Triadelphia pulvinata: A rare invasive fungal infection in a diabetic patient

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\textbf{A B S T R A C T}

Invasive fungal infections are a leading cause of morbidity and mortality in the immunocompromised patients. We report a case of \textit{Triadelphia pulvinata}, a rare dematiaceous fungi causing invasive fungal infection in a 68 year old diabetic Iraqi female. The diagnosis was made by combining the phenotypic findings and genome sequencing. There are only 4 case reports in literature and this is probably the first from India which was treated by Liposomal Amphotericin B.

1. Introduction

Invasive fungal infections are a leading cause of morbidity and mortality in the immunocompromised patients. While some fungi like \textit{Candida} and \textit{Aspergillus} are well defined to cause invasive infections in these hosts, a few are either unknown or often missed. \textit{Triadelphia pulvinata}, is a soil hyphomycete causing invasive fungal infection and there are only 4 cases documented in the literature. It is a rare dematiaceous hyphomycete first described by Maggi et al. in 1978 and the first case was following its isolation from the grass \textit{Loudetia simplex} in the Ivory Coast \cite{1}. The fungus was isolated in Saudi Arabia from soils contaminated with bat guano by Al Hedaiathy and Leathers in 1987 \cite{2}. In 2001, Al Hedaiathy reported the first and only case of human infection due to \textit{Triadelphia pulvinata} which was isolated from a superficial eczematoid lesion in an expatriate Indian laborer but the source of disease whether from Saudi Arabia or India is not established \cite{3}. Development of rapid and accurate amplification-based internal transcribed spacer (ITS) assays to diagnose invasive fungal infections could potentially impact care and improve outcome for affected patients \cite{4}. We report this first case of \textit{Triadelphia pulvinata} from India in a diabetic Iraqi female. This 68 year old diabetic patient presented with complaints of lower back ache, weight loss since 3 months. The diagnosis was made by combining the phenotypic findings and genome sequencing. The infection was successfully treated with Liposomal Amphotericin B.

2. Case

68 year old Iraqi female with a long standing history of diabetes mellitus of over 15 years visited our facility with chief complaints of lower back ache, weight loss, weakness and low grade fever since the past 3 months. No cough, shortness of breath, nausea, vomiting or any significant complaints. She was a house wife and was not employed. She denied any history of smoking or alcohol consumption. She denied any travel outside Iraq for the past 2 years.

She was evaluated thoroughly for all the suspected conditions and blood samples were sent for CBC, thyroid profile, HIV, HBV and HCV. The CBC showed a decreased RBC count (3.4 mil/L), Normocytic normochromic anemia and her thyroid functions (T3, T4 and TSH) were normal. The viral markers for HIV, HBV and HCV were non-reactive.

The radiological reports of MRI and PET CT findings showed D2-D7 enhancing lesions which were suggestive of malignancy with a possibility of a chronic disease like Brucellosis/ TB/ Malignancy/ Fungal infections.

Bone marrow biopsy was then performed to establish a diagnosis. The bone marrow sample was sent for histopathological examination and aerobic cultures.

The histopathology report revealed no signs of malignancy changes and was suggestive of inflammatory granulation tissue likely chronic infection. However, it was negative for AFB and GMS stain was positive for fungus.

The marrow was inoculated into BacTAlert bottles (bio Merieux) and incubated for 21 days. The bottles gave a positive signal on day 6 of inoculation and smears suggested branched filamentous hyphae. These were sub-cultured onto Sabouraud dextrose agar plates, which grew more than 30 small colonies that developed a waxy texture with a brownish color and a brown color on the underside of the culture plate as well. The colonies subsequently developed white velvety borders after around 2 weeks of incubation. (Picture 1: Sabouraud dextrose agar
plates with brown colonies

Lactophenol Cotton blue mounts were done from various parts of the colony. Brown, single-septate, cylindrical conidia, rounded at both ends and measuring approximately 3–3.5 by 9–10 µm, were formed throughout the culture (Fig. 1).

Also present were long (40- to 60-µm), thin, hyaline, multi-septate conidia as shown below in (Fig. 2). These characteristics are consistent with those previously reported in the literature for Triadelphia pulvinata.

Serum samples were screened with the galactomannan during the hospital stay. The serum galactomannan assay came positive ~ 0.54 (reference cutoff of 0.5).

Since the sequencing of the ribosomal genes is the diagnostic tool for the rapid detection and identification of fungi, regardless of whether morphologically distinct structures are produced [5], the samples were sent for sequencing. The fungal isolate from the bone marrow was sent to the SRL Reference Laboratory for Sequencing, where it was identified as Triadelphia pulvinata.

Sequence data was obtained by growing cells on potato dextrose agar (Difco, Detroit, MI) for 24 h at 30 °C. Template DNA was prepared and sequenced to yield the Internal Transcribed Spacer (ITS) and large subunit (LSU) D1/D2 sequences by amplifying template DNA with the ITS1, and NL4 primers and then sequencing with the same primers in addition to ITS4 and NL1 primers (4–7). The individual sequences were then compared to the ex-type strain of T. pulvinata R-4903. Results were considered significant at a query coverage of 94% and a percent identity of 99%. Thus, the identity of this strain was confirmed to be T. pulvinata based on the ITS sequence and consistency with the previously reported morphological features described above.

The isolate was confirmed as T. pulvinata by National Centre for Cultivation of Pathogenic Fungi (NCCPF), Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, India where it was sent for confirmation by Gene Sequencing.

The patient was then started on Liposomal Amphotericin B 5 mg/kg body weight as per the Infectious Diseases Society of America (IDSA) guidelines 2016.

The patient improved clinically on Liposomal Ampho B and repeat paired blood cultures were also sent from the patient were sterile even after 21 days of culture.

3. Discussion

This is the first case of invasive disease due to Triadelphia pulvinata, described from India which was treated successfully with Liposomal Amphotericin B.

Al Hedaithy in 2001 reported the first case in a 30-year old male who presented with eczematoid, scaly, grey lesions on the skin of both eyelids. Repeated KOH preparations of the skin scrapings showed presence of sclerotic, branched, septate hyphae. When cultured, skin scrapings from the lesion grew the dematiaceous fungus T. pulvinata [3].

Jameela et al., in 2012 reported the first case of T. pulvinata in a 58 year old Saudi woman with acute myeloid leukemia had a bloodstream infection with possibly both lung and brain involvement. Identification was by combined phenotypic features and fungal ribosomal DNA sequence analysis [6]. This is to be considered a hospital-acquired infection, as the woman was an inpatient for a month prior to the relapse of fever and the isolation of the fungus from blood. Immunocompromised patients including diabetics, patients on chemotherapy or ICU patients are more susceptible to these infections

Identification is not easy because of its pleomorphic nature as all species of the genus Triadelphia are pleomorphic, producing at least two forms of conidia from sporigenous cells that agglomerate in a sporodochium-like aggregate [7,8].

In conclusion we report a first case of this rare invasive Triadelphia pulvinata from India in an Iraqi female with history of Diabetes mellitus. Accurate and timely diagnosis are important in initiating appropriate therapy and genotypic detection methods are critical in making such rare diagnosis. Isolation from sterile sites like marrow or blood are essential to establish diagnosis. Rapid improvement on Liposomal
Amphotericin B and isolation from bone marrow culture as well as histopathology helped in establishment of the diagnosis. The isolate should be considered in the differential diagnosis especially in patients travelling from endemic countries like Saudi Arabia. Liposomal Amphotericin B remains the mainstay of management.

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Conflict of interest

The authors have no conflicts of interest to declare financially or otherwise.

Ethical form

The study received no funding and there are no potential conflicts of interest to declare. We obtained the consent to publish the case report from patient.

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