Prevalence and outcomes of patients with COVID-19-associated mucormycosis (CAM): A case series

Sangeeta Chakraborty, Prakash Shastry
Sir Ganga Ram Hospital, New Delhi, Delhi, India
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Background and objectives: Coronavirus disease-19 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus has been associated with increased secondary bacterial and fungal infections. A few centers from India have reported a high number of cases of COVID-associated mucormycosis (CAM). Depending on the anatomical site of infection, mucormycosis is classified as rhino-orbito-ethmoid, pulmonary, gastrointestinal, cutaneous, renal, and disseminated mucormycosis. Several risk factors such as uncontrolled diabetes mellitus, hematologic malignancies, renal disease, organ transplant, and corticosteroid therapy administrated for COVID-19 are implicated in CAM. In this study, we report a case series of CAM, presenting its prevalence, clinical features, risk factors, etiological agents, site of infection, and outcomes in a single center.

Methods: A retrospective data analysis of all proven mucormycosis cases among COVID-19 infected patients from September 1, 2020 to December 31, 2020, was carried out after approval from the institutional ethics committee. All proven cases of mucormycosis (either by culture from sterile site or histopathology), along with compatible clinical and radiological findings, in patients with positive real-time-polymerase chain reaction (RT-PCR) for SARS-CoV2 within 2 months of the diagnosis of mucormycosis were included in the study. All patients received treatment for COVID-19 and mucormycosis as per the institutional protocol. Data was collected in a proforma case record form developed for the study which included demographics, characteristics, risk factors, days to the diagnosis of mucormycosis after COVID-19, site of involvement by mucormycosis along with microscopy, culture and histopathology, treatment details and outcomes at 6 and 12 weeks.

Results: During the study period, a total of 19 patients were diagnosed with CAM. The major risk factors of the patients were type 2 diabetes mellitus (DM) (n = 15, 78.9%) and steroid therapy (n = 14, 94.7%). The other co-morbidities included hypertension (n = 7, 36.8%), chronic kidney disease (CKD) (n = 4, 21.1%) and chronic liver disease (n = 1, 5.2%). Rhino-orbital mucormycosis (ROM) was the most common form (n = 8, 47.3%). The prevalence of CAM (as calculated by the total number of cases of CAM divided by the number of COVID-19 cases treated) was 5.47/100 COVID patients during the study period. Majority (15, 78.9%) of the patients were successfully treated and discharged whereas three patients succumbed to infection and one left against medical advice. The mortality in this cohort (n = 4) was 21.05% as compared with 13.9% among all COVID patients (n = 9) admitted during the same time period in 2020.

Conclusions: Though sample size is small, the findings in our study suggest that the fatality from COVID-associated mucormycosis is high, though the risk factors remain the same. The incidence of mucormycosis was twice that in non-pandemic period. Early diagnosis is crucial as despite aggressive surgical medical therapy, mortality continues to be high.

Bazillusolobus maritispersus — new species on the block!
Ruchita Chabra, S. Rajkiran Raju, Priyadarshini Padaki, A.M. Shubha, Julian Canta, C Indumathi, Jayanthi Savio
St. John’s Medical College, Bengaluru, India
Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objectives: Entomophthorales, including genus Banzolobus, and Cordyceps are well-recognized cause of subcutaneous infections in immunocompetent hosts. genus Bazolobus is ubiquitous. All human infections except one reported so far have been due to B. maritispersus.

Here we present a case of an immunocompetent 5-year-old girl with a soft tissue swelling on the right upper buttom caused by B. maritispersus.
Methods: Tissue biopsy samples from swelling over right buttock were sent for microbiological and histopathological examinations. In microbiology, the samples were subjected to microscopy with Gram stain and KOH-calciofast, aerobic, anaerobic, and fungal culture. Additionally, CINNAAT was done to rule out Mycobacterium tuberculosis. Growth on fungal culture was identified by slide culture and microscopy. The isolate was sent to PGIMER, Chandigarh for characterization using whole genome sequencing. Environmental surveillance included surveillance of soil samples from in and around the patient's house was attempted to identify the source of infection.

Results: On physical examination, an indurated mass was noted on the right lower back with a scar of a previously attempted drainage. The surface over the lesion was erythematous, the skin was scaled with no discharging sinus.

Gram stain of the biopsy sample revealed few polymorphonuclear leukocytes and no microorganisms. KOH-calciofast revealed broad, partially septate, hyaline fungal hyphae. Aerobic and anaerobic cultures did not yield any pathogens. CINNAAT was negative for Mycobacterium tuberculosis. Fungal culture on SDA yielded growth of white, expanding colonies with cerebrofibrin-like, without aerial mycelium after 72 h of incubation, at both 25°C and 37°C. LPCR assay was prepared from the primary tubes and slide culture was performed. Preliminary identification was established as B. ranarum. VITEK MS (MALDI-TOF) did not identify the isolate as this is not available in the database. Molecular characterization and phylogenetic analysis were done and the isolate was identified as B. meristosporum. Soil samples from in and around the house did not yield fungal growth morphologically resembling Basidiobolus species.

Histopathological examination of the sample revealed Splendore-Hoeppli phenomenon with occasional broad, asperate hyphae.

The child was initially treated with oral potassium iodide and later initiated on oral tinidazole. The response was dramatic with 75% resolution of the lesion within 3 weeks of therapy and almost complete resolution after 6 weeks. The child is on regular follow-up since then and is doing well.

Conclusion: Subcutaneous entomophthoramycosis in children are rare tropical infections that can mimic malignancy and hence are often undiagnosed resulting in unnecessary pharmacotherapy or mutilating surgery. Diagnosis is established by isolation and correct identification of fungal species. To the best of our knowledge, only one clinical case of B. meristosporum has been reported so far. Identification of species is vital to understand factors governing pathogenicity, to establish epidemiological data, and probably resolve current controversial opinions on the pathogenic species of genus Basidiobolus. Speciation based on morphology alone is unreliable and molecular methods prove useful for confirmation of species. Therefore, it is imperative that all Basidiobolus isolates are sent to referral centers for speciation to build a strong reliable database.
Figure 1. Phylogenetic tree derived from neighbour joining method from ITS region of the *Basidiobolus* species

Figure 2. Phylogenetic tree derived from neighbour joining method from 28S region of the *Basidiobolus* species

Figure 3. Phylogenetic tree derived from neighbour joining method from 18S region of the *Basidiobolus* species