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Review Article

Can Clinical Staging of Primary Amoebic Meningoencephalitis be of any Therapeutic Benefit?

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Summary

Naegleria fowleri, is a free-living amoeba (FLA) known to infect humans and cause a fatal disease called primary amoebic meningoencephalitis (PAM). All of the patients are commonly admitted to the emergency room (ER). Often, treatment in the ER is delayed due to the rarity of disease leading to a delay in the diagnosis and late arrival of patients to the ER. The attempts to reduce raised intracranial pressure and subsequent herniation of the brain stem are challenging and become the cause of death in the affected patients during their stay in ER. Use of drugs like mannitol to reduce raised intra-cerebral pressure (ICP) could prove dangerous in the presence of cerebral haemorrhage and this fact could be overlooked during the management of patients with PAM. No precise therapy is followed for PAM, and most often a course of broad spectrum anti-protozoal drugs is employed. A CDC recommended drug miltefosine has show success in early diagnosed cases. So far, there is no clinical staging of PAM, and patients are managed for the complications that develop while their stay in the ER. Given the health scare associated with *N. fowleri* in countries with tropical climates, and its potential ability to cause severe meningoencephalitis that often progresses to lethal outcomes, we believe it is imperative to stage PAM into clear progressive stages to help its management in the ER and debate its therapeutic gains. Such a clinical staging could aid in efforts to diagnose and treat PAM. Furthermore, it will help in raising public awareness, in educating healthcare and allied personnel.

Keywords: *Amoebic Meningoencephalitis; Naegleria fowleri; Trophozoite*

Introduction

The genus *Naegleria* comprises of flagellate amoebas that are free-living protists and feed on bacteria. Of the entire genus, one species, *N. fowleri*, which is a free-living amoeba (FLA) is known to infect humans and cause a fatal disease called PAM. Other known FLA that produce a cerebral infection called granulomatous amoebic encephalitis (GAE), include *Balamuthia mandrillaris* and *Acanthamoeba* spp. [1,2].

PAM as a disease was first reported in 1965 [3]. It is rare, with less than 500 cases reported worldwide. No specific chemotherapy is available for *N. fowleri* infection, and most often broad spectrum antibiotics and antifungal are employed [4]. Often, treatment is delayed due to the rarity of disease leading to a delay in diagnosis, a mixed picture on biochemical analysis of CSF fluid and the fulminant nature of the disease [5]. The rise in intracranial pressure and subsequent herniation of the brain stem is usually the cause of death in the affected patients. Very few survivors are reported in medical literature, and those that do survive can present with neurological deficits on follow-up [6]. The only effective public health strategy so far to reduce the prevalence of *N. fowleri* in tap water has been chlorination of potable water, but careful quality control on the degree of chlorination is needed to successfully eradicate *N. fowleri* from the water supply [7].

Life Cycle of *N. folweri*

N. fowleri in the environment

N. fowleri exists as FLA in various morphological states (Figure 1A). The active replicating form is a trophozoite that can reproduce asexually. This 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 (Figure 1A) and withstand unfavorable conditions [8]. This enables the *N. fowleri* to survive cold temperatures, and nutritively hostile conditions.

Free-living trophozoite stage of *N. fowleri* and its detection

This form of the *N. fowleri* is seen to survive in warm waters at temperature ranges of 42-46°C. *N. fowleri* is a free-living parasite that has been reported in natural and artificial water bodies around the world (Figure 1B). *N. fowleri* has been found in sewage ponds, lakes, hot springs, rivers and swimming pools (Figure 1C). It has been shown to be present in both tropical as well as temperate climates. These water bodies may be utilized for recreational swimming and diving (Figure 1E), and thus serve as the likely source of infection for the majority of cases. Potable water supplies have also been documented to contain the *N. fowleri.* A recent study showed the presence of *N. fowleri* in 8% of water samples collected from various sites. This holds critical importance, as detailed later, and these water sources often form the basis of water ultimately destined for human consumption. This is the case in both developed settlements, where water is stored in under or above ground reservoirs, or in underdeveloped areas, where water reservoirs are often communal e.g. mosques, markets etc.

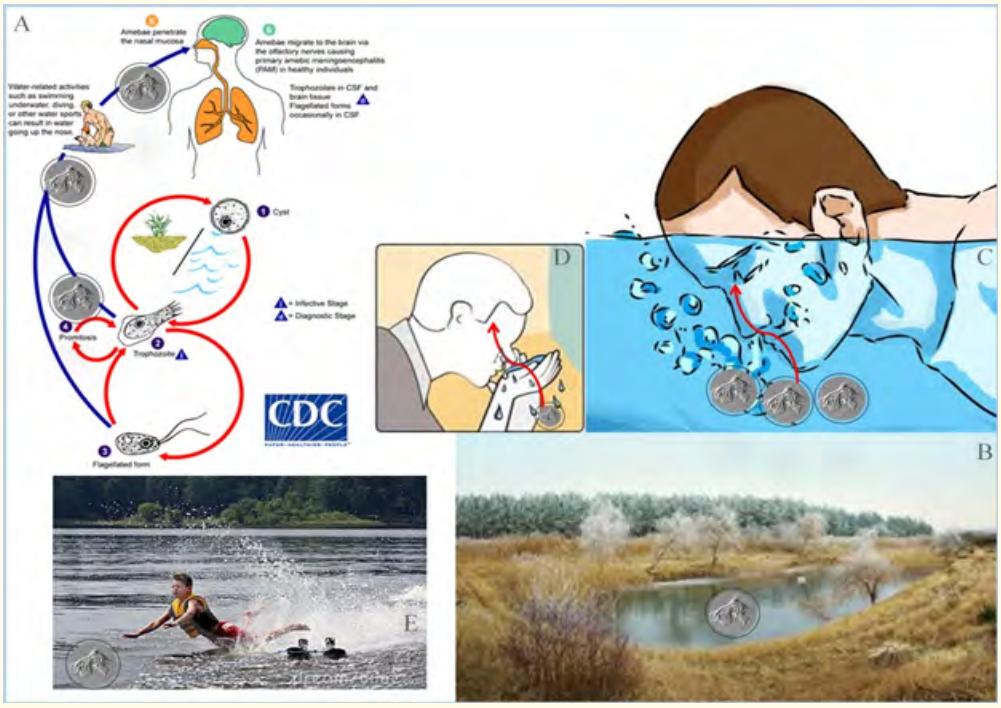

Figure-1: *Shows the life cycle and infection acquisition of Naegleria Fowleri (A). The organism lives in the form of a cyst, flagellate or trophozoite state. Freshwater lakes and ponds (B) in summers at high temperatures ~40OC favours the trophozoite forms of N. fowleri that can enter the nose during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

It is a highly phagocytic form of this pathogen that is responsible for the infection once it gets the chance to enter the nose [Figure1D]. Several methods exist to detect trophozoites of *N. fowleri* presence in environmental samples. Direct visualization under microscopy, aided by stains such as the Giemsa-Wright stain can be used to visualize amoebae in water and cerebrospinal fluid (CSF) samples. Amoebic antigens can also be detected via *N. fowleri* -specific antibodies using indirect immunoflorecence or immunohistochemistry techniques [9]. This technique can be employed for tissue and water samples suspected to contain *N. fowleri*. Polymerase Chain Reaction (PCR) techniques have been employed for the detection of *N. fowleri* nucleic acids in both tissue samples as well as in environmental samples [10-12]. This highly sensitive technique is helpful in samples where the amoebic concentration is low. Multiplex real-time PCR tests can perform rapid diagnostic assays within a few hours [10]. Non-nutrient media containing bacteria such as *E. coli* can be used to culture thermophilic protists such as *N. fowleri*. Specific testing with serological or PCR techniques can then confirm the presence of *N. fowleri* in the sample being tested [8].

Incidences and Prevention Strategies of PAM:

From 1962 - 2014, the CDC has reported 133 cases of *N. fowleri* infection [13,14]. The wide majority of these cases were described from temperate regions with a history of recreational swimming or diving, causing exposure of the patient to *N. fowleri*. However, documentation of cases in areas where a history of swimming was absent led to hypotheses that other practices may also be an additional risk factor [15]. Ablution practices involving the introduction of water into the deeper nasal passage (Figure 1D), as are employed in Ayurvedic, Yogic and the Islamic tradition of wudu (ablution) have also been hypothesized in several cases of *N. fowleri* infection, especially in cases where there was no history of the patient having partaken in swimming, or using neti pots [16].

The trophozoite stage is also important in planning the public interventions to reduce the prevalence of *N. fowleri* in water supplies used for human consumption. Chief among this is the chlorination of potable water provided to households, which must be overseen by an independent governing body that mandates and enforces standards for adequate water treatment. A possible intervention to prevent infection would be to discourage the practice of drawing water up the nose in ablution practices, especially in the summer months or in tropical climates. This would limit the risk of the protist coming into contact with nasal mucosa, and progressing to the next stage of infection. Another effective practice to combat the protist at this stage is the use of water that is either labelled sterile or has previously been boiled and then cooled for practices such as the use of neti pots, and in ablution. The use of nasal plugs and nose clips cannot be overemphasized as this completely eliminates the risk of infection. 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 [7]. The oral consumption of water contaminated with *N. fowleri* is not associated with symptomatic disease.

Evidence of different clinical stages of PAM:

Patients with PAM are mostly admitted in a pre-coma state, and no staging system currently exists to classify disease. With some of the recent cases of PAM presenting at an early stage of the disease, saving life has became possible (Florida hospital, News -2016). With almost all of the survivers of PAM in past 60 years, it appears that the disease was diagnosed and treated at a pre-cerebral stage. With greater awareness and public knowledge about the disease, cases are emerging that present in early stages of the infection, as detailed below. It appears to be very imperative to recognize these early staged *Naegleria* affected patients as an early diagnosis this could be life-saving, and beginning of an appropriate treatment could reduce the mortality seen in PAM patients.

Proposed Clinical Stages of *N. fowleri* infection:

Clinical Stage I: Nasal Stage (Pre-Crebral 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. Central to the understanding of the acquisition of *N. fowleri* at this stage is the fact that a deeper splash of contaminated water is needed for the trophozoite to enter into the nose in order to

cause the disease (Figure 2A). As this is a non-symptomatic nasal entry phase, the subsequent host-parasite interactions within the nasal cavity therefore cannot be studied. The human nasal cavity has been studded with vibrissae (thick hair) that cannot strangle *N. fowleri* because of being a microscopic organism. The nasal mucosa above these hairs also appear to have inadequate innate immune mechanisms to contain *N. fowleri* as evident by the fact that the pathogen proceeds towards the olfactory mucosa and olfactory bulb to establish the infection.

Diagnostic and Therapeutic Interventions:

As this stage is asymptomatic it is practically not possible to diagnose or treat the trophozoites that might have entered the nasal cavity (Figure 1C-D) (Figure 2A). With greater public awareness, if a patient who has accidentally fallen into the lake (as has happened in the case of the Ohio-USA patient in 2016) (Figure 1E) or water suspected of going deeper into the nose during ablution should be admitted to hospital with careful monitoring for the signs and symptoms of the disease for the next 48 - 72 hours. A nasal irrigation of such patients (Baig., *et al*. 2014) with saline could help obtain nasal fluid for tests and analysis mentioned above to diagnose the presence of a yet covert infection.

Clinical Stage II: Olfactory stage

The involvement of the olfactory mucosa and olfactory bulb is a transient stage before the involvement of the brain, which could be heralded by the changes in the olfaction sense (parosmia) that has been reported in some patients. 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 [17]. 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 exudate formation [17,18].

Diagnostic and Therapeutic Intervention:

The olfactory mucosal stage of infection occurs ahead of the involvement of the olfactory bulb for anatomical reasons., Parosmia and signs like apprehension and irritability if coupled with a history of swimming, ablution, use of neti pot and accidental fall in lakes with water splash into the nose should be an alert to admit the patient and begin treatment with anti-parasitic drugs without waiting for diagnosis. In cases of milder signs and symptoms, the diagnostic collection of fluid by nasal irrigation with saline or lumbar puncture should be done followed by treatment. If later the diagnosis turns positive, it could prove to be life saving and help in reducing the mortality rate in PAM.

Clinical Stage III: Meningoencephalitic stage

N. fowleri has been shown to cause neuronal injury, both through direct cell damage, and the release of cytotoxic proteins [19]. Cellular debris and these proteins lead to an inflammatory response involving cytokine release, which mediate further tissue damage and lead to a worsened neurological outcome (Figure 2C). The final stage in the pathogenesis of *N. fowleri* infection is a rapidly progressing meningoencephalitis disease known as PAM. The patient presents in this stage after an incubation time ranging from 1 to 2 days from the olfactory stage described above [4,20]. Usually, patients present with signs of meningitis such as a high fever, headache, nausea, projectile vomiting and altered sensorium. The Kerning and Brudzinski signs for meningeal irritation are found to be positive [19]. Radiological examinations reveal hydrocephalus and dilatation of ventricles, and the cisternae adjacent to the midbrain and the subarachnoid space may show a reduction in free space [5]. Herniation and cerebral edema occur due to a severe inflammatory response, and may present as photophobia and cranial nerve deficits [7]. The WBC count is markedly elevated, with leukocytosis seen in cerebrospinal fluid, along with increased protein levels, and reduced glucose levels. CSF examination under a phase contrast microscope reveals trophozoites when used with Giemsa-Wright stains [9]. Patients often deteriorate rapidly, with progressive cerebral deficits and worsening physical signs and symptoms. Death usually occurs less than five days after the onset of symptoms, with the most common cause of death being herniation secondary to cerebral edema [8].

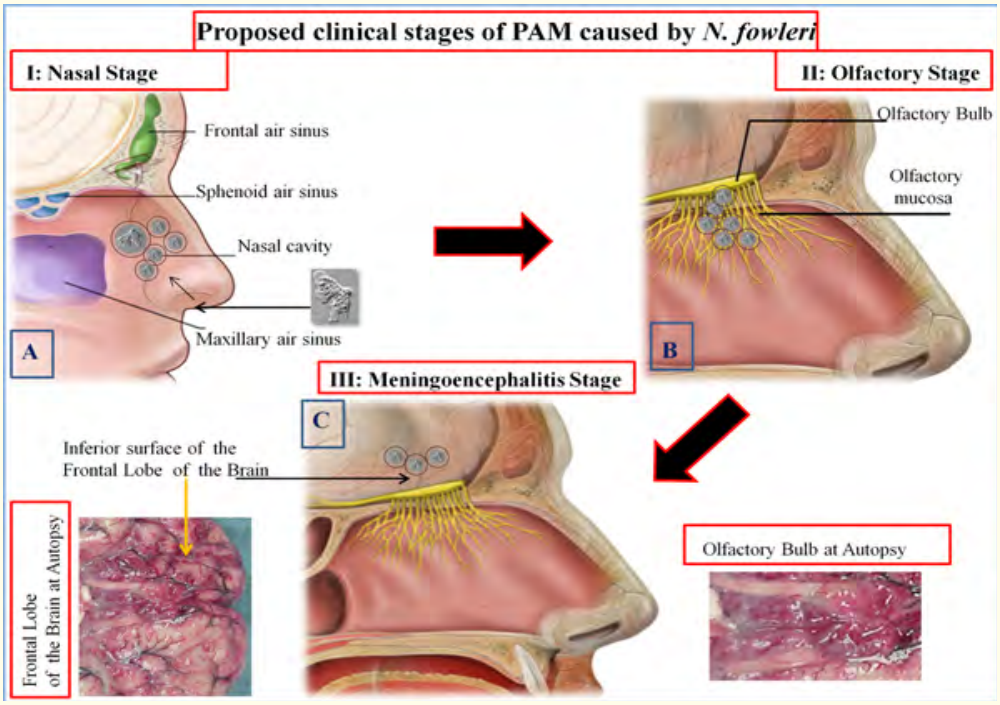

Figure 2: *Shows the proposed clinical staging of Primary Amoebic Meningoencephlitis (PAM). The different stages could help design diagnostic and therapeutic interventions at these stages. Note the nasal, olfactory and cerebral stages occur during the natural course of the disease and can herald signs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

Management of Meningoencephalitis stage

The current management of a patient with PAM involves a combination of antifungals, and antiprotozoals, given both through the intravenous and by intrathecal route. Azithromycin and amphotericin have been shown to be effective against *N. fowleri* in *in vitro* and mouse models, with conventional amphotericin shown to have a lower minimum inhibitory concentration (MIC) compared to liposomal amphotericin [21]. Further, a synergistic effect was reported between the two compounds against in vitro and mouse models of N. fowleri infection [22]. *In vitro* activity of miltefosine, an anti-cancer therapeutic agent, has been shown against *N. fowleri* as well [23]. In addition, miltefosine has been used in a drug combination to successfully treat a 12-year-old survivor [24]. Due to cerebral edema and herniation often accompanying meningoencephalitis disease, there is also a role for aggressive management of intracranial pressure (ICP). Dexamethasone has been successfully used in multiple survivors for lowering the ICP, and has been utilized in raised ICP in other infectious diseases as well [24].

Several novel agents have also been proposed as possible constituents of 'drug cocktails' to be administered to patients with *N. fowleri* infection. A study also demonstrated *in vitro* efficacy of procyclidine, an anti-cholinergic, digoxin, an Na+/K+ ATPase inhibitor, haloperidol, a dopamine antagonist, and amlodipine, a calcium channel blocker against *N. fowleri* isolates [25]. For drug delivery, a transcribrial device has been proposed that can be used potentially for drug delivery to the CNS, including drugs such as amphotericin B to have a better minimum inhibitory concentration (MIC), then when used by intravenous route [4]. In addition, since pro-inflammatory cytokines are abundant in PAM, anti-acute inflammatory drugs may be a useful adjunct in relieving neuronal loss and tissue damage associated with a hyperactive inflammatory response.

Steps that can prevent and improve the clinical outcome of PAM

The possibility of survival increases if there is an early diagnosis of disease and the patient receives medical attention promptly. The greatest hurdle in receiving care pertains to the long duration of symptoms before the patient receives any care. Unpublished CDC data suggest that the mean duration from initiation of symptoms to hospital admission is 2 days, which leads to often fatal outcomes due to the rapidly progressing nature of the disease. This duration is likely even higher in developing countries, and it is possible that many cases go unreported since the patient dies prior to receiving medical care. One solution is the widespread dissemination of public service messages in areas with a high incidence of *N. fowleri* infection, warning against recreational swimming in untreated water bodies, especially in the warm months. In addition, an early recognition of the signs and symptoms associated with meningoencephalitis can lead to fewer delays in seeking care, potentially increasing the chances of survival.

The second barrier to treatment is delayed diagnosis, and the non-availability of testing for the *N. fowleri*, as the infection frequently presents as similar to bacterial meningitis. Standard of care practice for suspected meningitis patients is a lumbar puncture (LP) with cerebrospinal fluid (CSF) examination. A CSF exam can reveal trophozoites under microscopy, and a PCR for *N. fowleri* can be performed in patients with early signs and symptoms consistent with meningoencephalitis.

Conclusion

The rate at which the aforementioned clinical stage progress from nasal to olfactory and finally to encephalitic stage is very fast, with an average window period of 24 - 72 hours in between them. A greater vigilance on history taking, anticipation of signs and symptoms in patients with an incidence of water entry into the nose, and alternate routes and methods of diagnosis in suspected patients could make this staging more worthy and help in saving lives of patients with PAM. Tackling *N. fowleri* infection requires a multi-faceted approach involving public authorities, hospital staff and allied healthcare personnel. There is a need to raise awareness among the public on the possible causes of *N. fowleri* infection, and the practices that can be adopted to reduce such occurrences. The public authorities need to ensure that potable water is treated before release to consumers, and that other sources of water, such as bored-wells are free of contamination. Proper training of allied and hospital healthcare staff can ensure an early diagnosis in patients with a high degree of suspicion of PAM, thus potentially increasing the possibility that early initiation of antimicrobial and adjunct therapy may result in patient survival. Our proposed staging can serve as the basis of public health education, and provide clinicians and para-medics with a plan to initiate a stage based treatment. The ability to categorize this infection can serve as a guideline to health professionals during diagnosis, and help when instituting therapy.

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