

Apophysomyces variabilis a flesh eating fungus – a case report

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Abstract:

Background: Modern therapeutic methods, addictive habits and increased usage of immunosuppressive medications have escalated human susceptibility to opportunistic fungal infections. Genus Apophysomyces is an opportunistic fungus causing infections in immunocompetent individuals. Apophysomyces variabilis is one of the four species often causing fatal cutaneous infections.

Case report: An elderly male presented with a painful necrotizing lesion on the anterior abdominal wall over right iliac fossa. Twenty days prior he was admitted to hospital for Zoster on the same spot and was treated with acyclovir. He had persisting post herpetic neuralgia. The pain became very intense about 5 days back and an analgesic patch was applied by the local practitioner. An erythema was noticed about 4 days ago, which developed to the present necrotizing lesion involving the entire abdominal wall.

Methodology: White cottony growth arising from the lesion grew a rapidly growing fungus which was confirmed by phenotypic and genotypic characters as A. Variabilis. Patient was treated with intravenous Amphotericin B and Voriconazole, but he succumbed. The isolate was deposited in the genebank (KF 147910)

Conclusion: Newer rapidly fatal fungal infections need to be included in the differential diagnosis of all necrotizing lesions. Awareness of such lesion among microbiologist is vital. A. Variabilis is a thermotolerant soil fungi capable of causing acute fatal infections in humans. Contamination of medications, dressings and also any exposed wound may end fatally.

Key words; Apophysomyces, varaiabilis, thermotolerant

I. Introduction

Intravenous drug use, haematological malignancies, long term steroid therapy and other immunosuppressive conditions have resulted in defective phagocytosis, inefficient intracellular killing and increasing the risk of acquiring opportunistic mycosis, some of which are often rapidly fatal. Apophysomycosis, is a rare opportunistic mycotic disease seen in immunocompetent persons(1) caused by one of the four species in the genus Apophysomyces. Apophysomyces species are worldwide in distribution. There are four species- A.elegans, A.variabilis ,A.trapezius and A.ossiformis which are thermotolerant, angio invasive soil inhabiting fungi. Apophysomyces infections are not very common² but invasive surgeries, steroids, old age and excessive usage of antibiotics³ makes the person highly vulnerable to Apophysomycosis .Only three case reports are published in India till date. Slide culture microscopic finding of mixed morphological spore forms, makes this the first fatal case report due to Apophysomyces Variabilis.

II. Case report:

64 years male patient with an anterior abdominal wall necrotizing fasciitis was brought to the surgery clinic by his wife. She informed that he was admitted in the same hospital about 20 days earlier for Herpes Zoster in the right iliac region. He was treated with parenteral & oral Acyclovir for ten days and was discharged. About four to five days after discharge from the hospital, patient developed intolerable post herpetic neuralgia. An analgesic patch was applied on to the site of zoster lesion by the local practitioner. During the next two to three days he noticed progressive redness leading to necrosis. That was when he was brought to hospital. Preliminary clinical diagnosis was “bullous fixed drug reaction”. On examination coarse crepitations were heard over the lungs. Wife also informed about chronic breathing problem and was taking a very tiny tablet daily for several years. His earlier (old) medical records showed that he was a case of Bronchiectasis and was placed on cortisone (5mg).

Preliminary laboratory results showed a total WBC count of 17,500 cells/ cu.mm, Blood urea 68 mg/dl, Serum creatinine 2.7 mg/dl; haemoglobin level dropped from 11.9 g/dl to 9 g/dl, and random blood sugar 300 mg/dl, although he was not a known diabetic. He was treated conservatively with antibiotics and analgesics. Next day the lesion had increased in size and the diagnosis was changed to 'necrotizing fasciitis'. An extensive debridement was immediately done. Twenty four hours later white cottony growth along the margin of the necrosed tissue was noticed. Emergency second debridement was done and the cottony growth was sent for fungus culture. It was cultured on Sabouraud's dextrose agar (SDA) and incubated at 25 degree Celsius. Following the second debridement he was also treated with intravenous Amphotericin B 70mg/day and voriconazole 200 mg/ day. Subsequently he developed respiratory distress and was given ventilator support. But his condition deteriorated and he succumbed on the 5th day of hospitalization.

III. Mycological Laboratory findings:

Potassium hydroxide preparation of the white growth showed sterile broad aseptate hyphae. Culture on Sabouraud's dextrose agar (SDA) yielded yellowish white tube filling powdery fungal growth within 48 h. Microscopic examination of lactophenol tease mount preparation showed broad aseptate sterile hyphae. Slide culture was put up and incubated at both 25°C and 40°C. Unbranched sporangiophores with prominent funnel-shaped apophyses (Fig 2) and pyriform sporangia along with few rectangular spores were observed in the slide culture after ten days. Variation in the size and shape of the sporangiospore and darkened area below the apophysis was also noticed. The culture was thermo-tolerant up to 40°C. Based on these phenotypic characters, we made a provisional identification of *Apophysomyces variabilis* (Fig. 2), which is referred to in this manuscript as JSS AP. Further confirmation was done by DNA extraction, gene sequencing of internal transcriber spiformisacer (ITS) regions and comparing the sequence with twenty eight sequences of *Apophysomyces* spp. deposits from Genbank.

IV. Genesequencing & Phylogenetic analysis

Genomic DNA from JSS AP isolate was prepared from mycelia grown in potato dextrose broth at 30°C for 72 hrs.⁶ PCR assays were performed using the ITS1 and ITS4 primers.⁷ The amplicon was purified with the Genelute PCR purification kit (Sigma, Bangalore, India) and cloned into pTZ57R/T TA cloning vector. The recombinant vector was transformed into *E. coli* DH5 α (Invitrogen, Bangalore, India) and clones were screened by PCR. Plasmids were extracted from three individual PCR positive clones using Genelute Plasmid extraction kit (Sigma, Bangalore, India) and the insert sequenced from Eurofins, Bangalore, India. The sequences were assembled using Gene Runner software⁸ and the resulting ITS1-ITS4 sequence was used to perform BLASTn searches.⁹ The search returned a total of 28 *Apophysomyces* sequences at average significant identities of $\geq 80\%$. All the FASTA sequences were retrieved from the blast hits to analyze the phylogenetic position of JSS AP isolate. Our sequence data was compared with those obtained in thirteen *A. variabilis*, eight *A. elegans*, five *A. trapeziformis* and two *A. ossiformis* to evaluate the effect of genetic admixture. The neighbour joining based phylogenetic analyses were performed using p-distance parameters in MEGA5¹⁰ and 1000 generations were run for bootstrap analysis. The results showed a pronounced divergence among these species. Phylogenetic analysis using ITS1-5.8S rRNA-ITS2 sequencing showed JSS AP isolate to be more closely related to *A. variabilis* than to the other three species, viz., *A. ossiformis*, *A. trapeziformis* and *A. elegans*. It can be observed from Figure 3 that the four major clades of identical or nearly identical sequences (Cluster 1 through 4) appeared as observed by Alvarez et al., 2010.⁵ In general, smaller genetic distances observed between *A. ossiformis* and *A. trapeziformis* pair (0.002-0.01) and *A. elegans* and *A. variabilis* pair (0.01-0.012) as observed in previous studies⁵ (Table 1). The JSS AP isolate was found to be morphologically and phylogenetically closer to *A. variabilis*, and due to high bootstrap value (80) of the novel clade, we are of the opinion that the isolate may be a new subspecies *A. variabilis* subsp. *Variabilis*.(22)

V. Discussion:

Apophysomyces variabilis is an angioinvasive fungus causing rapidly progressing necrotizing vasculitis.¹¹ *Apophysomyces* species are known immunosuppressants, which suppress the pathogen recognition genes and evade human defence resulting in fasciitis.¹² The incidence of *A. variabilis* in humans is unknown and difficult to ascertain due to lack of awareness and no data in the diagnostic laboratories. . Epidemiology of *Apophysomyces* infections is poorly understood and globally these infections are underreported solely due to delayed sporulation on routine media.¹⁶

5.1. Case reviews and Clinical types of Apophysomyces: Apophysomycosis was first detected in 1979,. Apophysomycosis can manifest as spontaneously healing cutaneous lesion or as a rapidly disseminating fatal infection involving renal or central nervous system¹⁸.inhalation may lead to fatal pulmonary and rhinocerebral type. It can be transmitted nosocomally through contaminated Medications and dressing materials.In the present case exact source for apophysomycosis could not be detected. Spores may have been from the environment,

scratching / rubbing of the area might have led to multiplication and necrosis, insect bites, burn wounds, topical lotions and hyperglycaemic status also favour transmission of apophysomyces. 20 . associated favourable conditions are diabetic ketoacidosis, increased iron uptake and natural disasters such as tsunami and tornado.²⁷

Even after molecular and ecological analysis, many questions regarding Apophysomyces such as epidemiology, pathophysiology, frequency of exposure to environmental source, geographical location and the infective dose remain unanswered. The present case study reported here is only the third case of apophysomycosis and the first case of *A. variabilis* infection in South India. This is the first case of fatal anterior abdominal wall necrotising fasciitis due to *A. variabilis* following application of an analgesic patch.³¹

VI. Summary:

In conclusion it can be said that suspicion of a possible fungal etiology in all cases of fasciitis must be looked for and early diagnosis and proper antifungal therapy will save such patients

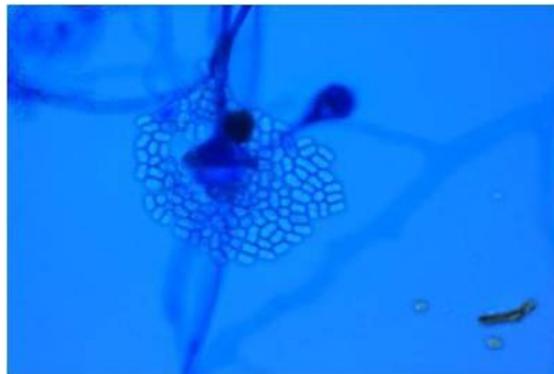


Fig 1 showing varying Sporangiospores

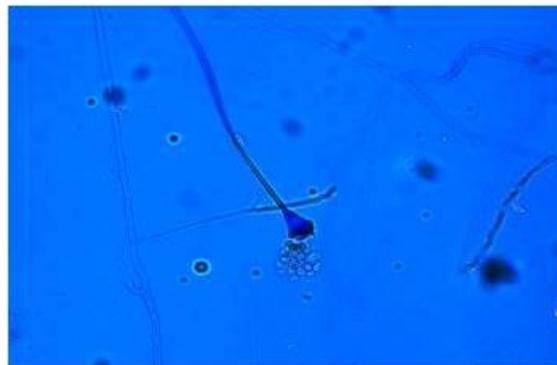


Fig 2 showing funnel shaped Apophyses

TABLE 2

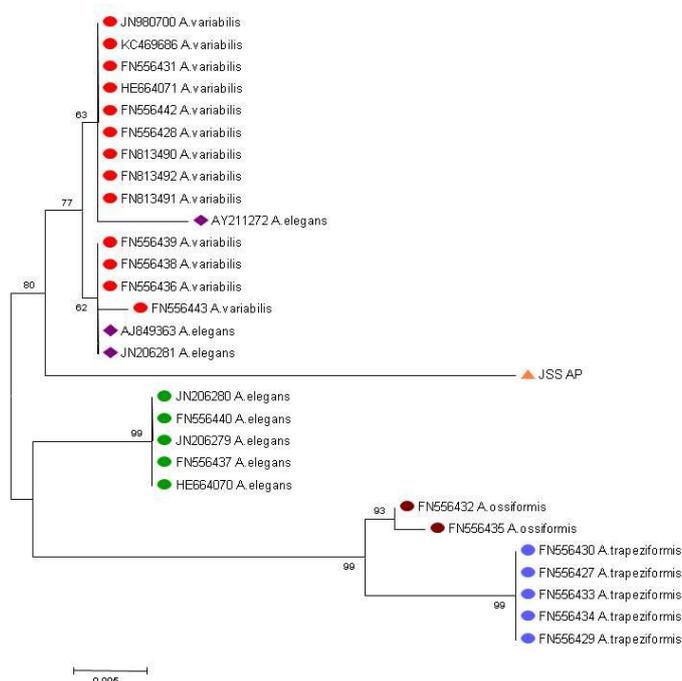


Table 2 : Neighbor-joining dendrogram of the genetic distances (DA) among various *Apophysomyces* species and JSS AP isolate.

VII. Conflict

All the authors have read the manuscript and have no conflict of interest. No funding has been received from any organisation

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