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REVIEW ARTICLE

Central nervous system infections caused by pathogenic free-living amoebae: An Indian perspective

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ABSTRACT

Pathogenic free-living amoebae (FLA), namely *Acanthamoeba* sp., *Naegleria fowleri* and *Balamuthia mandrillaris* are distributed worldwide. These neurotropic amoebae can cause fatal central nervous system (CNS) infections in humans. This review deals with the demographic characteristics, symptoms, diagnosis, and treatment outcomes of patients with CNS infections caused by FLA documented in India. There have been 42, 25, and 4 case reports of *Acanthamoeba* granulomatous amoebic encephalitis (GAE), *N. fowleri* primary amoebic meningoencephalitis (PAM), and *B. mandrillaris* meningoencephalitis (BAE), respectively. Overall, 17% of *Acanthamoeba* GAE patients and one of the four BAE patients had some form of immunosuppression, and more than half of the *N. fowleri* PAM cases had history of exposure to freshwater. *Acanthamoeba* GAE, PAM, and BAE were most commonly seen in males. Fever, headache, vomiting, seizures, and altered sensorium appear to be common symptoms in these patients. Some patients showed multiple lesions with edema, exudates or hydrocephalus in their brain CT/MRI. The cerebrospinal fluid (CSF) of these patients showed elevated protein and WBC levels. Direct microscopy of CSF was positive for amoebic trophozoites in 69% of *Acanthamoeba* GAE and 96% of PAM patients. One-fourth of the *Acanthamoeba* GAE and all the BAE patients were diagnosed only by histopathology following autopsy/biopsy samples. Twenty-one *Acanthamoeba* GAE survivors were treated with cotrimoxazole, rifampicin, and ketoconazole/amphotericin B, and all eleven PAM survivors were treated with amphotericin B alongside other drugs. A thorough search for these organisms in CNS samples is necessary to develop optimum treatment strategies.

Keywords: CNS infections; pathogenic free-living amoebae; *Acanthamoeba*; *Balamuthia mandrillaris*; *Naegleria fowleri.*

INTRODUCTION

Pathogenic free-living amoebae (FLA), namely *Acanthamoeba* sp., *Naegleria fowleri*, *Balamuthia mandrillaris*, *Sappinia* sp., and *Paravahlkampfia francina,* are distributed worldwide. Studies conducted in north Indian states show the presence of FLA in natural and artificial water bodies (Panda *et al*., 2015; Krishnamoorthi *et al*., 2022). These neurotropic amoebae can cause central nervous system (CNS) infections in humans, such as primary amoebic meningoencephalitis (PAM), chronic granulomatous amoebic encephalitis (GAE), and nonlethal encephalitis (Lorenzo-Morales *et al*., 2011; Król-Turmińska & Olender, 2017). There is no effective treatment for the CNS infections caused by these organisms, resulting in high mortality among patients with these infections (da Rocha-Azevedo *et al*., 2009). The present review deals with the demographic characteristics, symptoms, cerebrospinal fluid (CSF) and brain tissue characteristics, diagnosis, and treatment outcomes of patients with CNS infections caused by three pathogenic FLA reported in India. Infections due to *Sappinia* spp. and *P. francina* have not been reported to date.

Acanthamoeba granulomatous amoebic encephalitis

Acanthamoeba are widespread FLA capable of causing GAE, keratitis, pneumonitis, and cutaneous infection in humans. GAE, also known as *Acanthamoeba* granulomatous encephalitis, is an opportunistic and slowly progressive fatal disease produced by several species of *Acanthamoeba* (namely, *A. castellanii*, *A. culbertsoni*, *A. hatchetti*, *A. healyi*, *A. polyphaga*, *A. rhysodes*, *A. astronyxis*, and *A. divionensis*) (Visvesvara *et al*., 2007). These species can be isolated from the natural environment and from swimming pools, sewages, domestic tap water, contact lenses/cases, and so on (Bose *et al*., 1990; Devi & Mahanta, 2019). In hospital settings, these species have been isolated from dental treatment units, dialysis units, humidifiers, ventilators, eyewash stations, and water sources used for drinking, bathing, and handwashing by high-risk patients in ICUs (Khurana *et al*., 2015a; Visvesvara *et al*., 2007). The life cycle of *Acanthamoeba* includes trophozoite and cyst stages, and the trophozoites have

distinct, spiny surface projections called acanthopodia (Figure 1) (Marciano-Cabral & Cabral, 2003). Metagenomic analysis has shown that *A. castellani* has a mitochondrial genome of 41,591 bases, comprising 70.6% AT (Greninger *et al*., 2015). There are 21 different genotypes of *Acanthamoeba* based on nucleotide sequence variations in the 18S rRNA gene (Kalra *et al*., 2020). Genotypes T4, T10, and T11 have been shown to cause GAE, and genotype T4 has been predominantly associated with CNS infections in India (Behera *et al*., 2016; Megha *et al*., 2018). *Acanthamoeba* isolated from environmental or clinical specimens has been found to harbor pathogenic bacteria, such as *Rickettsia* and *Legionella pneumophila*, indicating that *Acanthamoeba* could serve as reservoirs for bacterial pathogens and may therefore be a source of bacterial infections (Kalra *et al*., 2020). *Acanthamoeba* GAE are usually identified in biopsies of brain lesions during the late stage of the disease process or in autopsy (da Rocha-Azevedo *et al*., 2009).

Acanthamoeba GAE was first reported in 1972 (Jager & Stamm, 1972). *Acanthamoeba* meningitis was first reported in 1984 in India; since then, 42 case reports (Table 1) and one retrospective study of *Acanthamoeba* GAE have been documented (Behera *et al*., 2016). The reported cases of *Acanthamoeba* GAE may be low due to the inherent difficulty in diagnosis and unfamiliarity with the illness. Of the 42 case reports of *Acanthamoeba* GAE, 17 were reported in New Delhi and the others were reported in different regions of India (Tables 1 & 4). Twenty-seven (64%) of these patients were males, and 27 (64%) were children and teenagers. Two of these patients had history of water-related activities and one had a history of sewage cleaning, during which amoebae might have reached the CNS directly through the olfactory neuroepithelium (Lalitha *et al*., 1985; Das *et al*., 2020; Singh *et al*., 2020). One of the patients had geophagia, and another had suffered a head injury (Singhal *et al*., 2001; Das *et al*., 2016). The route of this infection might occur by inhalation of cysts or trophozoites through the respiratory tract or breaks in skin and invasion of the CNS by hematogenous spread from the lungs or skin. *Acanthamoeba* GAE is often seen in immunocompromised individuals, in whom metabolic, physiological, or immunological integrity is compromised (Khan, 2008). This condition can occur in immunocompetent individuals as well. Table 1 shows that 83% of *Acanthamoeba* GAE cases were found to be immunocompetent, while only 17% (7/42) had some form of immunosuppression, such as leukemia, systemic lupus erythematosus (SLE; two patients each), AIDS, malignancy, or malnourishment (one patient each). One patient had an *Acanthamoeba* secondary infection in an underlying brain cyst (Ranjan *et al*., 2009).

Symptoms: The incubation period for *Acanthamoeba* GAE may vary from several weeks to months. This condition has a gradual onset with a subacute to chronic course of focal neurologic symptoms that can mimic other disorders - neurocysticercosis, toxoplasmosis, tuberculoma, brain tumors, viral encephalitis, and bacterial or tubercular meningitis (Visvesvara *et al*., 2007). Patients with *Acanthamoeba* GAE in this review presented with fever (64%), headache (64%), vomiting (54%), seizures (30%), and altered sensorium (21%), and a few have presented with loss of consciousness, stiff neck, photophobia, vertigo, ataxia, and lower limb weakness (Tables 1 & 4).

Diagnosis: Neuroradiologic findings of *Acanthamoeba* GAE are usually nonspecific. In this reveiw, neuroimaging showed multiple lesions with edema or hydrocephalus in 17 patients and hydrocephalus in four patients. CT/MRI results were normal in ten patients. CSF showed elevated protein and white blood cell levels in half of the patients, with mostly lymphocytes in 15 cases and polymorphonuclear leucocytes in seven cases. Definitive diagnosis of *Acanthamoeba* GAE is accomplished by direct microscopic demonstration of trophozoites in the CSF or brain tissue and growth on 2% non-nutrient agar overlaid with *Escherichia coli* (da Rocha-Azevedo *et al*., 2009). *Acanthamoeba* can be cultured axenically in a liquid medium with supplements and antibiotics (Megha *et al*., 2017). In this review, 29 CSF samples showed motile amoeba on a wet mount, five of which were confirmed by Giemsa staining. *Acanthamoeba* cultures were positive in 25 samples, and no growth

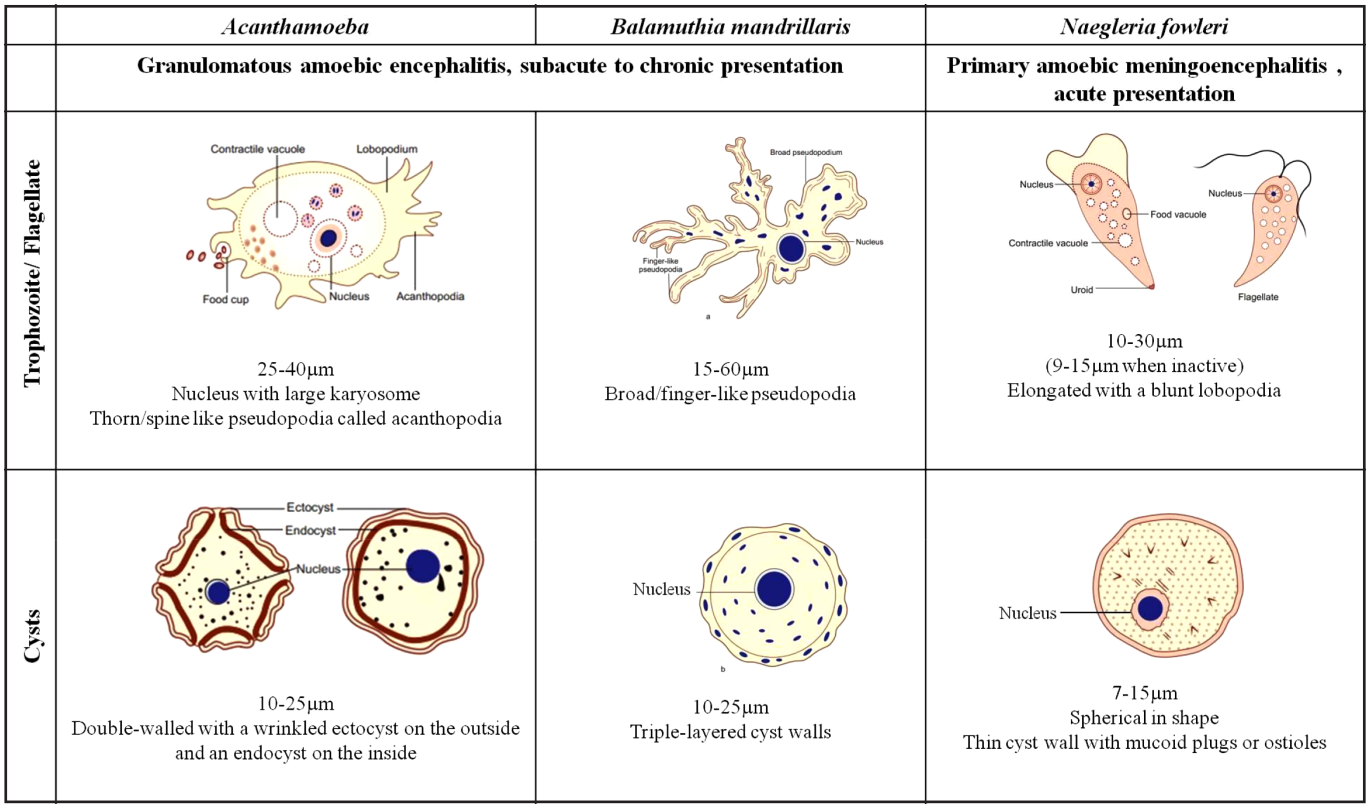

Figure 1. Schematic diagrams of Free-living amoebae (Khurana & Mewara, 2021). (Adapted with permission)

Table 1. *Acanthamoeba* granulomatous encephalitis in India

Place of study	Age in Yrs /Sex	Symptoms & *Risk factors	CSF biochemistry (mg/dl) & Cell count/cubic mm.	Diagnosis				Treatment Empirical/ Specific	Out-come
				Neuroimaging CT/MRI	Trophozoites/ Cysts-WM/S	Culture	Histopathology		
Lokamanya Tilak Municipal General Hospital, Sion, Bombay (Gogate *et al.*, 1984)	15/F	Fever Nausea Vomiting	Protein: 320 mg Glucose: 150 mg Cell count: 60 cells Polymorphs: 35% Lymphocytes: 60%	NA	Positive	Positive	Autopsy: Necrotising haemorrhagic lesions with amoebae	Ampicillin Neomycin	Died
	19/M	Fever, Drowsy Headache Vomiting	Protein: 170 mg Glucose: 90 mg Cell count:1700 cells	NA	Positive	Positive	Autopsy: Necroti-zing haemorrhagic lesions with amoebae	Chloramphenicol	Died
Christian Medical College and Hospital, Vellore. (Lalitha *et al.*, 1985)	40/M	Fever, Head-ache, Neck pain, lethargy *Bathed in a pond	Protein: 110 mg Glucose: 88 mg Cell count: 2960 cells Neutrophils: 94% Lymphocytes: 6%	Normal	Positive	Positive	Not done	Penicillin Chloramphenicol	Cured
Seth G.S. Medical College, Parel, Bombay (Karande *et al.*, 1991)	0.15 /M	Seizure Poor feeding Tonic spasms Loss of consciousness	Protein: 660 mg Sugar: 16 mg Cell count: 280 cells Polymorphs: 220 Lymphocytes: 60	Lesion in right frontal lobe with hydrocephalus	Positive	Positive	Not done	Cotrimoxazole 5-flurocytocine Sulphadiazine	Cured
Univ. College of Medical Sciences, Shahdara, Delhi (Sharma *et al.*, 1993)	12/F	Fever Headache Vomiting Stiff neck	Protein: 65 mg Sugar: 45 mg Cell count: 300 cells Polymorphs: 100%	Normal	Positive	Positive	Not done	Cotrimoxazole	Cured
Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi (Singhal *et al.*, 2001)	8/M	Headache Vomiting Neck stiffness	Protein:> 1 g Glucose: 18 mg Cell count: 250 cells Lymphocytes:100%	Hydrocephalus with basal exudates	Positive	Positive	Not done	Cotrimoxazole	Died
	8/F	Fever Headache Vomiting Seizures	Protein: 200 mg Glucose: 21 mg Cell count:150 cells Lymphocytes:100%	Multiple lesions	Positive	Positive	Not done	Cotrimoxazole Ketoconazole Rifampin	Cured
	3/M	Fever Seizure Ataxia *Head injury	Protein: 17 mg Glucose: 87 mg Cell count: 200 cells Lymphocytes: 180	Normal	Positive	Positive	Not done	Cotrimoxazole Ketoconazole Rifampin	Cured
Jawaharlal Inst. of Postgraduate Med.	45/F	Fever Malaise	Protein: 90 mg Sugar: 46 mg	Normal	Positive	No growth	Not done	Cotrimoxazole Fluconazole	Cured

Education & Research, Pondicherry (Hamide *et al*., 2002)		Neck stiffness	Cell count:1000 cells Neutrophils: 77% Lymphocytes: 23%				Rifampicin Ceftriaxone Albendazole	
Grant Medical College Byculla, Mumbai (Velho *et al*., 2003)	26/M	Headache Backache Weakness in lower limbs	NA	Lesion in R-temporoparietal L-cerebellar region, edema	NA	NA	Excised tissue biopsy: Trophozoites	ATT Steroid	Died
Hinduja National Hospital and Research Centre, Mumbai (Shirwadkar *et al*., 2006)	24/F	Fever Seizures Neck stiffness Loss of consciousness. *SLE	Protein: 174 mg Glucose: 42 mg Leukocytes: 1 cell	Multiple lesions in left cerebellar hemisphere	NA	NA	Autopsy:Necrotis-ing haemorrhagic lesions with cysts, & trophozoites PCR, brain tissue: Acanthamoeba	Ceftriaxone Phenytoin Pulse steroids	Died
Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh (Singh *et al*., 2006)	14/F	Seizure, Loss of conscious-ness, Neck rigidity, R-hemiparesis	Protein: 52 mg Glucose: 63 mg	Multiple lesions in L-parietal lobe with edema	NA	NA	Autopsy: Necrotic haemorrhagic lesion, amoebic trophozoites	NA	Died
	10/M	Headache, Vomiting	Protein: 64 mg	Lesion in L-fron totemporal lobe	NA	Positive	Not done	Ketoconazole Rifampicin	Cured
	19/F	Headache, Vomiting Unsteady gait	Protein: 60 mg Glucose: 91 mg	Multiple lesions with edema, cystic lesion in L-parietal lobe	NA	NA	Autopsy:Necrotic, haemorrhagic lesions, amoebic trophozoites	ATT	Died
	38/M	Headache,vom iting, Altered sensorium	NA	Lesions in left parietal lobe with edema	NA	NA	Autopsy: GAE	NA	Died
AIIMS, New Delhi (Kumar *et al*., 2007)	24/M	*AIDS	NA	NA	Positive	Positive	NA	NA	NA
AIIMS, New Delhi (Gupta *et al*., 2008)	17/M	Seizure Hemiparesis	NA	Normal	NA	Positive	NA	Cotrimoxazole Ketoconazole Rifampicin	Died
Dayanand Med. College & Hosp. Ludhiana Punjab (Kaushal *et al*., 2008a)	63/F	Headache Vomiting Altered sensorium	Protein: 26 mg Glucose: 96 mg Cell count: 10 cells Mostly lymphocytes	Normal	Positive	Positive	Not done	Cotrimoxazole Fluconazole Rifampicin Amphotericin B	Died
AIIMS, New Delhi (Radhakrishnan *et al*., 2009)	14/M	Mood swings *ALL	Protein and sugar - Normal	Normal	Positive	Positive	Not done	Cotrimoxazole Ketoconazole	Died
St Stephen's Hospital, New Delhi (Ranjan *et al*., 2009)	25/M	Seizure	Protein: 150 mg Glucose: 20 mg Cell count: 8 cells Lymphocytes: 90%	Cystic lesion	Positive	NA	Excised cyst: Glial ependymal cyst	Rifampicin Cotrimoxazole Fluconazole	Cured
Sita Ram Bhartia Institute, New	15/F	Headache, Fe-ver, vomiting	Protein: 199 mg Cell count: 50 cells	Normal	Positive	Positive	Not done	Cotrimoxazole Ketoconazole	Cured

Delhi. (Saxena *et al*., 2009)		Photophobia	Lymphocytes: 96%					Rifampicin	
Nizam's Institute of Medical Sciences, Hyderabad (Reddy *et al*., 2011)	38/M	Headache Fever, Seizure Weakness of left limbs	NA	Lesion in R-parietal lobe	Positive	No growth	Autopsy: Necrotic haemorrhagic lesions, amoebic cysts, trophozoites	ATT Steroids	Died
PGIMER, Chandigarh. (Khurana *et al*., 2012)	3/M	Fever, seizures Vomiting, Alt-ered sensorium *Malnutrition	Protein: 265 mg Sugar: 37 mg Cell count: 240 cells Polymorphs: 100%	Hydrocephalus	NA	Positive	Not done	Cotrimoxazole Ketoconazole Rifampicin	Cured
PGIMER, Chandigarh (Vyas *et al*., 2013)	18/M	Headache Vomiting Altered sensorium	Protein: 132 mg Glucose: 71 mg Cell count: 20 cells Lymphocytes: 100%	Lesion in L-frontoparietal lobe with edema	NA	NA	Autopsy: Haemorrhagic, necrotic lesions, Trophozoites	Vancomycin Ceftriaxone Steroids	Died
National Institute of Mental Health & Neurosciences (NIMHANS), Bangalore, Kar-nataka. (Chandra *et al*., 2014)	16/M	Headache Fever, Right hemiparesis, altered sensorium	NA	Multiple lesions in thalamus, brain stem, corticomedulla-ry junctions and L-basal ganglia	NA	NA	Autopsy:Necrotis-ing haemorrhagic lesions, cysts & trophozoites PCR, brain tissue: Acanthamoeba	ATT Steroids	Died
Kasturba Medical College, Manipal, Karnataka (Khanna *et al*., 2014)	30/M	Headache Fever Weight loss	Protein: 134 mg Glucose: 34 mg Cell count: 207 cells Mostly lymphocytes	Normal	Positive	Positive	Not done	Cotrimoxazole Rifampicin Fluconazole	Cured
University College of Med. Sciences & Guru Teg Bahadur Hospital, Delhi University, New Delhi (Das *et al*., 2016)	4/M	Headache, Fever, seizures Vomiting, *Geophagia	Protein: 16 mg% Glucose: 40 mg% Cell count:20 cells Mostly lymphocytes	Hydrocephalus	Positive	Positive cysts	Not done	Cotrimoxazole Rifampicin Amphotericin B	Died
	10/F	Fever, head-ache,Vomiting Neck rigidity *Malignancy	Protein: 9 mg% Glucose: 84 mg% Cell count: Lymphocytes: 4	NA	Positive	Positive	Not done	Cotrimoxazole Rifampicin Amphotericin B	Died
	0.75 /F	Fever Vomiting	Protein: 20 mg% Glucose: 65 mg% Cell count: No cells	NA	Positive	Positive	Not done	Cotrimoxazole Rifampicin Amphotericin B	Cured
	12/M	Headache Fever, Altered sensorium	Protein: 10 mg% Glucose: 81 mg% Cell count: No cells	NA	Positive	Positive	Not done	Cotrimoxazole Rifampicin Amphotericin B	Cured
	11/F	Fever, Neck rigidity, Head-ache, vomiting	Protein: 119 mg% Glucose: 23 mg% Cell count: No cells	NA	Positive	No growth	Not done	Cotrimoxazole Rifampicin Amphotericin B	Cured
	9/F	Fever, Seizure Vomiting Neck rigidity *TBM	Protein: 320 mg% Glucose: 16 mg% Cell count: 185 Mostly lymphocytes	NA	Positive	Positive	Not done	Cotrimoxazole Rifampicin Amphotericin B	Died

	12/F	Fever Vomiting	Protein: 234 mg% Glucose: 27 mg% Cell count: 50 cells Mostly lymphocytes	NA	Positive	Positive	Not done	Cotrimoxazole Rifampicin Amphotericin B	Cured
Kashibai Navale Med.College, Pune Maharashtra. (Ghadage *et al*., 2017)	2/M	Fever, Inability to stand and walk	Cell count:30, 000 Polymorphs: 95% Lymphocytes: 5%	Mild Hydrocephalus	Positive	NA	Not done	Vancomycin Ceftriaxone	Cured
PGIMER, Chandigarh (Nampoothiri *et al*., 2018)	19/M	Headache Fever *AML	Protein: 45 mg Glucose: 46 mg Cell count: No cells	Hypodense lesion in right parietal lobe	Positive	Positive PCR Positive	Excision: amoebic cysts	Cotrimoxazole Rifampicin Ketoconazole Azithromycin	Cured
NIMHANS, Bangalore (Nehete *et al*., 2018)	11/M	Headache Vomiting Unsteady gait	Protein: 4.5 mmol Cell count:12 cells Polymorphs: 2 Lymphocytes: 10	Lesion in vermis, para-vermian region hydrocephalus	NA	NA	Trophozoites	Cotrimoxazole Ketoconazole	Died
	30/M	Headache Fever, Altered sensorium Vomiting	NA	Lesion in basi-frontal, R- frontal lobe, edema	NA	NA	Biopsy: Trophozoites and cysts	NA	Died
Nizam's Institute of Med. Sciences, Hyderabad (Thamtam *et al*., 2016)	28/M	Headache Fever, Neck stiffness *SLE	Protein: 125 mg Glucose: 37 mg Lymphocytic pleocytosis	Multiple lesions in frontal, parietal lobe with edema	NA	NA	Autopsy: Haemorr-hagic necrosis, Trophozoites & cysts	Methyl-prednisolone	Died
Christian Medical College, Vellore (Das *et al*., 2020)	51/F	Fever, R-sided weakness *Fell into well	Protein: 86 mg/dl Cell count: 340 cells Lymphocytes: 80%	Cerebral arterial territory infarcts	Positive	Positive	Not done	Cotrimoxazole Rifampicin Fluconazole	Cured
	22/M	Headache	Protein: Normal Glucose: Normal Cell count: Lymphocytes: 2	Normal	Positive	Positive	Not done	Cotrimoxazole Fluconazole Rifampicin Miltefosine Metronidazole	Cured
Institute of Medical Sciences, Banaras Hindu University, Varanasi (Singh *et al*., 2020)	15/M	Headache Fever Neck rigidity *Sewage cleaning	Protein: 97 mg/dL Glucose: 30 mg/dL Cell count: 600 Polymorphs 90% Lymphocytes 10%	Hydrocephalus, periventricular ooze, Papilledema	Positive	NA	Not done	Cotrimoxazole Rifampicin Ketoconazole Amphotericin B	Cured
AIIMS, New Delhi (Siripurapu *et al*., 2021)	32/M	Headache Vomiting R- side weakness L- hemiparesis Sensorial decline	Protein: normal Sugar: normal Lymphocytes: 4/mL	Lesions in bilateral hemispheres	Positive	NA PCR (scalp aspirate) positive	Biopsy: Necrotic tissue, Inflammation, amoebic trophozoites	Cotrimoxazole Rifampicin Fluconazole Albendazole Azithromycin Flucytosine Miltefosine	Cured

ALL: Acute lymphoblastic leukaemia; ATT: Anti-tuberculous chemotherapy; CT: Computed tomography; AIDS: Acquired immunodeficiency syndrome; L: Left; MRI: Magnetic resonance imaging; ND: Not done; NA: Not available; PCR: Polymerase chain reaction; R: Right; S: Staining; SLE: Systemic lupus erythematosus; TBM: Tuberculous meningitis; WM: Wet mount; Yrs: Years

was observed in three samples. In ten patients, GAE was diagnosed postmortem in biopsy/autopsy samples, in which histopathology showed hemorrhagic necrotic lesions with amoebic trophozoites and cysts (Table 1). *Acanthamoeba* and *Balamuthia* trophozoites appear morphologically similar in tissue sections under a light microscope; therefore, electron microscopy, indirect immunostaining (IIF), or molecular analysis are required for confirmation (Feingold *et al*., 1998). Three culture-negative cases showed motile amoebae in wet mounts, which is a common occurrence in CNS infections caused by FLA (Parija *et al*., 2015). In recent years, PCR has been used to identify *Acanthamoeba* DNA in CSF and brain tissue samples and has been considered as an alternative to conventional methods (Qvarnstrom *et al*., 2006). In this reveiw, PCR was used effectively to confirm *Acanthamoeba* in the culture or brain tissue/scalp aspirate in four cases (Table 1). A prospective study showed that PCR is as effective as culture methods for the diagnosis of *Acanthamoeba* GAE (Behera *et al*., 2016).

Treatment: There is no effective drug regimen recommended for *Acanthamoeba* GAE. *In vitro* sensitivity studies with virulent *Acanthamoeba* have shown variable efficacy to ketoconazole, pentamidine, 5-fluorocytosine, polymyxin, sulfadiazine, cotrimoxazole, azithromycin, flucytosine, and amphotericin B (da Rocha-Azevedo *et al*., 2019). Twenty-one (50%) *Acanthamoeba* GAE patients in this review survived after antimicrobial treatment. One patient was successfully treated with penicillin and chloramphenicol. Others have reported favorable outcomes using combination therapy with cotrimoxazole, rifampicin, and ketoconazole/amphotericin B (Table 1). Miltefosine and albendazole were used in two cases and reported to be effective against *Acanthamoeba* (Aichelburg *et al*., 2008). Forty-eight percent of patients (n=20) with *Acanthamoeba* GAE died, and nine of these patients were treated with either empirical or anti-tubercular therapy. Eight other patients had unfavorable outcomes despite having been administered with cotrimoxazole, rifampicin, ketoconazole, and amphotericin B. Seven of these patients were below 20 years old, and one was above 60 years of age. No specific reason could be identified for the treatment failure in these patients. The duration of therapy ranged from two to nine months depending on the patient's clinical response and parasitological clearance. It has been suggested that corticosteroid therapy may exacerbate *Acanthamoeba* GAE and should therefore be avoided. Antibodies to *Acanthamoeba* have been demonstrated in serum samples from both healthy individuals and those with GAE. The role of these antibodies in protection against infection remains unknown. However, acquired protective immunity mediated by the T-cell response has been suggested as the reason for the low incidence of *Acanthamoeba* GAE in humans (Marciano-Cabral & Cabral, 2003).

***Naegleria fowleri*: Primary amoebic meningoencephalitis**

Naegleria fowleri is the only pathogenic species of *Naegleria* that causes PAM and is commonly found in fresh water sources, such as rivers, lakes, ponds, hot springs, domestic water supplies, sewage, heating and ventilation units, and swimming pools (Gogate & Deodhar, 1985; Panda *et al*., 2015). This species is thermophilic and tolerates temperatures up to 45˚C. Most cases of *N. fowleri* infections have been associated with bathing in streams, ponds, lakes, and indoor swimming pools during the summer months when the water is warm. Rare cases have occurred following inhalation of airborne cysts, nasal irrigation, and total immersion in bathwater (Stubhaug *et al*., 2016). Infection occurs when *N. fowleri* trophozoites enter the nasal passages, attach to the olfactory mucosa, migrate along the olfactory nerves, and cross the cribriform plate to the brain (Król-Turmińska & Olender, 2017). *N. fowleri* has three morphological forms: a trophozoite, a flagellate, and a cyst (Figure 1) with a genome size of 29.5 megabases comprising 36.9% GC (Liechti *et al*., 2019). Bioinformatic analysis revealed 208 predicted secretory proteins in the *N. fowleri* genome and some of them have hydrolyzing function that may contribute to pathogenicity of PAM and 245 predicted miRNAs with target genes involved in mitochondrial biological processes, including oxidation-reduction, dehydrogenase activity, and the electron transport chain (Padmashree & Swamy, 2015; Liechti *et al*., 2019).

N. fowleri infection is distributed worldwide, and PAM caused by *N. fowleri* was first reported in 1965 by Fowler and Carter in Australia (Fowler & Carter, 1965). PAM was first reported in India in 1971; since then, 25 cases have been documented, mostly in the northern states (Tables 2 & 4). A few cases of PAM have been reported in newspapers; however, these cases were not included in this review. PAM has most commonly been seen in males (80%), half of whom were children below 15 years. More than fifty percent (n=13) of PAM patients had a history of water-related activities. As predisposing factors, one patient had acute lymphoblastic leukemia (ALL) and another had type 2 diabetes mellitus. It is believed that infections by *N. fowleri* are frequently misdiagnosed and underreported, probably due to inadequate information regarding their pathologies and the fact that patients often die before a definitive diagnosis can be established.

Symptoms: PAM produced by *N. fowleri* has a short incubation period that varies from three to eight days with a sudden onset of neurological symptoms that progress rapidly, leading to coma and death if not treated early. This disease mimics acute bacterial, tuberculous, or viral meningitis/meningoencephalitis (da Rocha-Azevedo *et al*., 2009). In this review, PAM patients presented with fever (96%), headache (60%), vomiting (52%), altered sensorium (40%), and seizures (33%). A few had unconsciousness, photophobia, poor feeding, delirium, and slurred speech (Tables 2 & 4).

Diagnosis: Imaging studies of PAM have shown purulent exudate with hemorrhagic necrosis throughout the cerebral hemispheres, brain stem, cerebellum, and upper spinal cord (Visvesvara *et al*., 2007). Neuroimaging data was available in 14 patients, 11 of which showed lesions with hydrocephalus, edema and exudates, and cerebral/cortical atrophy in older patients. The CSF of PAM patients showed elevated protein levels (n=16) and increased numbers of white blood cells (n=18), comprising predominantly polymorphs in 11 and lymphocytes in six patients (Tables 2 & 4). PAM is suspected in patients with symptoms of encephalitis/meningitis and history of contact with freshwater with CSF showing increased levels of polymorphs, protein, and negative bacterial, fungal, tubercular, and viral infections. Diagnoses of PAM are made mostly by direct microscopic examination of CSF wet mounts that show actively motile trophozoites in a linear forward direction (da Rocha-Azevedo *et al*., 2009). Of the 25 cases reviewed in the present series, amoebic trophozoites were observed in the wet mounts of 24 cases (96%), and only one patient was identified through histopathology tissue section following the patient's autopsy. The wet mount preparation was confirmed by Giemsa staining in four patients and Romanowsky staining in one patient. Ten (42%) patient cultures were positive for *N. flowleri* on non-nutrient agar, and no growth was observed in five patient samples. In another ten patients, cultures were not carried out. Two patient CSF samples were confirmed by species-specific PCR (Tables 2 & 4). A triplex real-time TaqMan PCR assay for simultaneous identification of *Acanthamoeba spp., B. mandriallaris*, and *N. fowleri* can allow for rapid detection and identification of pathogenic FLA (Qvarnstrom *et al*., 2006). *Naegleria* genotypes can be identified by sequencing the 5.8S rRNA gene and the internal transcribed spacer 1 and 2 (ITS1 and ITS2) regions of *N. fowleri* (Visvesvara *et al*., 2007).

Treatment: The survival of PAM patients depends on prompt diagnosis and initiation of anti-*Naegleria* therapy coupled with intensive supportive care. Amphotericin B is the only agent with established clinical efficacy for PAM and can be used alone or in combination with drugs such as rifampicin, fluconazole, sulfadiazine, miconazole, cotrimoxazole, ketoconazole, ornidazole, and chloramphenicol (da Rocha-Azevedo *et al*., 2009). Table 2 shows that all the surviving cases of PAM (n=11, 44%) were treated

Table 2. *Naegleria fowleri,* Primary amoebic meningoencephalitis in India

Place of study	Age in Years/Sex	Symptoms & *Risk factors	CSF biochemistry (mg/dl) & Cell count/cubic mm	Diagnosis			Treatment Empirical/ Specific	Out-come
				Neuroimaging CT/MRI	Trophozoites /cysts in WM/S	Culture		
B. C. Roy Memorial Hospital for Children, Calcutta, West Bengal (Pan *et al.*, 1971)	3/M	Fever, Unconsciousness *Played in puddle	NA	NA	Positive	NA	Amphotericin B Sulphadiazine Dexamethasone	Cured
	0.5/M	Fever, Seizure Unconsciousness Neck rigidity	NA	NA	Positive	NA	Amphotericin B Sulphadiazine Streptomycin	Cured
Udaipur, Rajasthan (Bedi *et al.*, 1972)	45/F	Fever, Headache, Vomiting, Cough *Bathed in well water	NA	NA	Positive	NA	Penicillin Sulphadiazine	Died
AIIMS, New Delhi (Malhotra *et al.*, 1978)	F	Fever, Headache, Photophobia	NA	NA	Positive	NA	NA	Died
Lady Harding Medical College New Delhi (Singh *et al.*, 1998)	8/M	Fever Headache Vomiting	Protein: 97 mg Glucose: 28 mg Cell count:1600 cells Mostly neutrophils	Basal meningitis Hydrocephalus	Positive	*N. fowleri*	Amphotericin B Rifampicin	Cured
PGIMER, Chandigarh (Jain *et al.*, 2002)	26/F	Fever, Headache, vomiting Altered sensorium	Protein: 410 mg Glucose: 52 mg Cell count: 950 cells	Multiple lesions in brainstem, cerebell-um, temporal, parietal lobes	Positive	No growth	Amphotericin B Rifampicin Ornidazole	Cured
Kasturba Medical College,Mangalore, Karnataka (Shenoy *et al.*, 2002)	0.4/M	Fever, Vomiting, Seizure, Unconsciousness, Coma *Bathed in well water	Protein: 361 mg Glucose: 10 mg Cell count:130 cells Neutrophils: 80% Lymphocytes: 20%	Hypodense collection in bilateral frontal convexity	Positive (Giemsa)	*N. fowleri*	Amphotericin B Ceftriaxone	Died
Kasturba Medical College, Manipal Karnataka (Hebbar *et al.*, 2005)	0.5/M	Fever, Lethargy Poor feeding Altered sensorium *Bathed in well water	Protein: 4 gm Glucose: 2.4 mmol Cell count:1540 cells Neutrophils: 16% Lymphocytes: 83%	NA	Positive (Giemsa)	*N. fowleri*	Amphotericin B Chloramphenicol Metronidazole	Died
PGIMER, Chandigarh (Singh *et al.*, 2006)	40/M	Fever, Headache Vomiting Altered sensorium	Protein: 95 mg Glucose: 27 mg	R-basal ganglia infarction, exudates in perimesen-cephalic cistern	Autopsy: Multiple infarcts, meningeal thickening, exudates and trophozoites		ATT Ceftriaxone	Died

Govt. Medical College, Nanded, Maharashtra (Tungikar *et al.*, 2006)	30/M	Fever, Headache, Vomiting Generalized weakness	Protein: 226 mg Glucose: 26 mg Cell count: Pus cells, Occasional lymphocytes	Cerebral oedema	Positive	NA	Amikacin,Cefota-xime, Penicillin Metronidazole Azithromycin Cloxacillin	Died
Dayanand Medical College & Hosp., Ludhiana, Punjab (Kaushal *et al.*, 2008b)	36/M	Fever, Headache, Nausea, Vomiting, Seizure *Bathed in a pond	Protein: 110 mg Glucose: 36mg Cell count: 90 cells Mostly polymorphs	Ill-defined hypodensity in brain stem and hypothalamus	Positive (Giemsa)	*N. fowleri*	Amphotericin B Rifampicin Ceftazidime	Died
MLN Medical College, University of Allahabad, Allahabad, UP (Rai *et al.*, 2008)	0.6/M	Fever, Seizure Altered sensorium *Bathed in pond water	Protein: 63.9 mg Glucose:109.8 mg Cell count: 50 cells Polymorphs: 10% Lymphocytes: 90%	Lesion in left frontal lobe and cerebellar hemisphere	Positive	NA	Amphotericin B Rifampicin Chloramphenicol	Cured
Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP (Tilak *et al.*, 2008)	35/M	Fever, Headache, Vomiting, Altered sensorium, Coma. *Bathed in stagnant water, HIV, Pul. TB	Protein: 258 mg Glucose: 17 mg Cell count: 280 cells Neutrophils: 40% Lymphocytes: 60%	NA	Positive	*N. fowleri*	ATT Steroid Mannitol Amphotericin B	Died
National Institute of Communicable Diseases, New Delhi (Gupta *et al*., 2009)	20/M	Fever, Headache, Loss of vision & hearing, Slurred speech, Difficulty swallowing *ALL.	Protein: 56 mg Glucose: 185 mg Cell count:1500 cells Neutrophils:>90% Few lymphocytes	Normal	Positive	*N. fowleri*	Amphotericin B Rifampicin	Died
Dr. Rajendra Prasad Govt. Med. College, Himachal Pradesh (Angrup *et al*., 2010)	85/M	Fever, Headache, Delirium, Altered sensorium, *Bathed in a pond	Protein: 125mg Glucose: 80mg Cell count:15 cells Mostly polymorphs	Cortical atrophy	Positive	*N. fowleri*	Amphotericin B Rifampicin	NA
Military Hospital, Bhopal, Madya Pradesh (Ramanan *et al*., 2010)	46/F	Fever, Headache, Vomiting Photophobia	Protein: 2.2 gm Glucose: 20 mg Cell count:1200 cells Mostly neutrophils	NA	Positive (Romanowsky)	No growth	Amphotericin B Rifampicin Cotrimoxazole	Died
Kasturba Medical College, Manipal, Karnataka (Khanna *et al.*, 2011)	0.4/M	Fever, Vomiting Decreased feeding Abnormal body movements	Protein: 731 mg Glucose: 5 mg Cell count: 990 cells Mostly lymphocytes	NA	Positive	NA	Amphotericin Ceftazidime	Died
Dayanand Medical College and Hospital, Ludhiana, Punjab (Gautam *et al.*, 2012)	73/M	Fever, Neck pain, Seizures, Altered sensorium *Head injury, DM T2	Protein: 120 mg Glucose: 165 mg Cell count:70 cells Polymorphs: 92% Lymphocytes: 8%	Cerebral atrophy	Positive	*N. fowleri*	Amphotericin B Rifampicin	Cured
Lady Harding Medical College New Delhi (Yadav *et al*., 2013)	0.1/M	Fever, Seizures *Bathed in well water	Protein: 293 mg Glucose: 21 mg Cell count: 300 cells Polymorphs: 100%	Dilated lateral ventricles and hydrocephalus	Positive	*N. fowleri*	Amphotericin B Rifampicin Fluconazole	Cured

Narinder Mohan Hospital, Ghaziabad, UP (Meenakshi *et al*., 2014)	5 days /M	Seizure Respiratory distress	NA	NA	Positive	NA	Amphotericin B	Cured
Dr. Rajendra Prasad Govt. Med. College, Himachal Pradesh (Sood *et al*., 2014)	6/M	Fever, Headache Altered sensorium *Played with water in a cement tank	Cell count: 415 cells Mostly neutrophils	NA	Positive (Giemsa)	No growth	Amphotericin B Rifampicin Fluconazole	Cured
Netaji Subhash Chandra Bose Medical College, Jabalpur, Madhya Pradesh (Gupta *et al*., 2015)	40/M	Fever, Headache. Vomiting Slurred speech *Swimming pool	Protein: 76 mg Glucose: 44 mg Cell count:82 cells Polymorphs: 3% Lymphocytes: 95%	Normal	Positive	*N. fowleri*	ART, ATT Amphotericin B Fluconazole Metronidazole	Cured
Kothari Medical Centre Kolkata, West Bengal (Ganguly *et al*., 2017)	15/M	Fever, Headache, Altered sensorium, Seizure *Bathed in river water	Protein: 200 mg Glucose: 64 mg Cell count: 210 cells Polymorphs: 12% Lymphocytes: 88%	Normal	Positive CSF: Nested PCR *N. fowleri*	No growth	NA	Died
QRG Central Hosp. and Research Centre, Faridabad, Haryana (Mittal *et al*., 2019)	0.6/F	Fever, vomiting, Seizures, Abnormal body movements, Poor feeding	Protein: 363 mg Glucose: 54 mg Cell count:1020 cells Polymorphs 90% Lymphocytes 10%	Meningitis, Hydrocephalus	Positive CSF: PCR *N. fowleri*	NA	Amphotericin B Ceftriaxone Vancomycin Acyclovir	NA
Velammal Medical College Hospital & Research Institute, Madurai, Tamil Nadu (Perumal-samy *et al*., 2020)	47/M	Headache Vomiting Altered sensorium *Bathing in a river	Protein 166 mg/dl Glucose: 45 mg/dl Cell count: 30 Polymorphs 70% Lymphocytes 30%	Cribriform base plate defect	Positive	Negative	Amphotericin B Azithromycin	Cured

ALL: Acute lymphoblastic leukaemia; ATT: Anti-tuberculous chemotherapy; CT: Computed tomography; DM T2: Diabetes mellitus Type 2; HIV: Human immunodeficiency virus; MRI: Magnetic resonance imaging; ND: Not done; NA: Not available; PCR: Polymerase chain reaction; Pul.TB: Pulmonary tuberculosis; WM/S: Wet mount/staining

Table 3. *Balamuthia mandrillaris* encephalitis in India

Place of study	Age in Years /Sex	Symptoms& *Risk factors	CSF biochemistry (mg/dl) & cell count/cubic mm	Diagnosis			Treatment Empirical/ Specific	Out-come
				Neuro-imaging CT/MRI	Histopathology Autopsy/Biopsy	Molecular identification PCR		
AIIMS, New Delhi (Prasad *et al.*, 2008)	22 /M	Numbness in fingers and legs, vertigo, difficulty swallowing, right sided weakness, Seizure. *CSOM	Protein and glucose: Normal Lymphocytes: 5	Lesion in medulla Micro-haemorrhage	Acute haemorrhagic necrotising encephalitis	*B. mandrillaris* in paraffin block	ATT Methyl-prednisolone Amphotericin B Acyclovir	Died
PGIMER, Chandigarh. (Khurana *et al.*, 2015b)	18/M	Fever, headache, vomiting, slurred speech, right upper & lower limb weakness, altered sensorium	Protein:132 mg Glucose:71 mg Lymphocytes: 20	Multiple lesions in bilateral frontoparietal, left cerebellum, temporal & occipital lobes	Haemorrhagic necrotic lesions, granulomas with trophozoites	*B. mandrillaris* in fixed tissue section	ATT Ceftriaxone Vancomycin	Died
	18/M	Fever, headache, vomiting	Not done	Lesions in left parafalcine, frontoparietal, parietooccipital, right parietal lobe	Necrotic granulomatous inflammation with tropho zoites & cysts	*B. mandrillaris* in brain biopsy	ATT Albendazole Amphotericin B Clarithromycin	Died
Max Super Speciality Hospital, New Delhi (Tarai *et al.*, 2018)	57/M	Seizures, altered sensorium, unresponsive state *Sarcoidosis	Protein:247 mg Glucose:105 mg Lymphocytes: 5	Multiple lesions, in bilateral cere-bral hemispheres, with internal haemorrhages and edema	Necrotizing haemorrhagic inflammation, with amoebic trophozoites	*B. mandrillaris* in brain biopsy	Fluconazole Cotrimoxazole Voriconazole	Died

ATT: Anti-tubercular chemotherapy; CSOM: Chronic suppurative otitis media; CT: Computed tomography; MRI: Magnetic resonance imaging; PCR: Polymerase chain reaction

Table 4. CNS infections caused by free-living amoebae in India

	Acanthamoeba GAE (n=42)	*Naegleria fowleri* PAM (n=25)	BAE (n=4)
Place of study & No. of cases	New Delhi (n=17); Chandigarh (n=7); Maharashtra (n=6); Karnataka (n=4); Vellore (n=3); Hyderabad (n= 2); Pondicherry, Punjab and Uttar Pradesh (n=1 each)	New Delhi (n=4); Karnataka, Uttar Pradesh, West Bengal (n=3 each); Chandigarh, Himachal Pradesh, Madhya Pradesh, Punjab (n=2 each); Haryana, Maharashtra, Rajasthan, Tamil Nadu (n=1 each)	Chandigarh (n=2) New Delhi (n= 2)
Gender	Males (n=27, 64%); Females (n=15, 36%)	Males (n=20, 80%); Females (n= 5, 20%)	Males (n= 4)
Age in years	< 20 (n=27, 64%); >20 (n=15, 36%)	<20 (n=12, 48%); > 20 (n=12, 48%); NA (n=1)	<20 (n=2); >20 (n=2)
Risk factors	Leukaemia, SLE, History of water activity (n=2 each);, AIDS, Geophagia, Head injury, Sewage cleaning, Malignancy, Malnourished (n=1 each)	History of water activities (n= 13, 52%); Leukaemia (n=1); Diabetes mellitus Type 2 (n=1)	Sarcoidosis (n=1)
Symptoms	Fever (n=26, 64%);, Headache (n=27, 64%); Vomiting (n=22, 52%); Seizure (n=12, 30%); Altered sensorium (n=9, 21%); Neck stiffness (n=10, 23%)	Fever (n=23, 96%); Headache (n=15, 60%); Vomiting (n=13, 52%); Altered sensorium (n=10, 40%); Seizure (n=8, 33%)	Fever, Headache, Seizure, Vomiting, Altered sensorium (n= 2 each)
CSF profile	Protein: Elevated (n=24, 52 mg to >1g) Glucose: Decreased (n=9, 16 to 37 mg) Elevated (n=6, 87 to 150 mg) Cell count: Elevated (n=22, 8 to 1700 cells) Mostly lymphocytes (n=15); polymorphs (n=7)	Protein: Elevated (n=16, 95 mg to 4.0 g) Glucose: Elevated (n=4, 80-185 mg) Decreased (n=2, 5-10 mg) Cell count: Elevated (n=18,15-1600 cells) Mostly polymorphs (n=11); lymphocytes (n=6)	Elevated Protein (n=2) Range (132-247 mg/dl) Elevated cell count (n=1)
Diagnosis Neuroimaging	Multiple lesions in frontal, parietal, cerebellar, thalamus, vermis regions with edema or hydrocephalus (n=17); hydrocephalus (n=4); cystic lesion (n=1); Arterial infarcts (n=1); Normal (n=10); NA (n=9)	Hydrocephalus (n=3); Multiple lesions in brain stem, cerebellum, frontal lobe, cerebral hemisphere, hypothalamus, frontal convexity (n=4); Cortical /cerebral atrophy (n=2), Cerebral edema / exudates (n=2); Cribriform base plate defect (n=1); Normal (n=3); NA (n=10)	Lesions in medulla, cerebellum, temporal, occipital, parietal region, with edema / haemorrhage (n=4)
Wet mount	Trophozoites & cysts (n=29, 69%); ND (n=13)	Trophozoites (n=24, 96%); ND (n=1)	Not done
Culture	Positive (n=25, 60%); NG (n=3); ND (n=14)	Positive (n=10, 42%); NG (n=5); NA (n=10)	Not done
Histopathology	Haemorrhagic necrotic lesions with trophozoites and cysts (n=11, 26%); Trophozoites & cysts (n=4, 10%), Cyst (n=1); NA/ND: (n=25)	Multiple infarcts, meningeal thickening gross exudates, and amoebic trophozoites (n=1)	Haemorrhagic necrotic lesions with trophozoites (n=4)
PCR	Acanthamoeba (culture n=1; brain tissue n=2, scalp aspirate n=1)	*N. fowleri* in CSF (n=2)	*B. mandrillaris* (n=4)
Tx. outcome	Cured (n=21, 50%); Died (n=20, 48%);NA(n=1)	Cured (n=11, 44%); Died (n=12, 48%); NA (n=2)	Died (n=4)

ND: Not done NA: Not available. NG: No growth; Tx: Treatment

with amphotericin B along with rifampicin and fluconazole or sulphadiazine. Of the 12 (48%) patients who had fatal outcomes, three were treated with empirical therapy and seven received amphotericin B in combination with other drugs. Amphotericin B treatment failure in these patients could have occurred due to extremely young age (Shenoy *et al*., 2002; Hebbar *et al*., 2005; Khanna *et al*., 2011), immunosuppression (Tilak *et al*., 2008; Gupta *et al*., 2009), or incomplete treatment (Kaushal *et al*., 2008b). The duration of treatment for PAM varied from nine days to six weeks (da Rocha-Azevedo *et al*., 2009). Individuals with history of swimming in freshwater have been found to have IgM and IgG antibodies to *N. fowleri* that persist for several years following recovery from PAM. However, the role of these antibodies in protective responses is unknown. *N. fowleri* cause an acute inflammatory cytokine response that contributes to neuronal damage and irreversible brain damage (Visvesvara *et al*., 2007). Increased awareness is required for the prevention of PAM, as chlorination of water at 2 ppm is effective against *N. fowleri* trophozoites and warning signs can be placed in recreational waters during the hot summer months.

Balamuthia amoebic encephalitis (BAE)

B. mandrillaris causes GAE, also known as *Balamuthia* amoebic encephalitis (BAE), occurs in healthy and immunosuppressed individuals as well as in animals. This species was first identified as a pathogen in 1986 when it was isolated from the brain of a mandrill baboon at the San Diego Zoo. The life cycle of *Balamuthia* includes a trophozoite and a cyst stage (Figure 1) (Matin *et al*., 2008). The mitochondrial genome of *B. mandrillaris* strain 2046 has 41,656 bases with 64.8% AT (Greninger *et al*., 2015). Similar to *Acanthamoeba*, *L. pneumophilia* has been found to infect *B. madrillaris in vitro* (Shadrach *et al*., 2005). Contact with contaminated soil is a major risk factor for BAE and invasion of the CNS by hematogenous spread from primary sites, such as the skin and the respiratory tract, which are considered the routes of infection (Matin *et al*., 2008; Schuster *et al*., 2009). Several human cases of BAE have been reported worldwide, mostly in Latin America, the southwestern United States, and Australia (Jung *et al*., 2004; Schuster *et al*., 2009). Four fatal cases of BAE—two each from New Delhi and Chandigarh—have been reported in India (Table 3). All four cases were males, three were immunocompetent, and one had sarcoidosis. Given that 14 (33%) of the cases in the present review with *Acanthamoeba* GAE were not culture-confirmed, some of them could have been due to *B. mandrillaris*, as *Acanthamoeba* and *B. mandrillaris* have similar morphology in tissue sections (Booton *et al*., 2003). In fact, some cases reported as *Acanthamoeba* have subsequently turned out to be *B. mandrillaris* (Deetz *et al*., 2003).

Symptoms: BAE has a subacute to chronic course, which may vary from weeks to months or years. Similar to *Acanthamoeba* GAE, the symptoms of BAE may mimic neurotuberculosis, toxoplasmosis, or neurocysticercosis (Matin *et al*., 2008). Patients with BAE in this review have presented with fever, headache, vomiting, seizures, altered sensorium, right-side weakness, slurred speech, and vertigo (Table 3). However, the signs and symptoms of BAE are generally not initially evident, as BAE patients typically deteriorate days or weeks after becoming symptomatic (Schuster *et al*., 2009).

Diagnosis: BAE is difficult to diagnose due to its lack of specific characteristics and a lack of familiarity with amoeba morphology in microbiological and histopathological investigations (Yagi *et al*., 2005). BAE is usually preceded by an initial cutaneous infection, which may appear on the face, trunk, hands, or feet (Doyle *et al*., 2011; Król-Turmińska & Olender, 2017). MRI and CT of the brains of the BAE patients discussed in this review showed multiple lesions with internal hemorrhage and cerebral edema resembling brain tumors, abscesses, toxoplasmosis, or cysticercosis. The CSF of some of these patients showed elevated protein and lymphocyte levels. However, CSF was negative for trophozoites in all patients (Table 3). Wet mount preparations are often found to be negative for amoebic trophozoites in cases of GAE caused by *Acanthameoba* and *Balamuthia* (Parija *et al*., 2015). Autopsy and biopsy samples of BAE patients have shown granulomatous necrotizing hemorrhagic lesions with amoebic trophozoites and cysts in histopathology sections. In this review, two BAE patients were diagnosed antemortem, two were diagnosed postmortem, and all four cases were confirmed by species-specific PCR in brain tissue (Table 3). *B. mandrillaris* cannot be grown on *E. coli*-coated non-nutrient agar plates, but it is possible to isolate them using tissue cultures (Jung *et al*., 2004; Schuster *et al*., 2009).

Treatment: The optimal antimicrobial therapy for BAE remains to be determined. Currently, BAE treatment relies on empirical combination therapy consisting of pentamidine, ketaconazole, fluconazole, flucytosine, sulfadiazine and macrolide antibiotics, azithromycin, or clarithromycin (Schuster *et al*., 2009). All the patients in this review who were treated with empirical or specific drugs had unfavorable outcomes (Table 3). Antibodies to *B. mandrillaris* have been demonstrated in the serum samples of healthy individuals and BAE patients. However, the role of these antibodies in protective responses remains to be determined (Huang *et al*., 1999; Schuster *et al*., 2009). Increased levels of IL-6 production in tissue cultures infected with *B. madrillaris* suggest that IL-6 has a role in blood-brain barrier permeability (Jayasekera *et al*., 2005). Wearing protective clothing while working with soil might prevent infection by *B. mandrillaris*.

CONCLUSION

CNS infections caused by pathogenic FLA are underreported because only a few laboratories are capable of performing diagnostic tests for the detection of FLA. It is necessary to impart awareness of the diseases caused by these agents to healthcare workers. Research efforts are needed in the area of rapid diagnostics and optimum treatment of these diseases. Many FLA isolates from the hospital environment could not be identified using conventional PCR with species-specific primers (Khurana *et al*., 2015a). Next-generation sequencing is necessary to identify undiscovered FLA that may cause CNS infections.

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Conflicting Interest

The author declares that they have no conflict of interests.

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