Fatal amoebic meningoencephalitis caused by *Balamuthia mandrillaris* in a sarcoidosis patient

Bansidhar Tarai, Puneet Agarwal, Sivanantham Krishnamoorthi, Abhishek Mewara, and Sumeeta Khurana

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AUTHOR’S NAMES:

Bansidhar Tarai¹, Puneet Agarwal¹, Sivanantham Krishnamoorthi², Abhishek Mewara²*, Sumeeta Khurana²

RUNNING HEAD:

Fatal meningoencephalitis by *B. mandrillaris*

AFFILIATIONS AND ADDRESSES OF AUTHORS:

¹Max Super Speciality Hospital, 1, Press Enclave Road, Saket, New Delhi 110017, India;
²Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh – 160012, India.

CORRESPONDING AUTHOR:

*Dr Abhishek Mewara

Assistant Professor, Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh – 160012, India.

Phone No: 0172-275166; Fax No: 0172-2744401; Email: abhishekmewara@gmail.com

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TEXT

Introduction

*Balamuthia mandrillaris* is an emerging free living protozoan of public health importance causing infections worldwide. More than 200 human cases of fatal meningoencephalitis have been documented with a mortality exceeding 98 percent (1). Till date, three cases of amoebic encephalitis have been reported from India, all from North India and with a fatal outcome (2,3). Here, we report another case of fatal *B. mandrillaris* associated granulomatous amoebic encephalitis (GAE) from North India.

Patient report

A 57-year-old male presented with a history of altered sensorium of 7-day duration which started as a drowsy state for two days and progressed to secondary generalized seizures, followed by an unresponsive state. He was a known case of bronchial asthma for 16 years, sarcoidosis for seven years, and systemic hypertension with chronic glomerulonephritis for seven years. He was on dexamethasone 4 mg/d for the last seven years for sarcoidosis. On examination he was febrile with a Glasgow coma scale (GCS) of 6 (Eyes – 1, Movement – 4, Voice – 1), pupils were reactive and normal in size bilaterally, there was pedal edema and cushingoid features, but without any skin rashes or neck rigidity. The vital signs and cardiovascular system were normal. On respiratory system examination there were bilateral ronchi. Suspecting infective etiology, the patient was started on intravenous empirical antibiotics (cefepime-sulbactum 3 gm/d and teicoplanin 400 mg/d) and liposomal amphotericin B (150 mg/d) along with supportive therapy for seizures and ventilator support.
On laboratory investigations, the ESR was raised (103 mm/hr), serum angiotensin converting enzyme level decreased (11.6 U/L), and HbA1C level was raised (7.9%). The procalcitonin level was normal (1.06 ng/mL) and serological work up for HIV, HBsAg and HCV was negative. The CSF microscopy and cultures were negative for fungi, bacteria and mycobacteria and the CSF was also negative for antigen for Cryptococcus neoformans. Cultures of blood, urine and endotracheal tube aspirate also did not show any significant growth of bacteria or fungi. The CSF examination revealed a total leucocyte count of 5 cells/mm$^3$ (all lymphocytes) and elevated glucose (105 mg/dL) and proteins (247 mg/dL). Real-time polymerase chain reaction (PCR) for TB, HSV-1 and HSV-2 from CSF, and PCR for CMV from blood, were negative. On radio-imaging, the non-contrast computed tomography (NCCT) of brain demonstrated multiple abnormal hypodense lesions in bilateral cerebral hemispheres (Fig. 1a). The T1w, T2w, FLAIR, GRE sequences and T1w post-contrast images of magnetic resonance imaging (MRI) brain demonstrated multiple heterogenous ring enhancing lesions with internal hemorrhages and moderate peri-lesional oedema (Fig. 1b-f). MR venogram was not suggestive of cerebral venous thrombosis. The electro-encephalogram (EEG) was suggestive of generalized slowing of 4-5 Hz.

A brain biopsy was obtained on the seventh day of admission and subjected to histopathology which revealed diffuse areas of necrosis, hemorrhage, fibrinoid changes in vessels and superimposed infiltrates of neutrophils and histiocytes, i.e. necrotizing inflammation, with large collections of amoebic trophozoites around the blood vessels suggestive of GAE (Fig. 2a-c). The staining for fungal elements was negative. Further, DNA from the brain biopsy was extracted and subjected to PCR assay for Acanthamoeba spp. targeting 18S rRNA gene and B. mandrillaris targeting 16S rRNA gene as described previously (4,5). B. mandrillaris PCR
showed a specific band of 1075 bp (Fig. 2d). Following the diagnosis of BAE, intravenous fluconazole 400 mg/d and trimethoprim/sulfamethoxazole (160/800 mg twice/d) were added. On no clinical response, the patient was administered intravenous voriconazole (200 mg twice/d), but without any favorable response. The condition of the patient worsened and the relatives requested for a discharge from the hospital against medical advice after 17 days of hospital stay. He finally succumbed to the illness three weeks after discharge.

Discussion

GAE is a fatal illness of public health importance presenting with a subacute to chronic course of the disease and B. mandrillaris is an emerging cause of this malady. The disease is more commonly encountered in immunocompromised patients such as ours who was on steroids for more than seven years. Most of the cases reported in literature had a poor prognosis and majority could only be diagnosed after obtaining postmortem samples (6). Although we could achieve confirmatory ante-mortem diagnosis using molecular methods, it was still too late in the course of the disease. The earliest indication of GAE was radiological, however, a definite amebic etiology was established only after a brain biopsy was obtained and trophozoites were visualized, and despite administering a combination of antimicrobials the patient succumbed to the illness. An advanced stage of the disease at the time of hospitalization, underlying immunocompromised state, intrinsic multidrug resistance of B. mandrillaris and slow response to the treatment, and failure to deliver long term treatment could be the reasons of the poor outcome in this patient. It is also difficult to speculate whether addition of other drugs such as miltefosine or flucytosine could have bolstered the efficacy of the regimen.
Till date, only about a dozen of more than 200 reported *Balamuthia* cases have survived (6). Most of the survivors were administered varying combinations and dosages of 5-flucytosine, pentamidine, azoles like fluconazole, sulfadiazine, and macrolide antibiotics such as clarithromycin and azithromycin, while few also received albendazole, trimethoprim-sulphamethoxazole, liposomal amphotericin B, itraconazole, and miltefosine. In all the survivors, the multidrug combination therapy was continued for several months (range 4.2 to 60 months). Although multidrug combinations, dosages and duration has been reported in these survivors, no consensus guideline on the treatment has been established because of the rarity of the infection and lack of enough evidence (6).

In conclusion, considering the rarity and fatal nature of GAE, the earliest radiological suggestion of multiple hemorrhagic/necrotic lesions should be a cue for a prompt parasitology workup for the free living amoebae, especially nucleic acid detection, as a rapid diagnosis and early initiation of therapy might be the key to favorable prognosis in these patients.

**Acknowledgements**

None.

**Conflict of Interest**

None to declare.

**Ethical considerations**

Informed consent was obtained from family members.

**References:**

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Figure legends

**Fig. 1** Non-contrast computed tomography (NCCT) brain images of the patient. 

a. NCCT brain demonstrated multiple abnormal hypodense lesions in bilateral cerebral hemispheres. 

b-f. Respectively, T1w, T2w, FLAIR, GRE sequences and T1w post-contrast images of MRI brain demonstrate multiple heterogeneous ring enhancing lesions with internal hemorrhages and moderate peri-lesional oedema. No calcification is seen.

**Fig. 2** Histopathology of the brain biopsy and gel electrophoresis of *16S rRNA* gene polymerase chain reaction assay for *Balamuthia mandrillaris*. 

a. Haematoxylin and eosin (H&E) stain of brain biopsy under low power showing necrotic parenchyma (arrow head). 

b. H&E stain of brain biopsy under low power showing hemorrhage and hemosiderin pigments (thin arrow). 

c. H&E stain of brain biopsy under low power showing multiple trophozoites around the blood vessels (thick arrows). 

d. 1.5% agarose gel electrophoresis showing band at 1075 bp for *Balamuthia mandrillaris* by polymerase chain reaction amplification; M, 100 bp molecular ladder; NC, negative control (MilliQ water); P, DNA amplification product from brain tissue of the patient; PC, positive template control.