomyces* involve a wide range of patients, the majority of which are immunocompetent without any underlying comorbidities [6,17], as was observed in our case.

Diagnosis of mucormycosis can be challenging. In the present case, fungal elements were not detected in the wound swab sent on day 5. However, tissue specimen sent on day 21 showed broad, aseptate fungal hyphae in 20% potassium hydroxide mount and subsequently *Apophysomyces variabilis* was isolated in fungal culture. These findings highlight the importance of appropriate specimen collection as wound swab is not an ideal specimen for microscopy and culture. Multiple tissue specimens from different sites of the affected area need to be collected to obtain a diagnostic yield because it is often difficult to distinguish *Mucorales* from other filamentous fungi [18]. Infections due to *Apophysomyces* spp. complex are most often missed as the fungus does not usually sporulate on primary isolation media like SDA. Induction of sporulation on nutrient-deficient media such as corn-meal agar, water agar and Czapek Dox agar (CZA) is a standard practice to identify the agent phenotypically [19]. However, our isolate

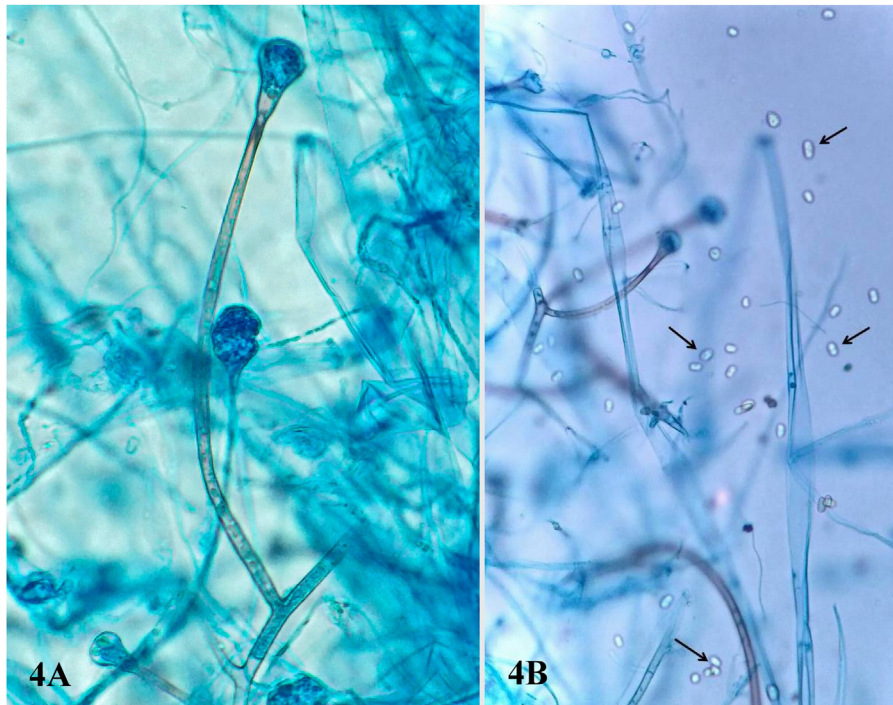


Fig. 4. Microscopic morphology of *Apophysomyces variabilis* in LPCB mount. **4A:** Presence of foot cell, sporangiophore (upto 400 μm long), pigmented subapical thickening, funnel-shaped apophysis and pear-shaped multisporangium (x 400). **4B:** Arrows showing smooth-walled, subhyaline sporangiospores of variable shape (x 400).

demonstrated production of asexual spores on SDA after 7 days of incubation at 25 °C. Though this is a rare finding, sporulation on conventional fungal culture media has also been reported by Alvarez et al. [8] and Chander et al. [11]. As per the first description by Alvarez et al. (2010) [8], colonies of *A. variabilis* on CZA are whitish, with scarce aerial mycelium; hyphae are branched, hyaline, smooth-walled, and 3–5.5 μm in diameter; reverse is concolorous. Sporangiophores are erect, arising singly, hyaline at first, soon becoming light greyish-brown, generally straight, slightly tapered towards the apex, unbranched, smooth-walled, measuring 100–400 μm in length and 2–3.5 μm in width. Sporangia are apophysate, terminal, pyriform, multisporangiate, white at first, becoming light greyish-brown when mature, measuring 15–50 μm in diameter. Apophyses are short, funnel-shaped, measuring 15–20 \times 15–20 μm . Sporangiospores are variable in shape, trapezoid, ellipsoid, subtriangular or claviform, hyaline to light brown in mass, smooth- and thin-walled, measuring 5–14 \times 3–6 μm . Colonies on SDA show similar features as CZA, but are more floccose, white, and with less sporulation [8]. Our isolate demonstrated similar morphological features as described by Alvarez et al. However, it sporulated well on SDA, a finding that is not commonly observed on routine fungal culture media. Early stages of growth of *Apophysomyces variabilis* may be mistaken for *Lichtheimia corymbifera*. But the former differs from the latter in having foot cell, prominent funnel-shaped apophysis, greyish brown subapical thickening in sporangiophore and resistance to cycloheximide. However, morphological characteristics overlap considerably and therefore molecular identification is required to accurately differentiate these species, particularly for poorly sporulating cultures [5,20]. Our isolate was submitted to NCCPF, Chandigarh which is the WHO Collaborating Center for Reference and Research on Fungi of Medical Importance, where ITS sequencing of the ribosomal DNA gene confirmed the isolate as *A. variabilis*.

Extensive surgical debridement and antifungal therapy with amphotericin B remain the mainstay of management of

Apophysomyces infections as reported in several studies [4,8,11,20,21]. Even surgical intervention alone has been reported to be curative in small lesions [4]. However, in our case surgical debridement offered no clinical benefit and the disease progressed, eventually requiring amputation. Posaconazole is recommended as salvage therapy for mucormycosis [22]. Although itraconazole is not the preferred drug for treatment of *Apophysomyces* infections, *in vitro* susceptibility testing of itraconazole against *A. elegans* showed minimum inhibitory concentrations (MIC) ranging from 0.25 to 2 $\mu\text{g}/\text{mL}$ [8,17]. In one study, *A. variabilis* strains isolated from environmental sources were shown to exhibit high MIC for amphotericin B (MIC₅₀ and MIC₉₀ at 1 and 4 $\mu\text{g}/\text{mL}$, respectively), but better susceptibility to posaconazole and itraconazole [5]. These findings suggest that itraconazole may be a treatment option for patients infected with a susceptible strain. The present case failed to respond to treatment as there was an initial assumption of bacterial infection and the fungal pathogen was isolated late in the course of the disease because of which no antifungal drug was added to the regime. As a consequence, the fungus propagated heavily and the clinical condition of the patient deteriorated. The patient left against medical advice before specific antifungal therapy could be instituted and eventually underwent below-knee amputation of the right leg at another city hospital a week later. Such devastating outcomes of *Apophysomyces* infection have also been reported by Pamidimukkala et al. [12] and Al-Zaydani et al. [23], thereby emphasizing the importance of early diagnosis and aggressive treatment in such cases.

Conclusion

Infection due to *Apophysomyces variabilis* should be considered in the differential diagnosis in patients with rapidly progressive necrosis of wounds, especially when there is lack of response to antibacterial agents in an otherwise healthy patient. Although trauma following road-traffic accident is a major risk factor, healthcare-related *Apophysomyces* infection should also be taken

into consideration. The present case highlights the importance of a high degree of clinical suspicion, early diagnosis and aggressive treatment in such cases which may prevent the disease progression and a possible amputation.

Authors' contributions

All authors have made pertinent contributions to the planning, conduct, and reporting of the work described in this article. All authors have read and approved the final manuscript.

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Ethical approval

The Ethical Committee of AIIMS Jodhpur, in which the work was done, has approved the work.

Consent for publication

Written informed consent for publication of the clinical details and/or images were obtained from the patient. A copy of the consent form is available for review by the Editor of this Journal.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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