Something Amiss: Acanthamoeba Meningoencephalitis: Report of Two Cases from Kolkata, India

Central nervous system infections due to free-living amoebas (FLA) namely *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea* are deadly, with mortality as high as 100%. They are underdiagnosed, and underreported, often, a diagnosis has been made postmortem.[1-3] The treatment strategy is not well defined.

Primary amoebic meningitis or meningoencephalitis (PAM), Granulomatous *Acanthamoeba* encephalitis, or disseminated disease affects the immunosuppressed, whereas keratitis is reported in immunocompetent persons. Coinfection by various organisms is also well known, adding to the dilemma. We report two cases mimicking pyogenic meningitis, where *Acanthamoeba* was isolated. One of them had a coinfection with *E. coli.* While gram-negative bacterial meningitis with mortality of >50% is well known after neurosurgery or traumatic brain injury, community-acquired *E. coli* meningitis is common in neonates. It affects adults with malignancy, immunosuppressive therapy, HIV infection, chronic alcoholism, or diabetes mellitus.[4,5]

Acanthamoeba acts as a co-host for several bacteria, including *L. pneumophila*, *C. burnetii, P. aeruginosa, V. cholerae*, *H. pylori*, *L. monocytogenes,* and *M. avium*. The endosymbiosis between amoeba and *E. coli* increases their virulence.[3,6] Among all of the genotypes of *Acanthamoeba* spp. reported to date, T4 appears as the most predominant genotype associated with meningitis/meningoencephalitis infections.[7]

Case One

A 54-year-old diabetic hypertensive female was admitted in late March 2023 with fever, headache, neck pain, vomiting, and convulsions of 3 weeks duration. On arrival, she was conscious, had neck rigidity, moving all four limbs equally. CT brain was normal. She was started on intravenous (IV) ceftriaxone, vancomycin, and dexamethasone. There was no papilledema on fundoscopy. Cerebrospinal Fluid (CSF) resembled pyogenic meningitis with 2250 cells (neutrophilic), low glucose (21 mg/dl), and high protein (374 mg/dl). Multiplex Polymerase Chain Reaction (PCR), stains, and cultures were negative [Table 1]. Magnetic resonance imaging (MRI) of the brain with contrast on March 31, 2023, was normal. On April 4, 2023, she became drowsy. Meanwhile, blood cultures returned negative. Antibiotics were escalated to meropenem and ampicillin after CSF worsened to 28800 cells (neutrophilic), glucose 0.7 mg/dl, protein 390 mg/dl, and multiplex PCR turning positive for *E. coli* [Table 1]. Vancomycin and ampicillin were stopped. High resolution Computed Tomography (HRCT) temporal bone revealed left-side otomastoiditis. A perforation in the left tympanic membrane was dry. She improved dramatically and was discharged after three weeks of meropenem. She was readmitted three days later with a severe headache, fever, and neck rigidity and restarted on meropenem. CSF revealed 1550 cells (neutrophilic), glucose 36 mg/dl, protein 186 mg/dl [Table 1], and culture grew *E. coli* sensitive to carbapenems. Meropenem was stopped after six weeks. Except for an occasional heaviness of the head, she remained well. As CSF remained cellular with 80 cells (lymphocytic), protein 86 mg/dl, glucose 77 mg/dl [Table 1], and the brain MRI showed patchy pachymeningitis with ventricular debris [Figure 1], a wet mount was done, which revealed FLA resembling the *Acanthamoeba* species. She was discharged on oral rifampicin (600 mg/day), fluconazole (400 mg/day), and cotrimoxazole (sulphamethoxazole 800 mg + trimethoprim 160 mg twice daily). She is stable after seven weeks of therapy and continuing the same.

Case Two

A 65-year-old diabetic man was admitted in early June 2023 with a two-day history of fever and dysuria, progressing to generalized weakness, photophobia, and a confused state. On admission, he was disoriented, with neck rigidity, photophobia, and moving all limbs well. Brain imaging revealed a left occipital hemorrhage with intraventricular extension [Figure 2]. Angiography of cerebral vessels was normal. He was started on I.V. piperacillin–tazobactam for urosepsis. He deteriorated with fever spikes, worsening sensorium, and papilloedema in 2 days and needed mechanical ventilation. CSF analysis resembled pyogenic meningitis with 4625 cells (neutrophilic), protein 1020 mg/dl, glucose 20 mg/dl [Table 2]. Multiplex PCR, stains, and cultures were negative. Antibiotics were escalated to meropenem and vancomycin. However, the repeat CSF on June 16, 2023, was worse with 6960 cells (neutrophilic 2335 mg/dl protein and glucose 64 mg/dl [Table 2]). The wet mount of CSF was teeming with *Acanthamoeba* [Figure 2]. Parenteral cotrimoxazole (sulfamethoxazole 800 mg + trimethoprim 160 mg twice daily), fluconazole (400 mg/day), and oral rifampicin (600 mg/day) were started. In a couple of days, he was communicating by gestures, weaned off the ventilator. Meropenem continued for 2 weeks. The CSF picture

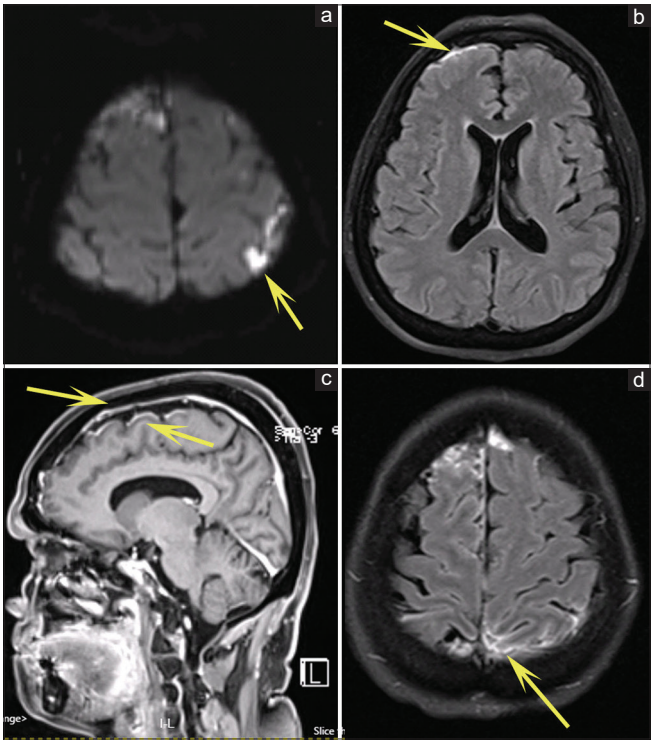

Figure 1: Contrast MRI of brain showing (a) bilateral cortical diffusion restriction suggestive of acute infarcts (arrow), (b and d) T2 Flair showing bilateral frontoparietal leptomeningeal enhancement suggestive of meningitis (arrows), (c) T1 sagittal section showing leptomeningeal enhancement and subdural collection (arrow)

Table 1: Serial CSF Analysis of Case One

Date	31.3.23	4.4.23	17.4.23	27.4.23	5.5.23	26.5.23	6.6.23	13.6.23
Cells (/mm)	2250 (N-90 %)	28800 (N -95 %)	82 (L)	1550 (N-80 %)	53 (L)	24 (L)	72 (L)	80 (L)
Protein(mg/dl)	374	390	127	186	106	90	98	86
Glucose(mg/dl) CBG-capillary blood glucose in parenthesis	21 (CBG -189)	0.7 (CBG-136)	105 (CBG- 140)	36 (CBG- 159)	78 (CBG-205)	75 (CBG-133)	71 (CBG-156)	77 (CBG-124)
MTB gene Xpert	Negative							
Stains (Gram, AFB, Fungus)	Negative							
Cryptococcal antigen	Negative							
cultures	Negative	Negative		*E. coli*				
Multiplex Polymerase Chain Reaction (PCR)		*E. coli*		*E. coli*		Negative		
Opening Pressure (mm Hg)	30				22	33	22	20
ADA (U/L)								0.8
Wet Mount								Plenty of FLA suggestive of *Acanthamoeba* spp.

N: Neutrophils, L: Lymphocytes

improved to 25 cells (lymphocytic) 130 mg/dl protein and 77 mg/dl glucose [Table 2]. However, the sensorium deteriorated in a week due to acute hydrocephalus [Figure 2], requiring external ventricular drainage (EVD). CSF from EVD (cells 1250, protein 633 mg/dl, and glucose 21 mg/dl) showed plenty of amoeba [Table 2]. Because of transaminitis, fluconazole, rifampicin, and cotrimoxazole were stopped. He improved with amphotericin B lipid emulsion (3 mg/Kg/day) daily along with intraventricular amphotericin B deoxycholate (0.1 mg) and methylprednisolone (5 mg) initially through EVD and subsequently in Ommaya on alternate days. However, in spite of initial good response, he succumbed in mid-July 2023.

Both our patients were HIV negative and had reasonably controlled diabetes. Case one had left-sided otitis media but no active ear discharge. This was probably the source of *E. coli.* However, the persistence of infection despite appropriate antibiotics prompted a search for an alternative diagnosis. *Acanthamoeba* coexisted with *E. coli* in this case. Case two again had pyogenic meningitis unresponsive to conventional antibiotics. Wet mount preparation of CSF clinched the diagnosis. FLA is diagnosed by hanging drop preparation of CSF. Tissue biopsy shows both cysts and trophozoites with the characteristic acanthopodia. Serum antibodies and PCR on CSF are not widely available. Brain imaging may show infarcts and hemorrhagic lesions[3,8] due to vasculitis or ring lesions in contrast resembling tuberculosis, fungal abscesses, or toxoplasmosis.[2] Case two presented primarily with fever and intracranial hemorrhage, probably due to necrotizing angiitis.[8]

Therapy should aim for amoebicidal drugs that cross the blood-brain barrier. Recommendations include combinations of amphotericin B, rifampicin with another drug like cotrimoxazole or a combination of amphotericin B, rifampicin, azithromycin, miltefosine, and either fluconazole or miconazole for PAM therapy.[9-11]

A recent review summarized the efficacy of different combination therapy used in FLA infections including miltefosine which has

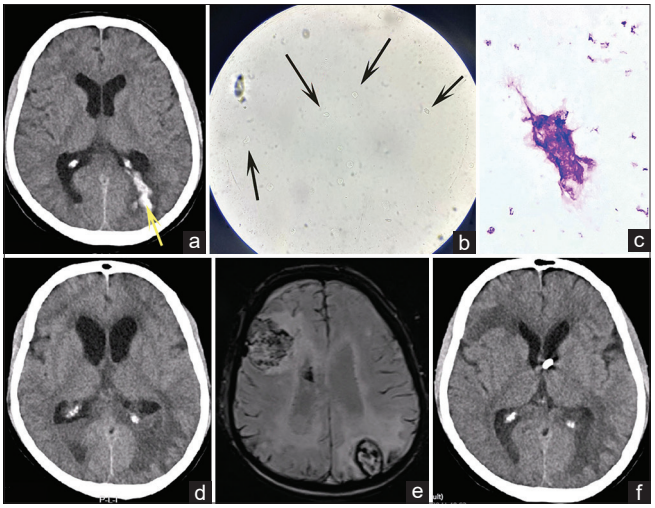

Figure 2: (a) CT scan of the brain showing intraparenchymal hemorrhage with surrounding perilesional edema in the left parietooccipital lobe with intraventricular extension (arrow). (b) Wet mount of CSF showing mobile free-living amoeba with a clear nucleus and acanthopodia (arrows) suggestive of *Acanthamoeba* species (40×). (c) Giemsa stain showing FLA having acanthopodia (100×). (d) CT scan of the brain showing acute hydrocephalus with ventricular debris. (e) Susceptibility weighted image showing hemorrhage. (f) CT scan of the brain after Ommaya placement showing normal ventricular size

shown promise.[9] The optimal duration of therapy is undefined. Steroids may be used for cerebral edema. Both parenteral and intraventricular amphotericin were used in case two.[12] Miltefosine was unavailable in the market. The source of *Acanthamoeba* is elusive in both patients who had diabetes,[13] and denied exposure to ponds or swimming pools. However, *Acanthamoeba*are is found in soil, fresh, and brackish water, cooling towers, heating, or air conditioning units. Both had suffered from COVID-19 illness. Whether this is predisposing to such outbreaks is yet to be explored.

Table 2: Serial CSF Analysis of Case Two

Date	9.6.2023	14.6.2023	26.6.2023	3.7.2023	7.7.2023
Cells(/mm)	4625 (N-90%)	6960 (N–85%)	25 (L-80%)	1250 (N-98%)	650 (N-85%)
Protein (mg/dl)	1020	2335	130	633	352
Glucose (mg/ dl)	20 (CBG-147)	64 (CBG-140)	77 (CBG-135)	21 (CBG-178)	73 (CBG-128)
Lactate (mmol/L)	23.72	1.87		18.23	12.24
ADA (U/L)		16			
MTB GeneXpert	Negative	Negative		Negative	
Stains (Gram, AFB, Fungus)	Negative	Negative	Giemsa- positive for *Acanthamoeba*	Negative	
Cultures	Negative	Negative		Negative	
Multiplex PCR	Negative			Negative	
Wet mount			Occasional motile FLA with *Acanthopodia* (Acanthamoeba spp)	Plenty of FLA resembling *Acanthamoeba*	

N: Neutrophils, L: Lymphocytes

Learning Points

Acanthamoeba causes fulminant meningitis and mimics pyogenic infections. Coinfections with other bacteria are possible.

A wet mount preparation of CSF must be a routine part of the CSF examination.

Due to lack of consensus on therapy, treatment has to be individualized.

The recent rise of these infections demands an epidemiological investigation in this post-Covid era.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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