ISSN 2075-5457 (print), ISSN 2220-9697 (online)

DOI: 10.26565/2075-5457-2024-42-2
UDC: 579

Molecular mechanisms and therapeutic strategies of *Naegleria fowleri* Carter (1970): a review of the fatal brain-eating amoeba

N. Datta

Naegleria fowleri is a thermophilic free-living amoeba that can cause a rare and fatal infection of the brain called primary amoebic meningoencephalitis (PAM). PAM is a serious public health concern, as it affects mostly young and healthy individuals who are exposed to warm freshwater environments, and has a mortality rate of approximately 98%. The infection occurs when the amoeba enters the nasal cavity during swimming or other recreational activities, and migrates to the brain through the olfactory nerve. In the brain, the amoeba causes extensive tissue necrosis, haemorrhage, and inflammation, leading to severe neurological symptoms and death within days. The pathogenesis of *N. fowleri* infection is not fully elucidated, but recent studies have shed some light on the molecular mechanisms that enable the amoeba to invade, proliferate, and evade the host immune system. These mechanisms include the expression of various surface molecules that mediate adhesion, motility, and phagocytosis of host cells, as well as the secretion of proteases and other factors that degrade host extracellular matrix and modulate host immune response. However, there are still many unanswered questions regarding the complex interactions between the amoeba and its host, which limit the development of effective diagnostic and therapeutic strategies. PAM is often misdiagnosed as bacterial meningitis, due to its nonspecific clinical presentation and lack of reliable diagnostic tests. This results in delayed or inappropriate treatment and poor prognosis. Currently, there is no specific or approved treatment for PAM, and the available options are based on empirical evidence or case reports. The survival rate of PAM remains very low, despite the use of multiple drugs and supportive care. Therefore, there is an urgent need for more research on the pathogenesis of *N. fowleri* and the identification of novel targets for intervention. With the advances in genomic and proteomic technologies, new opportunities have emerged to explore the molecular biology of *N. fowleri* and its host response. By identifying the genes and proteins involved in key processes such as adhesion, motility, and immune evasion, researchers can design targeted therapies to disrupt these essential functions and prevent or treat infection. This review provides a comprehensive overview of the current state of knowledge on *N. fowleri*, its pathogenic molecular mechanisms, and the biological processes involved in its infection, as well as the challenges and perspectives for future research.

Key words: *Naegleria fowlers, primary amoebic meningoencephalitis, pathogenesis, life cycle, molecular mechanisms, host immune system, diagnosis.*

Cite this article: *Datta N. Molecular mechanisms and therapeutic strategies of Naegleria fowleri Carter (1970): a review of the fatal brain-eating amoeba. The Journal of V. N. Karazin Kharkiv National University. Series "Biology", 2024, 42, p. 22-31. https://doi.org/10.26565/2075-5457-2024-42-2*

About the author:
Datta N. – Asutosh College (Affiliated to University of Calcutta), Shyama Prasad Mukherjee Road, 92, Kolkata, West Bengal, India, 700026, neelabhdatta@gmail.com, https://orcid.org/0000-0002-1577-5461

Received: 16.01.2023 / Revised: 27.02.2024 / Accepted: 18.03.2024

Introduction

Primary Amoebic Meningoencephalitis (PAM) is caused by a parasite named *Naegleria fowleri*, which freely lives within the host and results in death (Siddiqui et al., 2016). An autopsy is usually the only way to diagnose and determine the cause of death since symptoms typically appear rapid and the host rapidly deteriorates (Jamerson et al., 2017). A key feature of PAM is the rapid deterioration of brain tissue caused by the secretion of cytolytic factors by the amoeba, such as phospholipase A, phospholipase B, neuraminidase, and elastase (Visvesvara et al., 2007). This is due to the amoeba's mechanical consumption of living cells, which causes the host brain tissue to be damaged (Visvesvara et al., 2007). PAM can cause headaches, stiff necks, fevers, and nausea, which are commonly mistaken for bacterial meningitis (Marciano-Cabral, 1988). After to 10 days the death of the host will occur in absence of treatment (Evdokiou et al., 2022). As a result of contact with contaminated freshwater, amoebas enter the nasal passages and attach to the nasal mucosa (Marciano-Cabral, 1988). In addition to migration up olfactory nerves, replication occurs within the frontal lobe via the cribriform plate (Siddiqui et al., 2016). The initiation of replication in the brain activates a strong immune response involving macrophages, neutrophils, and eosinophils (Jamerson et al., 2017). By secreting cytolytic factors and proteases, *N. fowleri* lyses cells

(Jamerson et al., 2017), causing hemorrhagic necrosis of the central nervous system (CNS). There have been several high-profile cases of *Naegleria fowleri* over the past five years that have engendered mass news information. As a result of mass news attention, public fear was produced rather than public education. The discussion below examines the biology, pathogenesis, mechanism of pathogenesis, pathophysiology and diagnosis options of *N. fowleri*.

Taxonomy

The ultrastructural characteristics of protozoan unicellular eukaryotes were used by Levine et al. (1980) to propose a classification system for this diverse group of organisms. However, this system was revised by Adl et al. (2005) based on molecular phylogeny, biochemical pathway analysis, and morphological criteria. Among the protozoan unicellular eukaryotes, the genus *Naegleria* has attracted considerable attention due to its medical importance and evolutionary significance. Several studies have focused on the molecular and genetic features of different *Naegleria* species, aiming to elucidate their phylogenetic relationships and taxonomic status (Martinez-Castillo et al., 2016). The origin and evolution of *Naegleria* have also been investigated by comparing the genome and transcriptome of *Naegleria gruberi*, a non-pathogenic species, with those of other eukaryotes (Fritz-Laylin et al., 2010). These studies have revealed that *Naegleria gruberi* possesses a complex molecular machinery and biochemical pathways that are shared with animals and fungi, suggesting that it represents an ancient lineage of eukaryotes. However, the taxonomy of *Naegleria* is still unresolved, especially for the pathogenic species *Naegleria fowleri* (De Jonckheere, 2011). It belongs to the phylum Percolozoa and is technically classified as a shape-shifting amoeboflagellate excavate, rather than a true amoeba. Although there are more than 40 species in the genus *Naegleria*, only *Naegleria fowler*i has been found to cause disease in humans. The identification and differentiation of *Naegleria fowleri* from other *Naegleria* species is challenging due to their morphological similarity and genetic variability.

History

Naegleria fowleri was first identified in 1965 by Fowler and Carter in Australia (Fowler, Carter, 1965). A year later, three fatalities were reported in Florida. It appeared that swimmers were the source of all cases in either Australia or Florida, since *Naegleria fowleri* thrives in warm and fresh water. In addition to United States tourists, *Naegleria fowleri* has also infected tourists from Japan, Italy, Thailand, New Zealand, Belgium, England, Californian hot springs, and United States freshwater lakes (Seidel et al., 1982). One must first appreciate the biology of *Naegleria fowleri*, its pathogenic mechanism, and the disease it causes, primary amoebic meningoencephalitis, in order to better comprehend the spread of this organism (Ladki et al., 2017). *Naegleria fowleri* species falls under the phylum Percolozoa. The microbe is a thermophylic free living organism that is most dynamic above 86°F and can endure temperatures over 113°F (Heggie, 2010). Thus, locals and tourists pursuing recreational water activities in the summer are most likely to find the amoeba at its peak during this time (Ladki et al., 2017).

Life cycle

The free-living amoeba *Naegleria fowleri* flourishes in freshwater sources such as lakes, ponds in hot climates (Marciano-Cabral, 1988). It has a complex life cycle that involves three morphological stages: (i) the trophozoite or amoeboid form, which is the active and feeding stage that reproduces by binary fission; (ii) the flagellate form, which is a temporary and non-feeding stage that develops in response to adverse environmental stimuli such as food depletion and enables the amoeba to swim and disperse; and (iii) the cyst form, which is a dormant and resistant stage that forms under harsh conditions such as low temperature, high osmolarity, or chemical stress (Siddiqui et al., 2016). The transition between these stages is regulated by environmental cues and molecular mechanisms that are not fully understood. The trophozoite stage is responsible for causing primary amoebic meningoencephalitis (PAM), a rare but fatal infection of the central nervous system (CNS) in humans and animals. The trophozoites are 10-35 μm in length, with a granular cytoplasm, a single nucleus with a prominent nucleolus, and various organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, and lysosomes (Khan et al., 2022). The trophozoites are usually the predominant form of *N. fowleri* in nutrient-rich environments, such as warm freshwater habitats or human body fluids. The flagellate form is 10-16 μm in length, with two anterior flagella and no visible organelles. The flagellate form is occasionally found in cerebrospinal fluid (CSF) of infected patients, but it is not considered to be pathogenic. The flagellate form can revert back to the trophozoite

form when favourable conditions return. The cyst form is 7-15 μm in diameter, with a smooth and thick cell wall, a single nucleus with a condensed chromatin, and a reduced cytoplasm. The cyst form is environmentally resilient and can survive for long periods of time until better conditions occur. The cyst form is not seen in brain tissue of infected patients and does not contribute to the pathogenesis of PAM. The process of forming cysts from trophozoites or flagellates is called encystation, while the process of forming trophozoites from cysts is called excystation (CDC, 2021). *Naegleria fowleri* infects humans or animals by entering the nose during water-related activities such as swimming or nasal irrigation. The trophozoites then penetrate the nasal mucosa and migrate along the olfactory nerves to reach the brain, where they cause extensive tissue damage and inflammation by feeding on neurons and glial cells, secreting proteases and phospholipases, inducing apoptosis and necrosis, and eliciting immune responses (microbeonline.com, 2022). The infection usually results in death within one to two weeks after exposure.

The pathogenicity of *N. fowleri* is associated with its trophozoite stage, which is the only form detected in brain tissue of infected patients and never as a cyst. A good strategy against PAM includes finding drugs that can kill the parasite selectively and effectively as well as those that induce encystation. A compound that induces dormancy can be used to develop novel therapeutic strategies since *N. fowleri* is inactive once encysted. Killing the parasite is much tougher than bringing its phenotypic transformation. Exposure to physiological, chemical, environmental and radiological changes, for example, can induce encystation, as opposed to the high concentrations of drugs used to kill the parasite, which can cause damage to host cells. In other words, any circumstance that encourages receptor conformational changes will trigger encystation. External stimuli may also affect cell surface receptors, causing them to undergo structural and conformational changes. Since *Naegleria* are not a threat once encysted, it could be extremely useful to identify compounds that can induce *Naegleria* encystation as a means of developing new therapeutic strategies. The proposed strategy could facilitate alternative therapeutic measures and/or immunity-driven extermination of the parasite in light of the acute nature of PAM that can lead to death within a few days. By inducing encystation in *N. fowleri*, virulent amoebas will be altered into avirulent forms and infection will be delayed. Virulent amoebae can be made dormant by using ligands (molecules, in addition to antibodies or osmolarity) that induce conformational changes that cause cyst formation (Khan et al., 2022). The large molecular mass of antibodies makes it improbable for them to cross the blood-brain barrier, so substances that can pass the barrier would be required to reach the infection site in the CNS and elicit encystation. The use of this method can be used to design combinatorial chemotherapies against this devastating infection if small molecules that can pass through the blood–brain barrier are identified. It may be possible to identify specific receptors involved in the differentiation process using differential gene expression analysis using RNA-seq in response to encystation (Khan et al., 2022). To determine an appropriate treatment regimen against brain-eating amoebas, a range of chemical libraries would need to be tested using bioassay-guided testing, along with a thorough understanding of their structure, composition, and permeability. In order to succeed in these prospects, rigorous research must be conducted.

Pathogenesis

As an amphizoic amoeba, *N. fowleri* can live spontaneously in water, soil, or the host in the central nervous system (CNS) of humans (De Jonckheere, 2011). Water activities, including swimming and diving, have been associated with *N. fowleri* infections in healthy children and adults. When water sploshes or wallows itself into the nasal cavity, *N. fowleri* is believed to enter the human body through the nose (Grace et al., 2015). As the infection enters the CNS, it attaches to the nasal mucosa, traverses the olfactory nerve to reach the olfactory lobes, and then travels through the cribriform plate (which is more porous in children and young adults (Jarolim et al., 2000). By activating macrophages and neutrophils in the olfactory lobes, *N. fowleri* induces a substantial immune response in the human body (Marciano-Cabral, Cabral, 2007). It enters the body as trophozoites. The pathogenicity of *N. fowleri* is also reliant on the release of cytolytic molecules that damage nerves and host cells (De Jonckheere, 2011). These molecules, including acid hydrolases, phospholipases, and neuraminidase, spread throughout the body. The adherence of the pathogen to its host cell is one of the most serious steps in any microbial infection (Güémez, Garcia, 2021). Pathogenic amoebas like *N. fowleri* have been found to have higher attachment levels. According to studies conducted in vitro, *N. fowleri* is capable of attaching to basement membrane components such as collagen I, laminin-1, and fibronectin to move through the nasal epithelium (Jamerson et al., 2012). They have been found to be capable of adhesion by co-localizing integrin-like proteins with actin filaments and binding

fibronectin (Jamerson et al., 2012). In addition, kinases C found in *N. fowleri* have been shown to enhance amoebic adhesion and cytotoxicity towards host cells (Jamerson et al., 2012). In theory, *N. fowleri* adhering to extracellular matrix constituents could trigger signalling transduction pathways that activate protein and protease expression that facilitate amoeba entry and multiplying into the CNS (Güémez, Garcia, 2021). A group of proteins associated with cytoskeletal reorganisation and stability was found in mice when they were passed through their brains, making them almost 100-fold more virulent (Güémez, Garcia, 2021). *N. fowleri*, which is highly virulent, contains a protein homologous to Rho guanine nucleotide exchange factor 28, which proposes that cytoskeletal rearrangement and stabilisation are important for pathology of the amoeba (Güémez, Garcia, 2021; Jamerson et al., 2017). Several studies have indicated that the amoeba's locomotion and pathogenesis are affected by the Nfa1 gene, which encodes for the Nfa1 protein (Güémez, Garcia, 2021). The use of anti-Nfa1 antibodies has been shown to decrease the amoeba's cytotoxic effects, suggesting that this protein plays an essential role in its pathogenesis (Kang et al., 2005). In addition, transfection of *N. fowleri's Nfa1* gene into nonpathogenic *N. gruberi* heightened cytotoxicity against Chinese hamster ovary cells (CHO), as equated with naive *N. gruberi* (Jeong et al., 2005; Güémez, Garcia, 2021). The amoeba has also been shown to contain a protein encoded by the *Nf*-actin gene in its cytoplasm, and pseudopodia which has been linked to increased cell adhesion, phagocytosis, and cytotoxicity (Sohn et al., 2019). *N. fowleri's* pathogenicity united with the concentrated immune response that follows its presence results in significant nerve and CNS tissue damage, which often leads to fatal conditions.

Mechanisms of pathogenicity

One of the crucial steps in the pathogen invasion process is adhesion, which involves the recognition and binding of specific molecules on the surface of *N. fowleri* to the host cells. Several studies have attempted to identify the adhesion molecules that *N. fowleri* uses to attach to the host cells, such as a 60 kDa integrin-like protein on its outer membrane (Han et al., 2004), and various extracellular matrix proteins, such as collagen type I, fibronectin, and laminin-I (Cervantes-Sandoval et al., 2010). Moreover, it has been shown that *N. fowleri* and *N. gruberi* differ in their expression of carbohydrates, and that mannose residues are essential for *N. fowleri* to adhere to the mouse nasal mucosa (Carrasco-Yepez et al., 2013). Another important factor in the pathogenesis of *N. fowleri* is the secretion of proteases, which are enzymes that degrade proteins and other macromolecules. Proteases play a role in the development of PAM, even though *N. fowleri* is not considered a strict parasite, by causing tissue damage and inflammation in the olfactory epithelium and the brain (Martinez et al., 1971; Chang, 1979). The first protease secreted by *N. fowleri* that was isolated and characterized was a 30 kDa cysteine protease, which exhibited cytopathic effects on BHK cells and was inhibited by Z-Phe-Ala fluoromethyl ketone (Aldape et al., 1994). Subsequently, other proteolytic proteins were identified, such as naegleriapores A and B (N-A and N-B), which are harmful to human cells and are processed by cysteine proteases (Martinez-Castillo et al., 2016). Additionally, two cysteine proteases, cathepsin B and cathepsin-B-like, were cloned and purified, and were found to degrade several human substrates, such as immunoglobulins, collagen, fibronectin, haemoglobin, and albumin (Lee et al., 2007). These findings suggest that *N. fowleri's* proteases may be potential targets for chemotherapeutic drugs to treat this infection (McKerrow et al., 2008). Furthermore, two other pathogenic processes that are associated with *N. fowleri's* ability to penetrate the CNS are dynamic locomotion and phagocytosis, which enable the amoeba to move and engulf a variety of host cells, such as erythrocytes, microglia, and neuroblastoma cells (Martinez-Castillo et al., 2016). A link between phagocytosis and Nf-actin was established by a study, indicating that actin polymerization is involved in the phagocytic activity of *N. fowleri* (Lee et al., 2007).

The phagocytosis of human cells by *N. fowleri* involves the formation of phagosome-cup structures, also known as amoebostomes, where a specific protein is predominantly localized, along with the cytoplasm and pseudopodia of the amoeba. *N. fowleri* trophozoites can also induce contact-independent lysis of human cells by secreting proteins that disrupt the cell membrane and reduce the viability of human microglial cells, as evidenced by morphological changes observed in a non-contact system (Kim et al., 2008; Martinez-Castillo et al., 2016). Moreover, the non-contact system showed a higher percentage of apoptotic cells (16%) compared to the *N. fowleri* lysates (Kim et al., 2008). Another important molecule that has been identified is Nf-cHSP70, which is hypothesized to have a protective role for the amoeba against environmental stress, especially high temperature. Nf-cHSP70 has also been associated with the pathogenicity and growth of *N. fowleri* (Song et al., 2007). Furthermore, *N. fowleri* can evade the immune response of the host by avoiding immunoglobulins (IgA and IgG) through capping mechanisms and by

resisting complement-mediated lysis through the expression of a "CD59-like" protein (Martinez-Castillo et al., 2016; Ferrante, Thong, 1979). All these factors contribute to the virulence of *N. fowleri* and its ability to cause primary amoebic meningoencephalitis (PAM), a fatal central nervous system infection. More research on this amoeba is needed to elucidate the physiopathology of PAM and to develop effective strategies for its prevention and treatment.

Pathophysiology and Clinical Features

Primary amoebic meningoencephalitis (PAM) is a fatal central nervous system (CNS) infection caused by the free-living amoeba *Naegleria fowleri*. The infection occurs when the amoeba enters the nasal cavity under pressure, such as during swimming or diving in warm freshwater, or through the use of contaminated water in a neti pot. The amoeba then invades the respiratory epithelium and olfactory mucosa, and reaches the CNS through the cribriform plate (Jarolim et al., 2000). Once in the brain, *N. fowleri* causes extensive damage to the brain parenchyma, resulting in cortical haemorrhages, tissue necrosis, and oedema. The most affected regions are usually the cerebellum, olfactory bulbs, and a basilar region of the frontal cortex. The infection also triggers a strong innate immune response, which contributes to the inflammation and tissue destruction (Marciano-Cabral, Cabral, 2007). The pathogenicity of *N. fowleri* is mediated by several factors, such as nitric oxide production, the protein Nfa1, pore-forming proteins, cysteine proteases, phospholipases, and phospholipolytic enzymes. These factors enable the amoeba to adhere to and phagocytose target cells, secrete cytolytic molecules that disrupt the cell membrane, and evade the host immune response by avoiding immunoglobulins and complement-mediated lysis (Pervin, Sundareshan, 2022; Martinez-Castillo et al., 2016). The clinical manifestations of PAM are usually evident within the first week of infection. However, PAM can be difficult to differentiate from other forms of meningitis due to the lack of characteristic clinical features. The initial symptoms include anorexia, vomiting, irritability, photophobia, diplopia, lethargy, seizures and coma. These are followed by severe headaches, high fevers, and neck stiffness. Death ensues between the third and seventh days after symptom onset (Martinez-Castillo et al., 2016). The post-mortem examination of PAM patients reveals severe tissue mutilation in the area of infiltration, along with ulceration of the olfactory mucosa and necrosis of the olfactory nerves (Visvesvara et al., 2013). There is also extensive haemorrhaging from necrotic blood vessels, and a considerable amount of amoeba invasion is observed in the adjacent frontal cortex via microscope (Hannisch, Hallagan, 1997). The pathogenesis of PAM poses several challenges for diagnosis, treatment, and prevention. The early symptoms of PAM are nonspecific and can be confused with other CNS infections, leading to delays in diagnosis and treatment. The current treatment for PAM consists of aggressive supportive care and the administration of amphotericin B, an antifungal drug that has limited efficacy and high toxicity. Most patients die within days of symptom onset. New potential treatments are being investigated, such as miltefosine, an oral agent that has shown some activity against other pathogenic amoeba in vitro and in vivo (Debnath, 2021; Cope et al., 2016). The pathogenesis of PAM is also poorly understood at the molecular level. The specific mechanisms by which *N. fowleri* causes disease are not known, and more research is needed to elucidate them. For example, it is not clear how the amoeba evades the host immune response or how it causes the inflammation and destruction of brain tissue. A deeper understanding of these mechanisms could potentially lead to the development of new treatments for PAM. This could lead to the development of new therapeutic targets and strategies for PAM. Moreover, the prevention and control of PAM is difficult due to the widespread presence of *N. fowleri* in various water sources and its ability to survive in high-saline environments. The risk of infection can be reduced by avoiding activities that expose the nose to water pressure or sediment in warm freshwater bodies (Cooper et al., 2019; Ropar et al., 2013).

Diagnosis

Symptoms and signs of infection with *N. fowleri* usually appear between two and eight days after infection, although some cases have been reported within 24 hours. Computerized tomography (CT) and magnetic resonance imaging (MRI) examinations of the brain generally show cerebral oedema, sulcal effacement, and cisternal obliteration near the midbrain and subarachnoid space during the initial stages of infection. These conditions usually deteriorate as the infection develops, illuminating necrotic areas, stenosis, and aneurysms (Güémez, Garcia, 2021). There are no precise signs or symptoms that indicate *N. fowleri* infection, but the most common symptoms are headaches, fevers, chills, Brudzinski signs, Kernig signs, photophobia, confusion, seizures, and possible comas (Grace et al., 2015). A few cases have also

been reported with anomalous cardiac rhythms and myocardial necrosis (Martinez, 1985). A direct relationship has been found between death and an increase in intracranial pressure and cerebral spinal fluid pressure (CSF) (600 mm H_2O pressure or higher). The presence of polymorphonuclear leukocytes and *N. fowleri* trophozoites in cerebrospinal fluid (CSF) can be definitively identified with a lumbar puncture (Siddiqui et al., 2016).

Various irregularities in the colour of CSF have been found, fluctuating from grey in premature stages of infection to red in late stages due to an increase in red blood cells to about 24,600/mm^3 (Hebbar et al., 2005). Midbrain and subarachnoid spaces are two areas of the brain where magnetic resonance imaging (MRI) often shows irregularities (Martinez, 1985). CSF can be used to nurture *N. fowleri* by adding a few drops to a non-nutrient agar plate seeded with live or heat-killed bacteria. In addition to be autoclaved for 25 min at 121°C, sterile, heat-inactivated fetal calf serum should always be incorporated into the medium before use. Augmenting the medium with 1% peptone also progresses amoeba growth (Zaongo et al., 2018). Trophozoites grow within three days of being incubated at 37°C, and they instigate to form encyst after seven to ten days as their food source declines (Zaongo et al., 2018). The flagellation process of transforming the trophozoites into flagellates can help distinguish *N. fowleri* from other pathogenic amoebae occurs by mixing one drop of amoeba culture or sedimented CSF with one mL of distilled water during 1-2 hours (Siddiqui et al., 2016). Growing *N. fowleri* on solid non-nutrient agar has many advantages, but there are a few limitations, including bacterial contamination and a lower yield of cysts (Güémez, Garcia, 2021). To induce encystment, cells are washed twice in PBS (pH 7.4) followed by incubation in the encystment medium in 24-well plates at 37°C for 20 hours. It may be possible to check the manifestation of the amoeba using microscopy, immunofluorescence assays (IF), enzyme-linked immunosorbent assays (ELISAs), or flow cytometry (FC) (Siddiqui et al., 2016). It is also acclaimed to perform a reverse transcription polymerase chain reaction (RT-PCR) to identify the species and genus of the amoeba (Bellini et al., 2018). There is also a wide range of white blood cell concentrations (WBC) ranging from 300 to 26,000 mm3, whereas protein concentrations can be between 100 mg/100 mL and 1000 mg/100 mL, while glucose levels can be between 10 mg/100 mL and less than 10 mg/100 mL (Visvesvara et al., 2007). The amalgamation of clinical features and a history of contact with water confirm infection with *N. fowleri*. To increase the chance of survival, the patient must obtain medical treatment promptly (Siddiqui et al., 2016).

Pathological changes in brain due to *N. fowleri* infections

Brain hemispheres with *N. fowleri* infection are often mushy, noticeably enlarged, oedematous, and very congested. The leptomeninges (arachnoid and pia mater) have little purulent exudate inside sulci, the base of the brain, brainstem, and cerebellum but are severely clotted, diffusely hyperemic, and opaque. The olfactory bulbs typically have purulent secretions around them and exhibit haemorrhagic atrophy (Zumla, 2010). There are also many peripheral haemorrhage patches visible in the cortex. The base of the orbitofrontal and temporal lobes, the hypothalamus, the midbrain, the pons, the medulla oblongata, and the upper part of the spinal cord are where the majority of lesions are located (El-Maaty, Hamza, 2012). The subarachnoid space above the cerebral hemispheres and the cisternae around the midbrain may have been destroyed, according to CT images. Following intravenous contrast medium delivery, these areas may exhibit considerable diffuse augmentation (Visvesvara et al., 2007). Microscopically, fibrino-purulent leptomeningeal exudate is found throughout the cerebral hemispheres, brain stem, cerebellum, and upper section of the spinal cord. This effusion is primarily composed of PMNs, with a small number of eosinophils, macrophages, and lymphocytes. It is common to see clusters of many amoebic trophozoites in necrotic and oedematous neural tissue, frequently without PMNs (El-Maaty, Hamza, 2012). Trophic amoebae are additionally discovered deep within Virchow-Robin gaps, typically near blood arteries and devoid of any acute inflammation. Particularly lacking are the amoebic cysts (Visvesvara et al., 2007).

Conclusion

Naegleria fowleri is a free-living amoeba that can cause primary amoebic meningoencephalitis (PAM), a rare but fatal infection of the central nervous system. This infection occurs when *N. fowleri* enters the nasal cavity through contaminated water and reaches the brain through the olfactory nerves. The pathogenesis of PAM involves the destruction of brain tissue by *N. fowleri* and the inflammatory response of the host. Several factors have been identified that influence the transmission, proliferation, and virulence of *N. fowleri*, such as environmental conditions, host susceptibility, and molecular mechanisms. However, many aspects of the biology and pathogenicity of *N. fowleri* remain unknown, and more research is needed

to elucidate them. The diagnosis and treatment of PAM are challenging due to the nonspecific symptoms, rapid progression, and limited efficacy of available drugs. Therefore, there is an urgent need to develop new strategies for prevention and treatment of PAM, such as improved surveillance, rapid detection, novel therapeutics, and vaccines. To verify the diagnosis, methods based on molecular biology are suggested. Increasing incidence of PAM in both developed and underdeveloped countries is a serious public health concern that requires awareness and action from the health community. In the past 50 years, significant advances have been made in understanding the biology and pathogenesis of *N. fowleri*, which could facilitate the development of new interventions to combat this deadly infection. However, further research is required to overcome the challenges posed by this emerging pathogen.

References

Adl S.M., Simpson A.G., Farmer et al. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *The Journal of Eukaryotic Microbiology, 52(5),* 399–451. https://doi.org/10.1111/j.1550-7408.2005.00053.x

Aldape K., Huizinga H., Bouvier J., Mckerrow J. (1994). *Naegleria fowleri*: characterization of a secreted histolytic cysteine protease. *Experimental Parasitology, 78(2),* 230–241. https://doi.org/10.1006/expr.1994.1023

Bellini N.K., Santos T.M., da Silva M.T.A. (2018). The therapeutic strategies against *Naegleria fowleri*. *Experimental parasitology, 187,* 1–11. https://doi.org/10.1016/j.exppara.2018.02.010

Cooper A.M., Aouthmany S., Shah K., Rega P.P. (2019). Killer amoebas: Primary amoebic meningoencephalitis in a changing climate. JAAPA. *Official Journal of the American Academy of Physician Assistants, 32(6)*, 30–35. https://doi.org/10.1097/01.JAA.0000558238.99250.4a

Carrasco-Yepez M., Campos-Rodriguez R., Godinez-Victoria M. et al. (2013). *Naegleria fowleri* glycoconjugates with residues of α-d-mannose are involved in adherence of trophozoites to mouse nasal mucosa. *Parasitology Research, 112(10)*, 3615–3625. https://doi.org/10.1007/s00436-013-3549-2

Cervantes-Sandoval I., Serrano-Luna J., Judith Pacheco-Yépez et al. (2010). Differences between *Naegleria fowleri* and *Naegleria gruberi* in expression of mannose and fucose glycoconjugates. *Parasitology Research, 106*, 695–701. https://doi.org/10.1007/s00436-010-1727-z

Chang S.L. (1979). Pathogenesis of pathogenic *Naegleria amoeba*. *Folia Parasitologica, 26(3),* 195–200.

Cope J.R., Conrad D.A., Cohen N. et al. (2016). Use of the novel therapeutic agent miltefosine for the treatment of primary amebic meningoencephalitis: report of 1 fatal and 1 surviving case. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America, 62(6),* 774–776. https://doi.org/10.1093/cid/civ1021

Debnath A. (2021). Drug discovery for primary amebic meningoencephalitis: from screen to identification of leads. *Expert Review of Anti-infective Therapy, 19(9)*, 1099–1106. https://doi.org/10.1080/14787210.2021.1882302

De Jonckheere J.F. (2011). Origin and evolution of the worldwide distributed pathogenic amoeboflagellate *Naegleria fowleri*. *Infection, Genetics and Evolution, 11(7),* 1520–1528. https://doi.org/10.1016/j.meegid.2011.07.023

El-Maaty D.A., Hamza R.S. (2012). Primary Amoebic Meningoencephalitis caused by *Naegleria fowleri*. *Pakistan University Journal, 5(2),* 93–104.

Evdokiou A., Marciano-Cabral F., Jamerson M. (2022). Studies on the cyst stage of *Naegleria fowleri in vivo* and *in vitro*. *The Journal of Eukaryotic Microbiology, 69(2)*, e12881. https://doi.org/10.1111/jeu.12881

Ferrante A., Thong Y.H. (1979). Antibody induced capping and endocytosis of surface antigens in *Naegleria fowleri*. *International Journal for Parasitology, 9(6)*, 599–601. https://doi.org/10.1016/0020-7519(79)90018-3

Fowler M., Carter R.F. (1965). Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. *British Medical Journal, 2(5464),* 734. https://doi.org/10.1136/bmj.2.5464.734-a

Fritz-Laylin L.K., Prochnik S.E., Ginger M.L. et al. (2010). The genome of *Naegleria gruberi* illuminates early eukaryotic versatility. *Cell, 140(5)*, 631–642. https://doi.org/10.1016/j.cell.2010.01.032

Grace E., Asbill S., Virga K. (2015). *Naegleria fowleri*: pathogenesis, diagnosis, and treatment options. *Antimicrobial Agents and Chemotherapy, 59(11)*, 6677–6681. https://doi.org/10.1128/AAC.01293-15

Güémez A., García E. (2021). Primary Amoebic Meningoencephalitis by *Naegleria fowleri*: pathogenesis and treatments. *Biomolecules, 11(9)*, 1320. http://dx.doi.org/10.3390/biom11091320

Han K.L., Lee H.J., Shin M.H. et al. (2004). The involvement of an integrin-like protein and protein kinase C in amoebic adhesion to fibronectin and amoebic cytotoxicity. *Parasitology Research, 94(1)*, 53–60. https://doi.org/10.1007/s00436-004-1158-9
Hannisch W., Hallagan L.F. (1997). Primary amoebic meningoencephalitis: a review of the clinical literature. *Wilderness Environmental Medicine, 8(4),* 211–213. https://doi.org/10.1580/1080-6032(1997)008[0211:pamaro]2.3.co;2
Hebbar S., Bairy I., Bhaskaranand N. et al. (2005). Fatal case of *Naegleria fowleri* meningo-encephalitis in an infant: case report. *Annals of tropical paediatrics, 25(3)*, 223–226. https://doi.org/10.1179/146532805X58166
Heggie T.W. (2010). Swimming with death: *Naegleria fowleri* infections in recreational waters. *Travel Medicine and Infectious Disease, 8(4)*, 201–206. https://doi.org/10.1016/j.tmaid.2010.06.001
Jamerson M., da Rocha-Azevedo B., Cabral G.A., Marciano-Cabral F. (2012). Pathogenic *Naegleria fowleri* and non-pathogenic *Naegleria lovaniensis* exhibit differential adhesion to, and invasion of, extracellular matrix proteins. *Microbiology, 158(3)*, 791–803. https://doi.org/10.1099/mic.0.055020-0
Jamerson M., Schmoyer J.A., Park J. et al. (2017). Identification of *Naegleria fowleri* proteins linked to primary amoebic meningoencephalitis. *Microbiology, 163(3),* 322–332. https://doi.org/10.1099/mic.0.000428
Jarolim, K. L., McCosh, J. K., Howard, M. J., John D.T. (2000). A light microscopy study of the migration of *Naegleria fowleri* from the nasal submucosa to the central nervous system during the early stage of primary amoebic meningoencephalitis in mice. *The Journal of Parasitology, 86(1)*, 50–55. https://doi.org/10.1645/0022-3395(2000)086[0050:ALMSOT]2.0.CO;2
Jeong S.R., Lee S.C., Song K.J. et al. (2005). Expression of the nfa1 gene cloned from pathogenic *Naegleria fowleri* in nonpathogenic *N. gruberi* enhances cytotoxicity against CHO target cells in vitro. *Infection and Immunity, 73(7),* 4098–4105. https://doi.org/10.1128/IAI.73.7.4098-4105.2005
Kang S.Y., Song K.J., Jeong S.R. et al. (2005). Role of the Nfa1 protein in pathogenic *Naegleria fowleri* cocultured with CHO target cells. *Clinical and Vaccine Immunology, 12(7),* 873-876. https://doi.org/10.1128/CDLI.12.7.873-876.2005
Khan N.A., Muhammad J.S., Siddiqui R. (2022). Brain-eating amoebae: is killing the parasite our only option to prevent death? *Expert Review of Anti-infective Therapy, 20(1)*, 1–2. https://doi.org/10.1080/14787210.2021.1927712
Kim J.H., Kim D., Shin H.J. (2008). Contact-independent cell death of human microglial cells due to pathogenic *Naegleria fowleri* trophozoites. *The Korean Journal of Parasitology, 46(4)*, 217–221. https://doi.org/10.3347/kjp.2008.46.4.217
Ladki S.M., Samad J.A. (2017). Summer recreational water tourism: a deadly human encounter with *Naegleria fowleri*. *Journal of Tourism & Hospitality, 6(5)*, 306. https://doi.org/10.4172/2167-0269.1000306
Lee Y.J., Kim J.H., Jeong S. R. et al. (2007). Production of Nfa1-specific monoclonal antibodies that influences the *in vitro* cytotoxicity of *Naegleria fowleri* trophozoites on microglial cells. *Parasitology Research, 101(5)*, 1191–1196. https://doi.org/10.1007/s00436-007-0600-1
Levine N.D., Corliss J.O., Cox F.E. et al. (1980). A newly revised classification of the protozoa. *The Journal of Protozoology, 27(1)*, 37–58. https://doi.org/10.1111/j.1550-7408.1980.tb04228.x
Marciano-Cabral F. (1988). Biology of *Naegleria* spp. *Microbiological Reviews, 52(1)*, 114–133. https://doi.org/10.1128/mr.52.1.114-133.1988
Marciano-Cabral F., Cabral G.A. (2007). The immune response to *Naegleria fowleri* amebae and pathogenesis of infection. *FEMS Immunology and Medical Microbiology, 51(2)*, 243–259. https://doi.org/10.1111/j.1574-695X.2007.00332.x
Martinez A.J. (1985). Free-living amebas: natural history, prevention, diagnosis, pathology, and treatment of disease. CRC Press, Boca Raton, FL. 168 p.
Martinez A.J., Nelson E.C., Jones M.M. et al. (1971). Experimental *Naegleria* meningoencephalitis in mice. An electron microscope study. *Laboratory Investigation; a Journal of Technical Methods and Pathology, 25(5)*, 465–475.

Martinez-Castillo M., Cárdenas-Zúñiga R., Coronado-Velázquez D. et al. (2016). *Naegleria fowleri* after 50 years: is it a neglected pathogen? *Journal of Medical Microbiology, 65(9)*, 885–896. https://doi.org/10.1099/jmm.0.000303
McKerrow J.H., Rosenthal P.J., Swenerton R., Doyle P. (2008). Development of protease inhibitors for protozoan infections. *Current Opinion in Infectious Diseases, 21(6)*, 668–672. https://doi.org/10.1097/QCO.0b013e328315cca9
Pervin N., Sundareshan V. (2022). *Naegleria*. In: StatPearls [Internet].. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30571068.
Ropar J., Thomas A., Sharma P. (2013). Primary amoebic meningoencephalitis caused by *Naegleria fowleri*: Current status and future prospects. *Journal of Postgraduate Medicine, 59(4)*, 296–301.
Seidel J.S., Harmatz P., Visvesvara G.S. et al. (1982). Successful treatment of primary amebic meningoencephalitis. *The New England Journal of Medicine, 306(6)*, 346–348. https://doi.org/10.1056/NEJM198202113060607
Siddiqui R., Ali I.K.M., Cope J.R., Khan N.A. (2016). Biology and pathogenesis of *Naegleria fowleri*. *Acta Tropica, 164*, 375–394. https://doi.org/10.1016/j.actatropica.2016.09.009
Sohn H.J., Song K.J., Kang H. et al. (2019). Cellular characterization of actin gene concerned with contact-dependent mechanisms in *Naegleria fowleri*. *Parasite Immunology, 41(8)*, e12631. https://doi.org/10.1111/pim.12631
Song K.J., Song K.H., Na B.K. et al. (2007). Molecular cloning and characterization of a cytosolic heat shock protein 70 from *Naegleria fowleri*. *Parasitology Research, 100(5)*, 1083–1089. https://doi.org/10.1007/s00436-006-0404-8
Visvesvara G.S. (2013). Infections with free-living amebae. *Handbook of clinical neurology, 114*, 153–168. https://doi.org/10.1016/B978-0-444-53490-3.00010-8
Visvesvara G.S., Moura H., Schuster F.L. (2007). Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunology & Medical Microbiology, 50(1)*, 1–26. https://doi.org/10.1111/j.1574-695X.2007.00232.x
Zaongo S.D., Shaio M.F., Ji D.D. (2018). Effects of culture media on *Naegleria fowleri* growth at different temperatures. *Journal of Parasitology, 104(5)*, 451–456. https://doi.org/10.1645/18-6
Zumla A. (2010). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. *The Lancet. Infectious Diseases, 10(5)*, 303–304. https://doi.org/10.1016/S1473-3099(10)70089-X

Молекулярні механізми та терапевтичні стратегії *Naegleria fowleri* Carter (1970): смертельно небезпечна амеба, що пожирає мозок

Н. Датта

Naegleria fowleri - це термофільна вільноживуча амеба, яка може викликати рідкісну та смертельну інфекцію мозку, яка називається первинним амебним менінгоенцефалітом (PAM). PAM є серйозною проблемою для охорони здоров'я, оскільки вражає переважно молодих і здорових людей після перебування у теплому прісноводному середовищі, і має рівень смертності приблизно 98%. Зараження відбувається, коли амеба потрапляє в носову порожнину під час плавання або інших видів відпочинку і мігрує в мозок через нюховий нерв. У мозку амеба викликає великий некроз тканин, крововилив і запалення, що призводить до серйозних неврологічних симптомів і смерті протягом декількох днів. Патогенез інфекції *N. fowleri* повністю не з'ясований, але останні дослідження пролили світло на молекулярні механізми, які дозволяють амебі вторгатися, розмножуватися та уникати імунної системи господаря. Ці механізми включають експресію різних поверхневих молекул, які опосередковують адгезію, рухливість і фагоцитоз клітин господаря, а також секрецію протеаз та інших факторів, які руйнують позаклітинний матрикс господаря та модулюють імунну відповідь господаря. Однак досі залишається багато питань без відповіді щодо складних взаємодій між амебою та її господарем, які обмежують розробку ефективних діагностичних і терапевтичних стратегій. PAM часто помилково діагностують як бактеріальний менінгіт через його неспецифічні клінічні прояви та відсутність надійних діагностичних тестів. Це призводить до запізнілого або невідповідного лікування та поганого прогнозу. Наразі не існує спеціального чи схваленого лікування PAM, а доступні варіанти базуються на емпіричних даних або описах випадків. Рівень виживаності при PAM залишається дуже низьким, незважаючи на використання кількох препаратів і підтримувальну терапію. Таким чином, існує нагальна потреба в додаткових дослідженнях патогенезу *N. fowleri* та ідентифікації нових мішеней для втручання. З прогресом у геномних і протеомних технологіях з'явилися нові можливості для вивчення молекулярної біології *N. fowleri* та відповіді її господаря. Ідентифікувавши гени та білки, задіяні в ключових процесах, таких як адгезія, рухливість та імунне ухилення, дослідники можуть

розробити цільову терапію, щоб порушити ці важливі функції та запобігти або лікувати інфекцію. Цей огляд містить вичерпний огляд поточного стану знань про *N. fowleri*, його патогенні молекулярні механізми та біологічні процеси, пов'язані із зараженням, а також проблеми та перспективи майбутніх досліджень.

Ключові слова: *Naegleria fowleri, первинний амебний менінгоенцефаліт, патогенез, життєвий цикл, молекулярні механізми, імунна система господаря, діагностика.*

Цитування: *Datta N. Molecular mechanisms and therapeutic strategies of Naegleria fowleri Carter (1970): a review of the fatal brain-eating amoeba. Вісник Харківського національного університету імені В.Н. Каразіна. Серія «Біологія», 2024, 40, с. 22-31. https://doi.org/10.26565/2075-5457-2024-42-2*

Про автора:
Датта Н. – Коледж Асутош (Університет Калькутти), вул. Шьяма Прасад Мукерджі, 92, Калькутта, Західний Бенгал, Індія, 700026, neelabhdatta@gmail.com, https://orcid.org/0000-0002-1577-5461

Подано до редакції: 16.01.2023 / Прорецензовано: 27.02.2024 / Прийнято до друку: 18.03.2024