A Rare Case of *Apophysomyces variabilis* Skin and Soft Tissue Infection

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**Introduction**

A 60 year old male patient presented to the Department of Surgery, Dr Sampurnanand Medical College, Jodhpur, Rajasthan, India in September 2018 with complaints of pain on the back for one month (Day 0). On examination a boil of size (3x3 cm) was observed on the back, at medial side of left scapular region. He was prescribed amoxicillin-clavulanic acid 625mg three times a day and Non-Steroidal Anti-inflammatory Drugs (NSAIDs) for one week. No microbiological evaluation was requested by the treating surgeon on day 0. The patient presented again on day 5 with complaints of no improvement. The patient was hospitalized for three days. Incision and drainage of the wound was done and dressing with antibiotic coverage was given. On day 13, the patient came back again with complaints of no improvement. Wound debridement and dressing was done and the patient was called for follow-up after 7 days. The patient again presented on day 18 with a massive necrotizing wound on the back. Debridement of the dead tissue and toileting of the wound was done with antibiotic coverage. The patient was called for another follow-up after 7 days. On day 28, the patient presented to the emergency department with extensive necrotizing wound on the back extending up to the right arm with creeping wound margin in subcutaneous tissue [Figure1A]. The patient was febrile and well oriented to time, place and person at the time of presentation. The patient had no history of diabetes mellitus, hypertension or any other chronic illness.
Complete blood counts and blood glucose were within normal range. On wound examination, a clinical diagnosis of necrotizing fasciitis was performed. Differential diagnosis for etiological agents of mucormycosis, subcutaneous mycosis and entomophthoromycosis were made by the attending surgeon. Necrotic tissue was removed and a skin biopsy sample was sent for bacterial culture and sensitivity, potassium hydroxide (KOH) examination and fungus culture to the microbiology laboratory. On 10% KOH mount examination, hyaline, broad, aseptate fungal hyphae with wide angle branching were seen [Figure 1B]. Biopsy sample was subjected to bacterial and fungal culture.

Histopathology examination was not performed, and based on KOH examination findings, the patient was hospitalized and treatment of intravenous (i.v.) amphotericin B, 5 mg/kg/day with 5% dextrose was started immediately along with alternate day amphotericin-B soaked dressing of the wound. Intravenous amphotericin B 5 mg/kg/day was given for 4 weeks. Along with antifungal agent, antibiotic coverage of piperacillin-tazobactam 4.5g 8 hourly was also given for one week.

Bacterial culture was sterile but white cottony fungal growth with aerial mycelia was seen on blood agar after 48 hours of incubation. White cottony growth with aerial mycelial growth was observed On fungus culture within 2 days. Lactophenol cotton blue (LPCB) mount was prepared from the growth on day 33 (5 days old colony), day 38 (10 days old colony) and day 43 (15 days old colony) [Figure 2A]. In 5 days old colony, only broad aseptate fungal hyphae were seen; in 10 days old colony, scanty sporangia were observed; and in 15 days old colony, broad aseptate hyphae bearing sporangium with typical funnel shaped apophysis was observed at 400x magnification [Figure 2B]. Sporangiophores were seen to arise from foot cell. There was presence of dark thick brown structure just below sporangiophores. Sporangiospores were oblong in shape and 6-8µm in size. All these features were consistent with *Apophysomyces species complex*. Culture report was conveyed to the treating surgeon on day 44. Antifungal sensitivity testing was not performed for this isolate.

When fresh granulation tissue started to grow at wound margin, skin grafting was performed by the surgeon. Skin auto-graft was done on day 57. The patient was discharged after 3 days of skin-grafting and was kept on follow-up for one month. The skin graft healed well and there was no sign of recurrence of fungal infection or secondary bacterial infection.
The fungal isolate was identified as *Apophysomyces variabilis* by internal transcribed spacer (ITS-1) region sequencing at National Culture Collection for Pathogenic Fungi (NCCPF), Chandigarh, India. The GenBank accession number of the nucleotide sequence is MN317265.

The patient provided written informed consent before undergoing surgical procedures and also to report this case for scientific purposes.

**Comment**

Although *apophysomyces* spp. is an environmental fungus, human infection cases are being increasingly reported worldwide. *Apophysomyces* have been isolated from soil of tropical and subtropical regions. While most of the clinical cases are reported from India, this fungus has also been reported from Australia, the United States, South East Asia and South America.\(^1,2\) *Apophysomyces variabilis* was first described in year 2010 based on the analysis of the sequences of the histone 3 gene, the internal transcribed spacer (ITS) region of the rDNA gene, and domains D1 and D2 of the 28S rRNA gene. Based on these genetic studies, *Apophysomyces species* complex has been differentiated into *Apophysomyces elegans*, *A. variabilis*, *A. ossiformis*, and *A. trapeziformis*. Phenotypically, differences in apophysis and sporangiospores are observed among these species. *A. elegans*, shows bell and funnel shaped apophysies and ovoid, subspherical, broadly ellipsoidal to barrel shaped sporangiospores; *A. variabilis* shows funnel-shaped apophysis and clavate to ellipsoidal sporangiospores; *A. ossiformis* shows funnel-shaped apophysis and biconcave sporangiospores; *A. trapeziformis* shows funnel shaped apophysis and smaller trapezoid shaped sporangiospores in side view.\(^2\)

Although cases of sporulation of *Apophysomyces* spp. in primary cultures on SDA have not been reported in the literature, in one study sporulation was observed after 2 days of incubation on primary culture media like SDA and brain heart infusion agar (BHIA).\(^1\) The isolate in our study showed noticeable sporulation on SDA in primary culture only after 2 weeks of incubation. Subsequent subcultures on SDA also showed abundant sporulation after 2 weeks of incubation.

Necrotizing fasciitis is one of the severe soft tissue infections caused by bacterial as well as fungal pathogens. The necrotizing infections are found mainly in immunocompetent patients. Some fungi of the order Mucorales have invasive property and can produce this rapidly progressive infection, which destroys the soft tissues and spreads along the fascial planes and
invades deeper into the tissues.³ While most common fungal etiology of necrotizing fasciitis is linked to *Rhizopus spp.*, other mucorales which are associated with human infection, include *Mucor, Rhizomucor, Lichtheimia, Apophysomyces, Saksenaea, Cunninghamamella, Cokeromyces*, and *Syncephalastrum* spp.⁴ These fungal infections are often fatal even if treated in a timely manner.³ The introduction of soil contaminated with organism may serve as the primary means of inoculation of this organism at the trauma site. Iatrogenic soft tissue mucormycosis after skin graft, renal transplant, orthopedic surgery and lower segment caesarean section have been reported in the literature.⁵

The current case highlights that *Apophysomyces variabilis* infection may present as boils but possible inoculation of fungal agent iatrogenically cannot be ruled out in this case. Although the case in early stage was refractory to broad spectrum antibiotics, culture and sensitivity at this stage would have helped to conclude the exact etiology and progression of the case of boil to necrotizing fasciitis.

**References**

Figure 1: (A) Extensive necrotizing wound at the back of a 60 year old male patient presented to the Department of Surgery, Dr Sampurnanand Medical College, Jodhpur, Rajasthan, India in September 2018 with complaints of pain on the back for one month; (B) KOH mount from biopsy tissue showing broad aseptate hyphae with wide angle branching.

Figure 2: (A) Fungal colony on Sabouraud Dextrose agar showing aerial hyphae filling the culture plate and tube; (B) LPCB mount of 15 days old colony showing broad aseptate hyphae with funnel shaped sporangiospores arising from foot cell.