GOVERNMENT OF KERALA

Abstract

Health & Family Welfare Department - Technical Guidelines on Prevention, Diagnosis and Treatment of Amoebic Meningoencephalitis in Kerala - Orders issued.

HEALTH & FAMILY WELFARE (F) DEPARTMENT

G.O.(Rt)No.1760/2024/H&FWD Dated,Thiruvananthapuram, 20-07-2024

Read Proposal from the Chairperson, State Medical Board

ORDER

In the context of reporting Amoebic Meningoencephalitis cases in the State, for ensuring effective control and management of the disease, Government are pleased to issue the *"Technical Guidelines on Prevention, Diagnosis and Treatment of Amoebic Meningoencephalitis in Kerala"* as annexed to this order.

(By order of the Governor)
SHIJA C G
JOINT SECRETARY

To:
The State Mission Director - National Health Mission,
Thiruvananthapuram.
The Director of Health Services, Thiruvananthapuram.
The Director of Medical Education, Thiruvananthapuram.
The Chairperson, State Medical Board
All District Medical Officers (Health)
Principal Accountant General (A&E/Audit) Kerala.
Information & Public Relations (Web & New Media) Department
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Forwarded /By order

Signed by
Vilasini K V
Date: 20-07-2024 18:16:03

Section Officer

Annexure

TECHNICAL GUIDELINES ON PREVENTION, DIAGNOSIS AND TREATMENT OF AMOEBIC MENINGOENCEPHALITIS IN KERALA

Amoebic encephalitis is a rare but lethal central nervous system infection caused by free-living amoebae found in freshwater, lakes, and rivers. There are two types of amoebic encephalitis, namely primary amoebic meningoencephalitis (PAM) and granulomatous amoebic encephalitis (GAE). Primary amoebic meningoencephalitis (PAM) is a disease caused usually by infection with *Naegleria fowleri*, a microscopic amoeba commonly called a "brain-eating amoeba." This infection destroys brain tissue, causing severe brain swelling and death in most cases. PAM is rare and usually occurs in otherwise healthy children, teens and young adults. The initial symptoms of PAM are indistinguishable from bacterial meningitis, while the symptoms of GAE can mimic a brain abscess, encephalitis, or meningitis. These infections are almost uniformly fatal with only few reported survivors globally.

Kerala Scenario

Sporadic cases of PAM have been reported from many parts of Kerala in the last decade. As of July 7, 2024, over the last 2 months, five cases of PAM have been reported from North Kerala. Clinical features were suggestive of PAM. The cases over the last 2 months have been reported in children aged 5,13,12, 13 and 15 years respectively with a male-to-female ratio of 3:2 [n=5].

Introduction

Free-living amoebae are protozoan environmental parasites, found mainly in freshwater, ponds, lakes, and rivers. There are four main genera of amoebae implicated in human disease, namely *Naegleria* (only *Naegleria fowleri)*, *Acanthamoeba* (several species), *Sappinia* (only *S.pedata*), and *Balamuthia* (only *Balamuthia mandrillaris*). Rarely other free-living amoebae (FLA) such as *Vermamoeba [hartmanella] vermiformis* have also been implicated in human disease. The amoebae exhibit CNS tropism leading to meningoencephalitis. Acanthamoeba can also cause keratitis and disseminated infections in Immunocompromised.

Two distinct clinical syndromes are reported with free-living amoeba infections.

- Primary amoebic meningoencephalitis (PAM)
- Granulomatous amoebic encephalitis (GAE).

Naegleria fowleri is the causal agent of primary amoebic meningoencephalitis (PAM), which is an acute, fulminant, and rapidly fatal infection of the central nervous system (CNS). PAM develops following several days of exposure to the contaminated water source and typically causes death within 1-2 weeks of infection. Very few individuals survive the infection, because of its rapid onset and delayed diagnosis. Only 11 survivors of confirmed *N fowleri* PAM have been reported in the literature.

Naegleria species are ubiquitous in soil and fresh or brackish water (lakes, rivers, ponds). In general, they are sensitive to environmental conditions such as aridity and pH extremes and cannot survive in seawater. In humans, they are found in the throat and nasal cavity.*N fowleri* is heat-tolerant and can survive temperatures up to 45.8°C, pre-adapting the species to mammalian body temperature. Hence, an incubation temperature of 45°C is routinely used to isolate *N fowleri* from water samples, while suppressing the growth of other amoebae in the samples. As it grows best at elevated temperatures, *N fowleri* has been isolated from warm-water bodies, including man-made lakes and ponds, hot springs, and thermally polluted streams and rivers. It feeds on bacteria in the water bodies for survival. *N fowleri* is not found in saltwater sources such as seawater. Most *N fowleri* infections occur in the summer months.

Impact of Global Warming

The free-living amoeba with the most evidence of the effects of climate change is *N. fowleri*. . *N. fowleri* is thermophilic and one of its food sources is cyanobacteria, which flourishes in warmer waters. Climate change raising the water temperature and the heat driving more people to recreational water use is likely to increase the encounters with this pathogen. Furthermore, warming temperatures may be expanding the geographical range of *Naegleria* species. Many reported cases of PAM occurred in the weeks after an increase in air temperature, indicating a potential relation to climate change.

Lifecycle of *N.fowleri*

The life cycle of *N. fowleri* consists of 3 stages: trophozoite, a temporary flagellar stage known as amebo-flagellate, and cyst. The active replicating form is a trophozoite that can reproduce asexually. Trophozoite is the vegetative or feeding stage of the amoeba and is the infective form. In humans, this form is found in CSF or in tissue. Cysts are usually absent in clinical specimens, as the infection is so rapid and fatal that the patient typically dies before the trophozoites encyst. The trophozoite can transform into a flagellate stage, in which state it can survive without nutrition. This is also the stage in which the amoeba is distributed through water bodies. In harsher climates, the flagellated form can undergo encystation into a double-walled cyst and withstand

unfavourable conditions. This enables the *N. fowleri* to survive cold temperatures, and nutritively hostile conditions. (Figure 1)

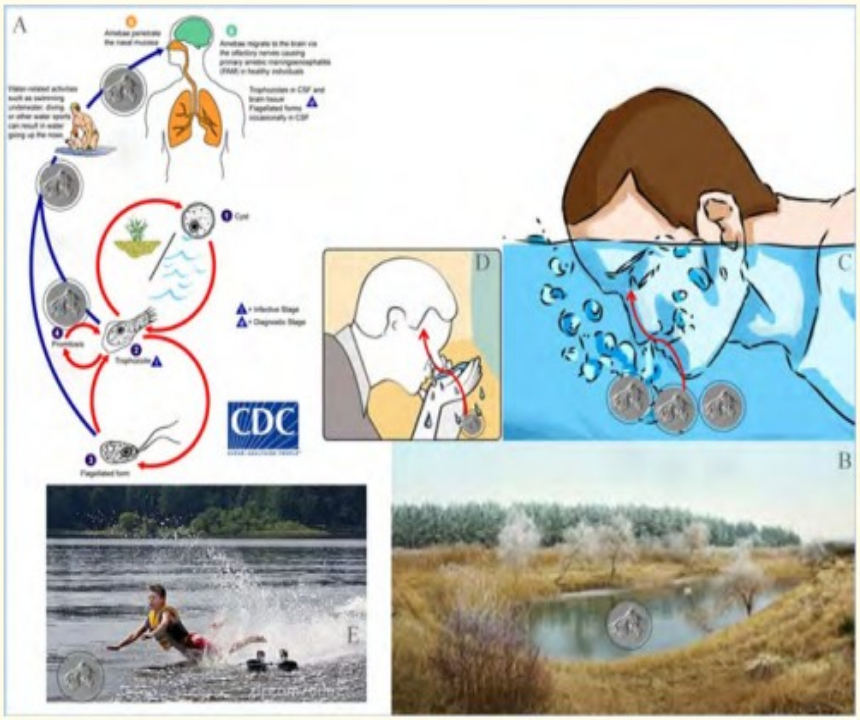

Figure-1: *Shows the life cycle and infection acquisition of Naegleria Fowleri (A). The organism lives in the form of a cyst, flagellate or trophozoite state. Freshwater lakes and ponds (B) in summers at high temperatures ~40OC favours the trophozoite forms of N. fowleri that can enter the nose during swimming (C), ablution (D) or the water sports of different types (E).*

Pathophysiology

PAM can occur in previously healthy young individuals exposed to warm, especially stagnant, fresh water. The portal of entry by the amoebae is through the olfactory mucosa and the cribriform plate. The oral consumption of water contaminated with *N. fowleri* is not associated with symptomatic disease.

The pathophysiology is thought to be due to an amplified host immune response causing an exaggerated inflammatory reaction, necrotizing encephalitis and subsequent parenchymal damage. It is thought that *N. fowleri* causes an acute inflammatory cytokine response, whereas Acanthamoeba and Balamuthia spp. cause a type IV hypersensitivity reaction. These inflammatory responses contribute to neuronal damage and subsequent irreversible brain damage.

Etiology

Primary meningoencephalitis

N fowleri is ubiquitous in soil and is also found in warm freshwater, particularly if the water is stagnant. Exposure to this amoeba is so common that studies have shown that Children younger than 2 years frequently carry the organism asymptomatically in their nose and throat, especially in warmer months and climates without getting infected. Infection with this pathogen occurs in both immunocompromised and immunocompetent individuals.

PAM is an exceptionally uncommon occurrence resulting from CNS invasion of the host by *N fowleri*. During a period of a few days to 2 weeks after inoculating a patient who had been swimming, diving, bathing, or playing in warm, usually stagnant, freshwater, the amoebae migrate through the cribriform plate, along the fila olfactoria and blood vessels, and into the anterior cerebral fossae, where they cause extensive inflammation, necrosis, and hemorrhage in the brain parenchyma and meninges.

Case reports have detailed rare infections following ritual ablution with tap water instilled into the nostrils. Similarly, sinus irrigation with contaminated tap (or other) water using neti pots (or similar devices) has been implicated as the mode of inoculation resulting in PAM.

Granulomatous amebic encephalitis

In contrast to PAM, GAE results from one of the following two pathways. With acanthamebic keratoconjunctivitis, amoebae may spread directly from the cornea to the CNS, though this is uncommon. More typically, GAE results from hematogenous seeding of the CNS following primary inoculation of the lungs or skin by *B mandrillaris, Acanthamoeba,* or *Sappinia* species. Once in the CNS, the pathogen results in focal granuloma and abscess formation. GAE more commonly occurs in immunocompromised hosts; however, GAE may also affect otherwise healthy hosts.

Table 1. Probable Water Exposures-Activity and Water Source-for Reported Cases of Primary Amoebic Meningoencephalitis

Exposure and Category	n (%)
Water activity (N = 247)	
Swimming/diving	143 (58)
Bathing[a]	40 (16)
Water sports (eg, waterskiing, wakeboarding, jet skiing)	24 (10)
Nasal irrigation	22 (9)
Splashing	12 (5)
Water festival	2 (1)
Other	4 (2)
Water source (N = 265)	
Lake/pond/reservoir	119 (45)
Swimming pool[b]	34 (13)
Tap water[c]	32 (12)
Canal/ditch/puddle	32 (12)
River/stream	21 (8)
Geothermal water	20 (8)
Water tank/cistern	5 (2)
Aquatic sports venue	2 (1)

[a.Depending on the setting, the term "bathing" may be used to refer to cleaning oneself or swimming. It was not possible to distinguish these definitions from case reports.
b.Detailed information regarding the operation, management, and condition of the pool at the time of the case-patient exposure was not reported for most cases.
c.Tap water includes water obtained through public water systems (n = 24), wells (n = 6), or boreholes (n = 2).]

In addition to swimming or diving in water bodies contaminated with *N. fowleri*, the use of neti pots for the treatment of sinusitis has been implicated in causing *N. fowleri* infection. The use of nasal plugs and nose clips completely eliminates the risk of infection.

Epidemiology

Although rare, cases of PAM and GAE have been reported worldwide, reflecting the ubiquity of the organisms. The first case of PAM was described by Fowler and Carter in Australia in 1965 followed by the first case described in the United States in 1966. PAM is more common in warmer regions and in the warmer months of spring and summer. There is no seasonal variation with GAE. The risk for infection has been estimated at 1 case per 2.6 million exposures to *N fowleri*.

Sex- and age-related demographics

The male-to-female ratio of PAM is 2:1 overall. PAM has been reported in infants as young as 4 months and is most commonly observed in the first 3 decades of life, with one study finding the median age of infection was 11 years (range: 4-56 years). The male-to-female ratio of GAE is 5:1 worldwide and can be seen at any age.

Clinical presentation and treatment

The diagnosis of PAM carries a high mortality rate of greater than 97%. The clinical presentation of PAM is often indistinguishable from bacterial meningitis with headache, fever, nausea, and vomiting being the most common presenting signs and symptoms. By the time other more common causes of meningitis are ruled out and the diagnosis of PAM is considered, it is often too late to save the patient from the cerebral edema that quickly develops and causes death. Studies show a median incubation period of 5 days and a median time from onset of symptoms to death of five days. Most patients present to medical care with signs or symptoms indicative of central nervous system involvement. Analysis of cerebrospinal fluid (CSF) usually reveals a high opening pressure, a predominantly neutrophilic pleocytosis, the presence of RBC, elevated protein concentration, and low glucose levels.

Table 2. Initial Cerebrospinal Fluid Laboratory Findings on Admission for Reported Cases of Primary Amebic Meningoencephalitis (N = 237) by Classification

Test (Reference Value)	Total (N = 237)		Confirmed (n = 118)			Probable (n = 80)			Suspect (n = 39)		
	n	Median (Range)	n	Median (Range)	*P* Value	n	Median (Range)	*P* Value[a]	n	Median (Range)	*P* Value[b]
Opening pressure (100–200 mm H_2O)	31	290 (36–570)	11	380 (36–530)	Ref	14	255 (138–570)	.261	6	215 (55–500)	.312
Red blood cell count (0 cells/μL, CSF)	124	212 (0–30 750)	87	212 (0–30 750)	Ref	29	350 (0–24 600)	.392	8	756 (0–2600)	.545
White blood cell count (0–5 cells/μL)	232	1238 (0–30 000)	117	1830 (10–29 000)	Ref	78	1510 (7–30 000)	.296	37	415 (0–22 000)	**< .001**
% Neutrophils (2% ± 5%)	181	82 (0–100)	97	80 (12–100)	Ref	64	90 (15–100)	**.002**	20	73 (0–100)	.104
% lymphocytes (62% ± 34%)	128	20 (2–100)	72	20 (2–90)	Ref	38	15 (2–85)	.691	18	65 (5–100)	**< .001**
Protein (15–60 mg/dL)	215	326 (20–1374)	109	326 (24–1342)	Ref	70	361 (20–1374)	.978	36	115 (20–731)	**< .001**
Glucose (40–80 mg/dL)	208	29 (0–223)	106	29 (0–180)	Ref	69	22 (0–223)	.401	33	42 (0–185)	**.035**

Only 27% of U.S. cases were diagnosed before death These findings indicate that the diagnosis is often considered too late or not at all in patients with meningitis.

Characteristics of PAM are as follows:

- PAM commonly affects children and young adults who have previously been healthy[immunocompetent].
- This disease occurs more often during the warmer months of the year and in warmer climates.
- Patients with PAM typically have a history of swimming, diving, bathing, or playing in warm, generally stagnant, freshwater during the previous 1-9 days.
- Rarely, patients with PAM may experience disordered smell or taste.
- Most often, the symptoms of PAM are indistinguishable from those of acute bacterial meningitis.
- The acute onset of PAM occurs over a period of hours to 1-2 days
- The neuro-olfactory route provides *N.fowleri* quick access to the brain and results in impaired adaptive immune response, causing a very rapid disease course.

- *N.fowleri* evokes a pro-inflammatory immune response with extensive brain-tissue damage whereas Acanthamoeba evokes a mixed immune response.

Granulomatous amebic encephalitis

The characteristics of GAE include the following:

- It affects individuals of all ages, although those at the extremes of age may be more susceptible
- Persons with debility or immunocompromise may be more susceptible to GAE.
- There is no seasonal variation because the causative pathogens are ubiquitous.
- Individuals with GAE may have keratoconjunctivitis or a skin ulcer or lesion preceding neurologic symptoms.
- A subacute or chronic presentation lasting days or weeks.

Clinical Stages of PAM

Patients with PAM are mostly admitted in a pre-coma state, and no staging system currently exists to classify the disease. Almost all the survivors of PAM in the past 60 years were diagnosed and treated at a pre-cerebral stage. This shows that early diagnosis of PAM and timely initiation of an antimicrobial cocktail might be lifesaving. To facilitate early diagnosis and timely initiation of treatment to utilize the therapeutic window of opportunity, clinical staging has been proposed by researchers from Aga Khan University Medical College [Fig 2]

Clinical Stage I: Nasal Stage (Pre-Cerebral Stage)

N. fowleri gains entry into the human host via the nose and follows the nasal passage. The introduction of water deep into the nasal orifice causes the protist to encounter the nasal mucosa, leading possibly to infection.

Clinical Stage II: Olfactory stage

In certain cases, *N.fowleri* proceeds from nasal mucosa to olfactory mucosa and bulb. The involvement of the olfactory mucosa and olfactory bulb is a transient stage before the involvement of the brain. This stage is heralded by changes in the olfactory perception (parosmia). Rojas-Hernandez., *et al.* have experimentally used immunohistochemistry in mice infected with *N. fowleri* to characterize the process by which the protist gains entry to the nasal mucosa. It reaches and crosses the cribriform plate, a sieve-like structure that supports the olfactory bulb and contains conduits between the anterior cranial fossa and the nasal passage. The protist reaches the olfactory bulb and begins to replicate. This induces an inflammatory response in patients that together with cytotoxic activity from the amoeba, results in necrosis, focal hemorrhages and exudates formation. Figure 2: Shows the proposed clinical staging of Primary Amoebic Meningoencephalitis (PAM).

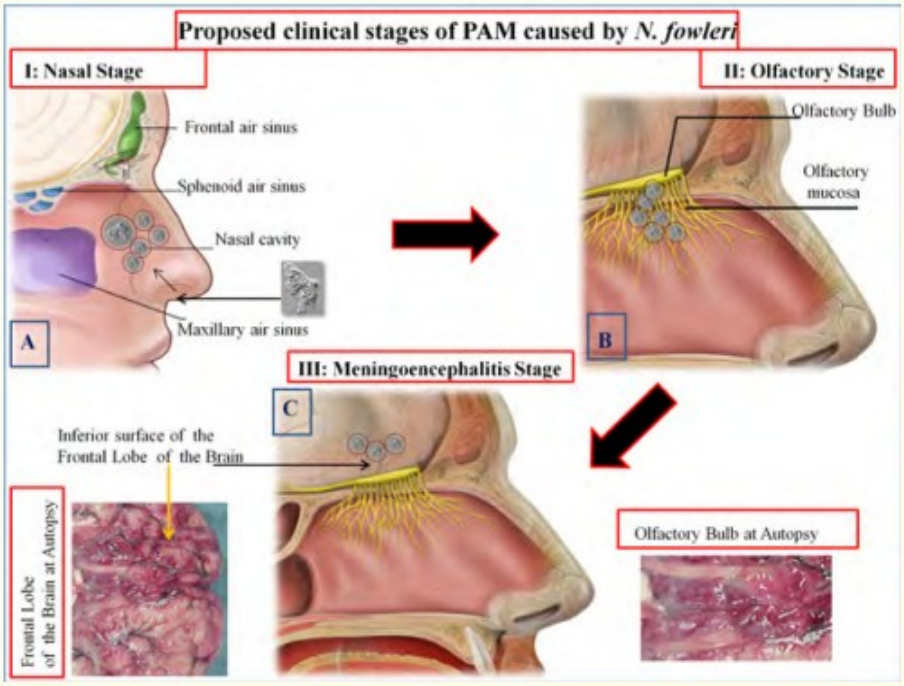

Figure 2: *Shows the proposed clinical staging of Primary Amoebic Meningoencephlitis (PAM). The different stages could help design diagnostic and therapeutic interventions at these stages. Note the nasal, olfactory and cerebral stages occur during the natural course of the disease and can herald signs that could help in rapid diagnosis and prompt treatment to improve the morbidity and mortality associated with PAM.*

Clinical Stage III: Meningoencephalitic stage

N. fowleri causes neuronal injury, both through direct cell damage, and by the release of cytotoxic proteins. Cellular debris and these proteins lead to an inflammatory response involving cytokine release, which mediates further

tissue damage. The final stage in the pathogenesis of *N. fowleri* infection is rapidly progressing meningo-encephalitis. The patient presents in this stage after 1 to 2 days from the olfactory stage described above. Herniation and cerebral edema occur due to a severe inflammatory response.

Diagnosis

Clinicians should have a high index of suspicion for PAM in the setting of acute meningoencephalitis with negative results for bacteria and common viruses, which should prompt the search for amoebae in the CSF. A history of exposure increases the likelihood of PAM, but it is not always obtained. In the current Kerala context, any person with an epidemiological link should be immediately suspected of having PAM.

The diagnosis of PAM is generally established via observation of motile trophozoites on examination of a CSF wet mount preparation immediately after obtaining the sample. A phase-contrast microscope is useful to optimize visualization of the amoeba. Trophozoites tend to lose motility and may resemble leukocytes. Giemsa or trichrome stains help in identifying morphologic features of the trophozoites: 10 to 25 microns in size with a single nucleus and a centrally located nucleolus with no peripheral chromatin.

A polymerase chain reaction (PCR) from the CSF facilitates rapid detection; it can identify the deoxyribonucleic acid (DNA) of *N. fowleri* and other free-living amoeba. *N. fowleri* can also be isolated in non-nutrient agar supplemented with enteric bacteria such as *E.coli* or Klebsiella. Serology is not useful for diagnosis.

The Centers for Disease Control and Prevention recommends microscopic examination of fresh, unfrozen, non refrigerated cerebrospinal fluid (CSF) for presumptive diagnosis. If amoebae are identified, the diagnosis should be confirmed through PCR.

Imaging findings on computed tomography (CT) and magnetic resonance imaging (MRI) are nonspecific. Brain edema, basilar meningeal enhancement, hydrocephalus, and areas of cerebral infarction have been described. Lesions in the brain tend to be located in the orbitofrontal and temporal lobes, base of the brain, cerebellum, and upper spinal cord. A purulent exudative inflammation

along the leptomeninges and extensive necrosis and hemorrhage of the parenchyma are characteristic pathological findings. Brain tissue can be examined for trophozoites, and brain sections can be stained with immunofluorescent anti-*N. Fowleri* antibodies

Differential diagnosis —

PAM must be distinguished from acute bacterial meningitis. The clinical manifestations and CSF findings of these two entities are similar. Epidemiological exposure may be useful in differentiating the two conditions. Usually, patients with PAM receive treatment for bacterial meningitis before the definitive diagnosis is established.

Treatment—

The optimal approach to treatment of PAM due to *N. fowleri* is uncertain; no studies evaluating the efficacy of single-drug or combination-drug regimens have been performed. The rarity of the disease, delay in diagnosis, fulminant clinical course, and the difficulties in making a rapid diagnosis have hampered the evaluation of drug regimens. In theory, the best drug regimen should include an amebicidal drug (or a combination of drugs) with good in vitro activity that is capable of crossing the blood-brain barrier.

Based on articles published following well-documented survivors as well as extrapolation from experience with treatment of infection due to *Acanthamoeba* and *Balamuthia* infection, the following combination of drugs (in addition to steroids to control cerebral edema) is expected to have maximum clinical efficacy.

●Conventional amphotericin B (1.5 mg/kg/day intravenously [IV] +/- intrathecally)

●Rifampin (rifampicin; 10 mg/kg/day orally in three divided doses or every 24 hours)

●Fluconazole (10 mg/kg/day IV or orally)

●Miltefosine (<45 kg: 50 mg orally twice daily; ≥45 kg: 50 mg orally three times daily)

●Azithromycin (500 mg IV or orally)

The optimal duration of therapy is uncertain; reports range from 9 to 30 days.

Amphotericin B deoxycholate demonstrates the most favorable in vitro activity (with minimum inhibitory concentrations [MICs] in the range of 0.018 to 1 mcg/mL) and is considered the drug of choice. Administration of amphotericin B is warranted as soon as the diagnosis is considered. Liposomal amphotericin B is less effective in animal models and has higher MICs than the conventional preparation. Acanthamoeba is not susceptible to Amphotericin B in in vitro studies.

Other active drugs in vitro include the azoles (fluconazole, voriconazole, ketoconazole, posaconazole, and itraconazole), rifampicin, miltefosine, and azithromycin. The in vitro activity of rifampicin is uncertain. Variations in virulence factors and sensitivity to antimicrobials have been postulated to explain clinical outcomes.

Approximately 11 survivors of PAM are reported in the literature. In most of these cases, an early diagnosis was made, and treatment with amphotericin B was initiated promptly; the most frequently used regimen was amphotericin B plus rifampicin (10 mg/kg/day). The synergy of amphotericin B with azoles (fluconazole 10 mg/kg/day) and rifampin has also been described. In one case, intrathecal administration of amphotericin B and miconazole was used. Another report documents five survivors in the United States. It is difficult to recommend a particular regimen but neuroprotective measures, reducing intracranial pressure, and hypothermia have been tried.

The use of miltefosine for the treatment of PAM has been extrapolated from favorable clinical experience with its use in the treatment of *Balamuthia mandrillaris* and *Acanthamoeba* spp infections.

Azithromycin has both animal and in vitro efficacy against *N. fowleri* and appears to be synergistic when administered with amphotericin B . Posaconazole has been found to inhibit the growth of *N. fowleri* in vitro within 12 hours, and experimental murine infection has been cured with posaconazole, suggesting that in the future this drug may replace fluconazole as the azole of choice; further study is needed to confirm these findings.

Table 3: Treatment of Confirmed Survivors of Primary Amebic Meningoencephalitis (n = 7)

Ref.	Country of Exposure	Year	Age (y)	Sex	Amphotericin B Route, Duration, days	Azole Route, Duration, days[a]	Azithromycin Route, Duration, days	Miltefosine Route, Duration, days	Rifampin Route, Duration, days	Dexamethasone Route, Duration, days	Symptom Onset to Start of Treatment, days
[19]	Australia	1971	14	M	IV (Unk.)[b] IT (Unk.)[b]	…	…	…	…	…	Unk.
[20]	United States	1978	9	F	IV (9) IT (10)	IV (9) IT (9)	…	…	PO (9)	IV (Unk.)[b]	3
[21]	Mexico	2003	10	M	IV (14)	IV/PO (30)[c]	…	…	PO (30)	IV (Unk.)[b]	0
[22]	United States	2013	12	F	IV (26) IT (10)	IV (26)	IV (26)	PO (26)	IV (26)	IV (4)	2
[23]	United States	2013	8	M	IV (19) IT (5)	IV (19)	PO (19)	PO (19)	PO (19)	IV (29)	5
[24]	Pakistan	2015	25	M	IV (Unk.)[b] IT (Unk.)[b]	Unk.[d] (Unk.)[b]	Unk.[d] (Unk.)[b]	PO (Unk.)[b]	IV (Unk.)[b]	…	3
N/A[e]	United States	2016	16	M	IV (14) IT (10)	IV (28)	IV (28)	PO (28)	IV/PO (28)[c]	IV (4)	2

Abbreviations: IT, intrathecal; IV, intravenous; N/A, not applicable; PO, oral; Unk., unknown.

[a]This included miconazole (n = 1 patient) and fluconazole (n = 5 patients).

[b]Duration of treatment unknown.

[c]Combination of intravenous and oral administration; specific duration of each route unknown.

[d]Route of administration unknown.

Table 4: Recommended Multidrug Regimen in Patients with PAM

Recommended treatment for PAM caused by *Naegleria fowleri* infection

A combination of medications is recommended for treatment of *Naegleria fowleri* infections. These drugs have been used in PAM survivors. Many have been found to have antiamebic activity against *Naegleria fowleri* in the laboratory.

Drug	Dose	Route	Maximum Dose	Duration	Comments
Amphotericin B*	1.5 mg/kg/day in 2 divided doses, THEN	IV	1.5 mg/kg/day	3 days	
	1 mg/kg/day once daily	IV		11 days	14-day course
Amphotericin B	1.5 mg once daily, THEN	Intrathecal	1.5 mg/day	2 days	
	1 mg/day every other day	Intrathecal		8 days	10-day course
Azithromycin	10 mg/kg/day once daily	IV/PO	500 mg/day	28 days	
Fluconazole	10 mg/kg/day once daily	IV/PO	600 mg/day	28 days	
Rifampin	10 mg/kg/day once daily	IV/PO	600 mg/day	28 days	
Miltefosine**	Weight<45 kg 50 mg BID Weight>45kg 50 mg TID	PO	2.5 mg/kg/day	28 days	50 mg tablets
Dexamethasone	0.6 mg/kg/day in 4 divided doses	IV	0.6 mg/kg/day	4 days	

*Conventional amphotericin (AMB) is preferred. When AMB was compared with liposomal AMB against *Naegleria fowleri*, the minimum inhibitory concentration (MIC) for AMB was 0.1 µg/mL, while that of liposomal AMB was 10x higher at 1 µg/mL. Liposomal AMB was found to be less effective in the mouse model and in in vitro testing than the more toxic form of AMB. AMB methyl ester was also found to be less effective in the mouse model. Because the prognosis of *Naegleria fowleri* infection is extremely poor, consider aggressive treatment.

Miltefosine is mildly nephrotoxic and the dosing may need to be adjusted for patients with impaired kidney function. However, few data are available

about the effective dose for amebic infection. The risk of nephrotoxicity should be balanced with the risk for death from PAM.

Acanthamoeba meningoencephalitis - a combination of Sulfadiazine + Pyrimethamine + Fluconazole + surgical resection of CNS lesion ± rifampicin. Intravenous/oral cotrimoxazole and flucytosine also may be tried.

Balamuthia mandrillaris

- Two cases: Flucytosine + Pentamidine + Fluconazole + Sulfadiazine + Azithromycin or Clarithromycin + surgical resection of CNS lesion
- One case: Pentamidine + Fluconazole + Sulfadiazine + Clarithromycin

Sappinia diploidea **(1 case)**

- Azithromycin + Pentamidine + Itraconazole + flucytosine + surgical resection of CNS lesion

Supportive treatment

Along with antimicrobial combination therapy, supportive therapy aimed at reducing intracranial pressure is equally important. In the survivors, along with antimicrobials, aggressive treatment for cerebral edema and the resulting elevated intracranial pressure with dexamethasone, CSF drainage via an external ventricular drain, hyperosmolar therapy with mannitol and 3% saline, moderate hyperventilation (goal PaCO2 30-35 mm Hg), and induced hypothermia (32–34°C) etc were administered. Consider the use of induced pentobarbital coma. The intracranial pressure goal is <20 mm Hg. Place nasogastric tube for miltefosine administration.

Raised ICP (suspect in altered sensorium, anisocoria, lateral rectus palsy, decorticate or decerebrate posturing, abnormal breathing pattern, hypertension etc.)

- Keep head in mid position and head end elevation 15-30 ^{0} to facilitate venous drainage.
- 3% saline 5ml/kg (especially if BP is low) or mannitol 2.5 to 5 ml per kg
- IF GCS <8 Intubate with Rapid Sequence Intubation raised ICP protocol

- Maintain euthermia/hypothermia [32-34°C], euglycemia, and normal Blood pressure.
- Avoid hypoxia and Hypercarbia.
- Adequate sedo-analgesia.

In case patient deteriorates: GCS < 8, features of raised ICP, Rapid fall in GCS, Intractable seizures
To consider necrotizing encephalitis with cytokine storm
May start pulse methylprednisolone 30mg/kg for 3 days*
Duration and dose of immunomodulators should be decided by institutional medical board
*Steroids/immunomodulators should be initiated after expert opinion from institutional medical board comprising of Paediatrician/Physician and Neurologist.

Prevention of amoebic meningoencephalitis

Measures to prevent primary amoebic meningoencephalitis include the following:

- Avoidance of diving and jumping into stagnant freshwater.
- Consider using nose plugs for unavoidable exposures or pinching your nose shut when diving or swimming in freshwater.
- Keep your head above water when swimming in freshwater, hot springs, and other untreated thermal bodies of water.
- When participating in water-related activities, avoid digging, or stirring up the sediment.
- Use boiled, filtered, or sterile water for nasal or sinus irrigation, not tap water.
- Wading pools should be emptied each day
- Swimming pools/water theme parks and spas should be kept clean, chlorinated and maintained correctly
- keep sprinklers and hoses away from noses.
- Flush still water from hoses before letting children play with them

If you are using unchlorinated water:

- Don’t let water go up your nose when showering or washing your face
- Watch children playing with hoses or sprinklers
- Teach children not to squirt water up their nose

Recommended SOP

- A history of nasal exposure to fresh water in the 14 days before symptom onset should be asked of any patient who presents with symptoms of acute meningitis.
- For patients with meningitis who have a history of recent nasal exposure to fresh water, the CSF specimen should undergo rapid testing for *N. fowleri/FLA* . Microbiologist should be immediately alerted about the clinical suspicion before sending CSF sample.
- In patients with clinical and CSF pictures suggestive of bacterial meningitis who are not responding to antibiotics or are rapidly deteriorating, consider PAM even in the absence of exposure to fresh water.
- CSF wet mount examination is to be done on all turbid/opalescent CSF samples.
- All cases diagnosed as PAM through CSF microscopy should be immediately initiated on the recommended multi-drug regimen and supportive therapy aimed at lowering intracranial pressure.
- All cases diagnosed by CSF microscopy should be immediately informed to DSO and the CSF sample should be sent to the reference lab for PCR and genomic sequencing [if needed].
- All cases of PAM should be treated by a multi-disciplinary team [Institutional Medical Board] comprising Physicians/ Paediatricians, intensivists, ID specialists, Neurologists and Microbiologists.
- Decisions on the use of intrathecal amphotericin B, immunomodulators, therapeutic hypothermia, pentobarbital protocol etc should be taken by the Institutional Medical Board.

Annexure A

Wet mount of CSF:

A wet-mount of the CSF should be examined immediately after collection under a microscope (preferably equipped with phase-contrast optics) for the presence of motile *Naegleria fowleri* /other *FLA* trophozoites.

1. Store CSF at room temperature (~25˚C) until examination. Do not refrigerate or freeze.
2. Gently agitate the CSF container to dislodge amoebae adhered to the container.
3. Apply a drop onto a microscope slide.
4. Observe cells for amoeboid movement(directional movements by means of blunt/bulbous pseudopodia) .

Wright stain - Apply a sample of the centrifuged CSF pellet (cytospin) to a slide and prepare a Wright stain using standard protocols. Look for the *N. fowleri*/FLA trophozoite, which can be differentiated from leukocytes by the nucleus that has a large, centrally-placed nucleolus.

References.

1. Clinical Care of *Naegleria fowleri* Infection, CDC USA.
2. Primary Amoebic Meningoencephalitis (PAM) Clinical Practice Guideline Johns Hopkins All Children's Hospital
3. Cope JR, Ali IK. Primary Amebic Encephalitis: What have we learned in the last 5 years? Curr Infect Dis Rep 2016;18:31.
4. Capewell LG, et al. Diagnosis, clinical course, and treatment of primary amoebic encephalitis in the United States, 1937-2013. J Ped Infec Dis Soc 2014;1-8.
5. Abdul Mannan Baig and Armaghan-e-Rehman. "Can Clinical Staging of Primary Amoebic Meningoencephalitis be of any Therapeutic Benefit?". *EC Microbiology* 5.3 (2017): 101-108.
6. Epidemiology and Clinical Characteristics of Primary Amebic Meningoencephalitis Caused by Naegleria fowleri: A Global Review Radhika Gharpure, John Bliton, Alexandra Goodman, Ibne Karim M. Ali, Jonathan Yoder, and Jennifer R. Cope: Clinical Infectious Diseases · May 2020