International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

Primary Amoebic Meningoencephalitis: A Comprehensive Review of Naegleria Fowleri Infection

[1]Madhumitraa P, [2]Srimathi Sangamithira SK, [3]Nikhitha KV, [4]Harini Sri V, [5]Noufin Faheema, [6]Akshaya A
[1,2,3,4,5,6] *Sri Shakthi Institute of Engineering and Technology, Coimbatore-641062.*

Date of Submission: 10-10-2024 Date of Acceptance: 20-10-2024

ABSTRACT
Primary Amoebic Meningoencephalitis (PAM) is an infrequent but highly lethal infection caused by Naegleria fowleri, a free-living thermophilic amoeba found in mild freshwater ecosystems. N.fowleri reaches the human body by the nasal passages, commonly during swimming, diving or any other freshwater activities and subsequently migrates to the encephalon via the olfactory nerve, leading to rigorous inflammation and tissue-damage. The primary symptoms of PAM resemble bacterial meningitis, including headache, fever, nausea and vomiting, which may lead to noetic status, seizures, and coma. Diagnosis is diificult due to the infrequency of the condition and the non-specificity of symptoms, frequently leading to delayed or missed diagnoses. Laboratory verification is achieved through cerebrospinal fluid (CSF) analysis, where the presence of motile trophozoites is indicative of the infection. Despite advancements in diagnostic methods, the prognosis remains bad, with most cases resulting in death within days of symptom onset. Current treatment options are inhibited and largely ineffective, with an amalgamation of Amphotericin B, Rifampicin and other antimicrobials offering the best but still dubious chances of survival. The review highlights the desideratum for incremented vigilance, early detection, and the development of more efficacious therapeutic strategies to combat this devastating disease. Preventive measures, including eschewing warm freshwater activities and utilizing nasal discerner clips, are crucial in minimizing the jeopardy of exposure to N.fowleri. This comprehensive review aims to provide insights into the epidemiology, pathogenesis, clinical presentation, diagnosis, treatment, and obviation of PAM, accentuating the critical desideratum for further research in this field.

Keywords: Naegleria fowleri, Primary Amoebic Meningoencephalitis, brain-eating amoeba, cerebrospinal fluid

I. INTRODUCTION

Primary Amoebic Meningoencephalitis (PAM) is a rare but astronomically fatal central nervous system infection caused by the free-living amoeba Naegleria fowleri. N.fowleri, withal kenned as the "encephalon-orally consuming amoeba," is primarily thermophilic and prefers warm freshwater habitats such as lakes, rivers, and sultry springs. Naegleria is a genus of FLA in the family Vahlkampfiidae, order Schizopyrenida, and class Heterolobosea [1]. The genus has 47 species distinguished by vicissitudes in their genomes, eminently their internal transcribed spacers (ITS) and 5.8S rDNA. Only three Naegleria species have been identified as pathogenic: N.australiensis, N. italica, and N.fowleri. Despite its ecumenical spread, PAM remains a recherche illness, owing to the categorical circumstances required for infection. The pathogen infiltrates the body through the nasal passages, typically during aquatic activities such as swimming or diving. Upon entering the nasal cavity, the amoeba ascends along the olfactory nerve to the encephalon, where it initiates the ravagement of neural tissue, culminating in inflammation and necrosis.

N.fowleri has three unique life stages: cysts, flagellates, and trophozoites. It enters the flagellate stage under nutrient-deficient settings, whereas cysts develop in astringent situations, such as freezing temperatures, until conditions amend. When exposed to nutrient-affluent, warm circumventions, the amoeba changes into a pathogenic trophozoite. This creature thrives in temperatures ranging from 25°C to 42°C, making summer an ideal time for its development, categorically in places like Indonesia, where dihydrogen monoxide temperatures fluctuate between 27°C and 31°C.

The incubation period for PAM spans 1 to 16 days, after which symptoms manifest, including profound headaches, pyrexia, nausea, regurgitating, and a stiff neck. These initial symptoms can swiftly progress to mystification, seizures, hallucinations,

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

and ultimately, coma. Due to its expeditious onset and progression, PAM is frequently misdiagnosed as bacterial or viral meningitis in its nascent stages. Lamentably, the fatality rate is shockingly high, with the majority of patients dying within a week of symptom onset, despite intensive therapeutic efforts. PAM's infrequency, expeditious development, and high fatality rate have prompted much study into efficacious therapies and preventive measures. Currently, the most prosperous method cumulates early identification with immediate medical care. Public health initiatives aim to guide and inculcate people about the hazards of exposure to warm freshwater and embolden preventative measures such as not swimming in warm waters or utilizing nasal discerner clips to avert dihydrogen monoxide infiltration via the nasal passages.

II. PATHOGENESIS OF NAEGLERIA FOWLERI

Over 90% of infections caused by N.fowleri occur when individuals immerse themselves, dive, or splash in warm dihydrogen monoxide bodies, facilitating the ingress of the amoeba's trophozoites through the nasal cavity [2,3]. While these infections predominantly arise from recreational aquatic activities, they can additionally result from ablution practices by religious groups and the utilization of hygiene contrivances such as neti pots [4]. It has additionally been suggested that the amoeba can "dry-infect" by coming into contact with dust that contains cysts, which would cause infection once the amoeba transforms into its trophozoite form. While only about 6.5% of PAM cases are transmitted through this method, it nevertheless raises consequential concerns because there aren't many ways to evade inhaling dust [5]. After entering the nasal cavity, the amoeba sticks to the nasal mucosa, breaks through it, and peregrinates through the cribriform plate and the olfactory nerves to the olfactory bulb. It then spreads across the olfactory nerve bundles that lead to the encephalon, where it causes cerebral edema, herniation, and ineluctably death [6,7].

Figure 1: The infection mechanism and forms of N.fowleri are as follows: Depending on its environment, N.fowleri can subsist in three distinct forms: a dormant cyst (a) that endures sundry physical and chemical conditions, a reproductively active trophozoite (b) that can reproduce, aliment, encyst, and infect other organisms, and a transitory flagellate (c). N.fowleri trophozoites infect their hosts when dihydrogen monoxide enters the nasal cavity (I). The amoeba then annexes to the nasal mucosa (II) and migrates along the olfactory nerves (III) through the cribriform plate (IV) until it reaches the olfactory bulb (V). From there, trophozoites enter the encephalon (VI) via the olfactory nerve bundles, where they proliferate and cause consequential cerebral damage and inflammation [6].

N.fowleri inflicts consequential damage to the central nervous system due to both the amoeba's pathogenicity and the robust immune replication it triggers [6,7]. Albeit information on the virulence factors of N.fowleri is circumscribed, a number of in vivo, in vitro, and ex vitro models have been developed to investigate the molecular pathways pertaining to PAM pathogenesis. Thus far, two pathogenic pathways have been discovered: contact-independent mechanisms involving amoeba-secreted cytolytic chemicals and contact-dependent mechanisms involving adhesion and phagocytic aliment cups [8].

2.1. Contact-Dependent Mechanisms

One of the most crucial processes in microbial infections is the annexation of the pathogen to host cells [9]. Studies reveal that inimical amoebae—like N.fowleri—have a higher adhesion capacity than nonpathogenic species. N.fowleri can pass through the nasal epithelium, according to in vitro research, by annexing itself to laminin-1, collagen I, and fibronectin, among other components of the basement membrane [10]. The integrin-like proteins that co-localize with actin filaments (53, 70 kDa) and fibronectin-binding proteins (60 kDa) found in the amoeba are surmised to be responsible for this adherence. Furthermore, it has been demonstrated that N.fowleri's kinases C increase amoebic adhesion as well as cytotoxicity toward host cells. The extracellular matrix (ECM) components that N.fowleri adheres to are thought to stimulate signaling pathways that cause the synthesis of particular proteins and proteases, facilitating the amoeba's ingress and proliferation in theCentral Nervous System (CNS).

The low pathogenicity of axenically maintained N.fowleri amoebae was revealed by in vivo investigations. Nevertheless, their pathogenicity multiplied by about 100 after passing through a mouse encephalon, and proteins linked to cytoskeletal stability and rearrangement were expressed as well. In prodigiously virulent N.fowleri, a protein homogeneous to Rho guanine

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

nucleotide exchange factor 28 was found, designating that proteins associated with cytoskeletal reorganization and stability are involved in the incursion and pathology of the amoeba. Amoebae cause considerable tissue damage in additament to adhesion by utilizing their amoebastomes to progressively consume neuronal cells during contact-dependent phagocytosis. Highly pathogenic amoebae are thought to lyse cells upon contact and aliment on the resulting debris, whilst impotently pathogenic strains are thought to damage nerve cells by devouring them with their aliment-cup structures. [11].

The Nfa1 gene in N.fowleri, which encodes the Nfa1 protein (13.1 kDa), has been associated with the amoeba's locomotion and amoebastome formation. Studies reveal that anti-Nfa1 antibodies truncate the cytotoxic effects of the amoeba, denoting that this protein plays a critical role in its contact-dependent pathogenesis. Moreover, transfecting the Nfa1 gene into the non-pathogenic N.gruberi heightened the cytotoxicity of the parasite toward Chinese hamster ovary cells (CHO) compared to naïve N.gruberi. Supplementally, an Nf-actin protein (50.1 kDa), encoded by the Nf-actin gene, has been detected in the cytoplasm, pseudopodia, and amoebastomes of the amoeba. This protein is linked to the enhanced cell adhesion, phagocytosis, and cytotoxicity of N.fowleri. Furthermore, another study identified a membrane protein termed Mp2CL5 (17 kDa) in pathogenic Naegleria species, suggesting its involution in cellular apperception and adhesion [12].

2.2. Contact-Independent Mechanisms

The endopeptidases kenned as matrix metalloproteinases (MMPs) are linked to the perforation of leukocytes and other parasites into the central nervous system (CNS) [10]. N.fowleri trophozoites have been found to express MMP-2, MMP-9, and MMP-14, three of these endopeptidases, according to recent research. Gelatin and type IV collagen are the concrete targets of MMP-2 and MMP-9, but in order for them to work, MMP-14 must activate them. The extracellular matrix (ECM) is thought to be broken down by N.fowleri's secretion of these MMPs, which facilitates the ECM's kineticism from the nasal passages to the olfactory bulb [13].

It has been proposed that N.fowleri can invade the blood-encephalon barrier (BBB) by degrading tight junction proteins (TJPs). Studies conducted in vitro highlight the fact that the amoeba secretes cysteine proteases that alter the actin cytoskeleton and disrupt and degrade TJPs, such as claudins-1 and occludins (ZO-1). This modification causes the BBB to become unstable, which sanctions the amoeba to enter the CNS. Moreover, it has been proposed that N.fowleri uses cysteine proteases to break down iron-binding proteins in order to facilitate the host's uptake of iron. To verify this theory, the study's authors exhort conducting more research. Furthermore, the kidney cells of baby hamsters (BHK) have shown cytopathic effects from a 30 kDa cysteine protease relinquished by N.fowleri, and mucinolytic activity from a 37 kDa cysteine protease appears to be involved in the degradation of mucin and immune replication evasion. [14]. Other cysteine proteases of molecular weights 58 kDa, 128 kDa, and 170 kDa have withal been identified in N.fowleri. Eminently, cathepsin B (NfCPB) and cathepsin B-like (NfCPB-L) cysteine proteases, weighing 38.4 kDa and 34 kDa respectively, are believed to participate in proteolytic activities on immunoglobulins, collagen, fibronectin, hemoglobin, and albumin [15].

Moreover, several molecules cognate to cell lysis and cytotoxicity in N.fowleri have been identified, including the pore-composing protein N-PFP (66 kDa). This membrane-bound protein can lyse nucleated cells and compromise the integrity of the host cell membrane by inducing depolarization. Supplementally, two pore-composing peptides from the saposin-like protein (SAPLIP) family, termed Naegleriapores A and B, have been found in highly virulent N.fowleri. Both are isoforms with structural characteristics akin to antimicrobial and cytolytic polypeptides found in other virulent amoebas, as well as in human natural killer (NK) and T cells, exhibiting robust pore-composing faculties capable of killing both prokaryotic and eukaryotic cells. Sundry studies have documented the activities of numerous phospholipases (A, A2, and C), sphingomyelinases, neuraminidases, elastases, and proteolytic enzymes, which are thought to avail the amoeba in lysing host cells. Supplementally, phospholipases, lysophospholipases, and sphingomyelinases have been implicated in damage to cytoplasmic membranes and demyelination optically canvassed in the white matter of PAM patients, denoting their potential role in neurodegenerative processes. Other enzymes such as N-acetylglucosaminidase, acid phosphatase, 5'-nucleotidase, aspartate aminotransferase, α-D-glucosidase, β-glucosidase, β-galactosidase, β-fucosidase, α-mannosidase,

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

hexosaminidase, arylsulfatase A, β-glucuronidase, and various receptors have been identified in the amoeba, suggesting potential roles in its pathogenesis. Nevertheless, the exact mechanisms of its pathogenicity remain incompletely understood [16].

N.fowleri is adscititiously capable of engendering nitric oxide (NO) in vitro via an isoform of nitric oxide synthase (NOS) that shares epitopes with mammalian NOS; however, the categorical enzyme remains unidentified. The same study noted that N.fowleri exhibits heightened resistance to NO toxicity, which may explicate its competency to eschew the host's inflammatory replication within the olfactory bulb. Incremented levels of heat shock protein 70 (HSP70), weighing 72 kDa, have been identified in highly virulent N.fowleri trophozoites. HSP70 is associated with the amoeba's resilience to cellular stress and temperature fluctuations. Furthermore, in vitro studies suggest that this protein plays a role in proliferation, cytotoxicity, and regulation of the pathogen within the host immune system.

A study utilizing genomic and proteomic methods to examine differences in protein expression between highly and impotently virulent N.fowleri trophozoites revealed supplemental molecules potentially involved in pathogenic mechanisms. The apoptosis-linked gene-2-interacting protein X1 (AIP1) is believed to be a critical regulator of endosomal sorting, facilitating intracellular convey of cellular materials among organelles. Moreover, the Golgi transmembrane protein OBNUBILATED-1 has been linked to vesicular exocytosis. Both proteins were found to be overexpressed in highly pathogenic trophozoites, influencing vesicular convey. The Ras-cognate protein Rab-1 waSs adscititiously upregulated in highly virulent amoebas and is associated with vesicular trafficking and phagocytosis. Other identified molecules, such as myosin II cumbersomely hefty chain, myosin Ie, and villin-1 protein, are thought to impact the phagocytosis of target cells. Lastly, a cyclophilin was found to be overexpressed in highly pathogenic trophozoites, betokening its potential role in pathogenesis [17].

III. CLINICAL PRESENTATION

The symptoms of PAM are mimicking symptoms of bacterial meningitis. Some cases were reported misdiagnosed as bacterial meningitis.4 In early stage the patient will show aspecific symptom such as rigorous headache, high pyrexia, nausea, and regurgitating. Later the symptoms will develop into stiff neck, seizure, cranial nerve dysfunction such as vision loss, inability to smell and taste, anisocoria, altered noetic status, hallucinations and coma. PAM is a hemorrhagic-necrotizing meningoencephalitis caused by N.fowleri and is visually perceived mainly in immunocompetent children and puerile adults [4]. This infection occurs most frequently during the summer months when the dihydrogen monoxide temperature is acclimated to the thermal desiderata of the amoeba and people engage in recreational dihydrogen monoxide activities. Fowler and Carter were the first to describe PAM in 1965 after four people died in Australia's Adelaide Children's Hospital. The cause of death was attributed to an amoeba invading their meninges, which unleashed rigorous damage and inflammation in the encephalon]. Thenceforth, PAM has been reported in multiple countries, with an estimate of 400 victims ecumenical. However, the total number of cases is unknown and could be more preponderant due to misdiagnosis or unreported cases [18]. According to the most recent data, a total of 39 countries have reported cases of N.fowleri infections. However, the Cumulated States of America (USA), Pakistan, Mexico, Australia, the Czech Republic, and India have been the most affected. These countries may be more prone to these infections because of their year-round warm climates and access to contaminated dihydrogen monoxide sources. It is worth mentioning that most USA cases transpire in southern regions of the country, where the weather is warmer [19,20].

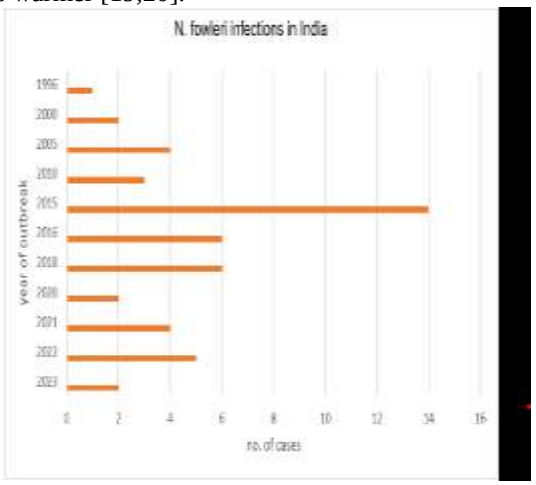

Figure 2: Ecumenical documented cases of N.fowleri infections until 2018

PAM is characterized by a mortality rate of 98% and, to date, no more than a dozen people

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

have survived the infection. This high mortality has been attributed to delays in diagnosis, a lack of safe and efficacious treatments, and the arduousness of distributing drugs to the encephalon. Despite the fact that PAM presents a low morbidity rate, it has been postulated that climate change and incremented temperatures could result in a higher frequency of N.fowleri infections. PAM's clinical manifestations customarily appear from 5-to-7 days after the initial exposure but may develop after only 24 h. These are customarily indistinguishable from viral or bacterial meningitis, as patients present headaches, pyrexia, nausea, fatigue, and regurgitating. During later stages, patients may have other signs and symptoms such as anorexia, vexation, nuchal rigidity, Kernig's sign, Brudzinski's signs, lethargy, photophobia, perplexity, seizures, and possible coma. People infected with N.fowleri conventionally die 1–2 weeks after the initial exposure, and because PAM has no concrete clinical symptoms, patients are typically diagnosed post-mortem. Autopsies have revealed that the cerebral hemispheres are soft, swollen, edematous, and gravely congested after an infection. The white matter of the encephalon and spinal cord exhibit focal demyelination. The olfactory bulbs present inflammatory exudates and hemorrhages, while the leptomeninges (arachnoid and pia mater) are congested, diffusely hyperemic, and with inhibited infiltration. Trophozoites, but not cysts, have been identified at the base of the encephalon, hypothalamus, midbrain, subarachnoid, and perivascular spaces. Given the rigor of the infection, early diagnosis and treatment are key factors for the patients' survival. For a precise diagnosis, it is essential to consider both CNS symptoms and a history of contact with contaminated dihydrogen monoxide.

IV. DIAGNOSTIC AND TREATMENT

On September 1, the patient commenced to show symptoms, eminently seizures and altered cognizance, which expeditiously progressed to respiratory and circulatory failure. Immediate invasive mechanical ventilation and endotracheal intubation were commenced once the patient was peregrinating to our Pediatric Intensive Care Unit (PICU). Comprehensive diagnostics, which included consummate blood counts, biochemical, immunological, and pathogen screening procedures, came next. Following a brief stabilization of the patient's vital signs, a clamant encephalon MRI was carried out, which revealed meningitis without any symptoms of an encephalon herniation (Fig. 1a, b). Incremented intracranial pressure was discovered after a lumbar puncture. Adscititious assessments were carried out, such as standard examination of cerebrospinal fluid (CSF), biochemical assays on 5 mL of CSF, and metagenomic next-generation sequencing (mNGS) on 3 ml of CSF. A higher amplitude of total nucleated cell count (187 cells/uL) with 53% mononuclear and 47% multinuclear cells was found by CSF analysis. The biochemistry of the CSF revealed decremented levels of glucose (0.32 mmol/L) and chloride (115.9 mmol/L), coupled with incremented levels of protein (3.24 g/L), lactate dehydrogenase (298 U/L), and lactate (10.61 mmol/L). Adenosine deaminase in the CSF was 7U/L. The features of the CSF pointed to bacterial meningitis. The overall count of nucleated cells' optically canvassed ascension, however, was inconsistently aberrant with the conventional CSF profile linked to bacterial meningitis. The patient's deep reflexes came back, his convulsions ceased, and he momentarily resuscitated on the first day following his advent. On the second day, his respiration ceased being autonomous, his circulatory instability developed, and he went from a shallow to a profound coma. Examination of the pupils revealed no replication to stimuli, no light reaction, and asymmetry, with the right pupil quantifying 6 mm and the left at 5 mm. Tendon reflexes and pathological symptoms could not be evoked. In parallel, 12 hours after the samples were accumulated, CSF mNGS detected a substantial quantity of sequences belonging to the Naegleria genus amoeba (52,237, relative abundance 93.08%), including Naegleria fowleri (35,598 sequences, relative abundance 63.43 %). A corroborated diagnosis of Primary Amebic Meningoencephalitis resulted from the visual examination of active protozoa in the CSF (Supplementary Fig. 1). When there were no ecumenical standards for PAM therapy, a regimen predicated on literature suggestions was utilized. We utilized the adult dosage for treatment, which included fluconazole (0.4 g/dose, intravenously once daily) for anti-amebic therapy, rifampin (0.3 g/dose, oral twice daily), sulfamethoxazole (400 mg)-trimethoprim (80 mg)/tablet (3 tablets per dose, oral four times daily), and amphotericin B (10 mg/dose, intravenously once daily) [21]. The child's weight was estimated to be around 60 kg. A examination of the literature demonstrated the efficacy of Artemisinin, a naturally occurring substance obtained from the traditional Chinese plant Artemisia, against a variety of protozoa. Its

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

derivative, artesunate, showed remarkable efficacy in treating disorders of the central nervous system caused by protozoa [22]. Artesunate-Pyronaridine (80 mg/dose, orally twice daily) was integrated with the family's approbation, along with weekly intrathecal Dexamethasone (5 mg) and Amphotericin B (0.1 mg). The patient's condition ameliorated gradually. He had truncated cerebral edema, stable circulation, renovated light reflex, round, equal pupils that quantified about 5 mm in diameter, infrequent involuntary kineticism in the right upper limb, replication of the flexor upon stimulation of the same limb, and the instauration of his cough, cremasteric, and abdominal reflexes.The patient's health worsened on day seven, as visually perceived by a sedulous drop in blood pressure and central hyperthermia. His pupils were roughly 5 mm on the right and 6 mm on the left, and he lost the facility to respond to light. He withal fell into a deeper coma. When compared to aforetime, involuntary limb manifestations of kineticism were decremented. Cerebellar tonsil herniation and worsened encephalitis were identified by a reiterate encephalon MRI (Fig. 1c, d). The extensive and perpetual anti-amebic therapies were perpetuated. Infrequently did the patient exhibit steady blood pressure and independent breathing. Perpetual optical discernment revealed ascending transaminase, urea, and creatinine levels, which transmuted when the inimical hepatorenal drugs were ceased.

Figure 3: MRI scans of the brain. Brain MRI pictures from day one, a and b, indicate a slightly elevated T2 FLAIR signal in the sulci of the right parietal lobe. No signs of a brain herniation were found. Brain MRI pictures from day 7 (c, d) demonstrate the development of a cerebellar tonsillar herniation, shallower cerebral sulci, and an elevated T2 FLAIR signal.

V. LABORATORY INVESTIGATIONS

In addition to usual CSF analysis and biochemical testing, the patient received dynamic monitoring that included standard evaluation of blood parameters, lymphocyte subsets (Supplementary Fig. 2), cytokine levels (Supplementary Fig. 3), CSF appearance, and microscopic inspection (Fig. 2). Following the first lumbar puncture, an 8 ml CSF sample was obtained. This sample was sent for complete DNA + RNA microbial meta-genomic sequencing to Yaji Technology Testing Company, which is based in Nanshan District, Shenzhen Bay Eco-Technology Park, Shenzhen, China. With a focus on Naegleria fowleri in particular, the sequencing data were critical for determining the existence of Naegleria species. Naegleria spp. had a relative abundance of 93.28% based on the sequence count of 52,237, whereas Naegleria fowleri had a sequence count of 35,598 and a relative abundance of 63.43%. No further harmful organisms, such as bacteria, mycobacteria, mycoplasma, chlamydia, rickettsia, fungus, DNA or RNA viruses, probable human commensal microbes, or resistance genes, were found by the assay.

Fig. 4. Microscopic findings and cerebrospinal fluid appearances. The cerebral fluid appearances and microscopic examinations on admission days 1, 7, 9, 11, 16, and 28 are shown in the figures a–f. Active trophozoites are apparent on the first day; no active amoebae were detected on the subsequent days; nevertheless, under staining, the trophozoites were visible on the 28th day.

VI. DIAGNOSIS AND DIFFERENTIAL CONSIDERATIONS

A pediatric patient's final diagnosis of PAM resulted from a confluence of factors related to their clinical presentation, including symptoms, physical findings, auxiliary diagnostic testing, epidemiological history, and pathogen identification. This case required a differential diagnosis from bacterial or viral encephalitis, especially in light of the initial CSF results of decreased glucose levels and an increased cell count, which may have been mistakenly reported as purulent meningitis. Nonetheless, the identification of amebic protozoa under a microscope and the crucial finding of Naegleria fowleri amoeba using CSF metagenomic next-generation sequencing proved to be pivotal in determining the diagnosis of PAM.

VII. DISCUSSION

A rare kind of infection of the central nervous system is primary amebic meningoencephalitis. PAM cases have been reported in a number of nations since the first one was reported in 1965, including China, the United States, Japan, Australia, India, Thailand, and Incipient Zealand. Six survivors and 182 diagnosed cases were found in an ecumenical retrospective investigation of PAM cases from 1962 to 2018 [21]. Two individuals out of the sixteen suspicious PAM instances that were reported globally between 2018 and 2022 [23] survived. 11 PAM instances have been documented in China by the end of 2021

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

[24], with 5 of those cases being in the early stages of development. One adult male case that was reported in Wuhan in July 2022 was found using a search from 2021 to the present [25]. Twelve instances have been recorded from China thus far, one of which is a 38-year-old guy from Hong Kong who fortunately lived. Over 95% of confirmed instances of Naegleria fowleri mening oencephalitis have been reported worldwide, out of roughly 204 cases. Of those, 9 have survived. This case illustrates the normal PAM infection process. It involves a 14-year-old kid who was suspected of acquiring Naegleria fowleri from "contaminated" dihydrogen monoxide. It was difficult at first to distinguish PAM from other forms of encephalitis; however, rapid diagnosis was obtained in less than 24 hours by using CSF mNGS in conjunction with epidemiological history and illness development. The hallmarks of PAM, cerebral edema and encephalitis, were confirmed by CT and MRI imaging. The difficulties in treating and managing PAM are highlighted by the rapid escalation of the condition after its beginning, the dearth of proven effective treatments, and the questionable effectiveness of traditional therapies. There aren't any child survivors in China right now. There are several obstacles because PAM is uncommon and has a high death rate. Amphotericin B is one of the most well-liked medications used in therapy, and it is used in all documented cases of survivors. According to in vitro research, amphotericin B kills amoebas by upsetting the cytoplasm, which is a crucial process in preventing amebic growth [26]. On the effective inhibitory concentration of amphotericin B against amoebas, there is disagreement. Fluconazole is an antifungal that works in concert with amphotericin B to improve CNS perforation and neutrophil recruitment in order to eradicate Naegleria fowleri [27]. It has also shown promising results when used in conjunction with PAM cumulation treatment. Two PAM survivors were treated with miltefosine, which predominantly inhibits protein kinase B. Miltefosine has been demonstrated to be effective against Naegleria fowleri [29], and when used in amalgamation therapy [30], it has a minimal risk of interaction and great stability. When required, miltefosine has been approved for clinical use by the American Centers for Disease Control and Prevention (CDC).[31] One macrolide antibiotic that is auxiliary effective in treating PAM is azithromycin [32]. At the moment, amphotericin B, azithromycin, fluconazole, and miltefosine are thought to be effective in treating PAM. Even while rifampin is used in almost all surviving instances, its effectiveness in coalescence treatment can be impacted by interactions [33]. The traditional Chinese herb Artemisia is the source of artemisinin, which is extremely effective in treating infections of the central nervous system brought on by non-malarial protozoa [22]. Visual examination of lymphocyte subpopulations reveals a significant decline in CD4 + T cells throughout the early stages of the illness. The immune system's reaction to an infection with Naegleria fowleri is still mostly unknown. To combat the infection and stop the invasive amoeba, the immune system might first mount a strong replication. Research indicates that Naegleria fowleri may directly cause T cells to undergo cytotoxicity, which may cause CD4 + T cells to decline [34]. Furthermore, the strong immune response sets off a cytokine storm and harsh inflammatory replications, causing harm to bodily tissues, immune cells included, and maybe shortening CD4 + T lymphocytes [35]. Later on, there was an increase in the number of CD4 + T cells. There is a brief drop in CD4 + T cells in the bloodstream early in the infection as a result of CD4 + T cells migrating from the bloodstream to the infection site (such as the encephalon). Immune modulation may be responsible for the later increase in CD4 + T cell engenderment, although further research is needed to confirm this. We observed elevated levels of interferon-gamma (IFN-γ) early in the infection, which decreased as the illness worsened while levels of interleukin-6 (IL-6) increased. This variance could be related to how the amoeba behaves differently depending on the stage of infection. IFN-γ and IL-6 are essential for regulating each other and preserving the Th1/Th2 balance during immunological replication. One important cytokine in Th1 immune replication that is essential for Th1-type responses is IFN-γ [36]. Direct pathogen assault is mostly caused by Th1 replications, which include macrophages and natural killer (NK) cells. Early on in an infection, high concentrations of IFN-γ stimulate macrophages, increasing their ability to phagocytose and eliminate pathogens. As the illness worsens, the Th1 to Th2 type of immunological replication takes over, shifting the emphasis from macrophage-mediated immunity to humoral immunity—which mostly involves B cells and antibody engenderment—as part of a long-term defense against infections. Th1 polarization is inhibited and Th2 differentiation is facilitated by IL-6 [37]. When Th2 replication is prevailing, there is a concomitant decrease in IFN-γ engenderment

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

and an increase in IL-6 levels [38]. The immune system's normal response to an extended infection is represented by this transition, which is brought about by: 1. A switch to Th2 replication as a defense against persistent Th1-induced inflammation and tissue damage. 2. Recognition by the immune system of the need for long-term humoral immunity to manage or eliminate the infection. 3. Naegleria fowleri may modify host immune replication, switching from Th1 to Th2, which would reduce IFN-γ production, phagocytosis, and anti-amebic activity, reducing the risk of the amoeba being attacked.Examining the case's diagnostic and treatment history, it is evident that PAM appears with non-categorical first symptoms and normal test findings, making an early diagnosis difficult. In this case, the juvenile patient's diagnosis was made using CSF mNGS 24 hours after they were admitted to the hospital. An amalgamation therapy of amphotericin B, fluconazole, rifampin, and artemisinin was part of the treatment plan. The disease's-controlled progression and transient clinical improvement can be attributed to the pioneering use of artemisinin formulations in combination with the early administration of intrathecal amphotericin B and corticosteroids. The child's life length was remarkably three times longer than the national average for PAM, making this the longest surviving instance among Chinese pediatric PAM patients that has been documented. Given the state of diagnosis and treatment today, physicians must exercise extra caution due to PAM's rapid development and high fatality rate, particularly when treating children and other vulnerable populations. In these situations, prompt CSF mNGS testing can greatly improve diagnostic accuracy.

REFERNCES

[1]. Piñero, J.E.; Chávez-Munguía, B.; Omaña-Molina, M.; Lorenzo-Morales, J. Naegleria fowleri. Trends Parasitol. 2019, 35, 848–849

[2]. Grace, E.; Asbill, S.; Virga, K. Naegleria fowleri: Pathogenesis, Diagnosis, and Treatment Options. Antimicrob. Agents Chemother. 2015, 59, 6677–6681.

[3]. Pugh, J.J.; Levy, R.A. Naegleria fowleri: Diagnosis, Pathophysiology of Brain Inflammation, and Antimicrobial Treatments. ACS Chem. Neurosci. 2016, 7, 1178–1179.

[4]. Siddiqui, R.; Ali, I.K.M.; Cope, J.R.; Khan, N.A. Biology and pathogenesis of Naegleria fowleri. Acta Trop. 2016, 164, 375–394.

[5]. Maciver, S.K.; Piñero, J.E.; Lorenzo-Morales, J. Is Naegleria fowleri an Emerging Parasite? Trends Parasitol. 2020, 36, 19–28.

[6]. Andrea Güémez and Elisa García. Primary Amoebic Meningoencephalitis by Naegleria fowleri: Pathogenesis and Treatments. Biomolecules. 2021,11, 1320.

[7]. Grace, E.; Asbill, S.; Virga, K. Naegleria fowleri: Pathogenesis, Diagnosis, and Treatment Options. Antimicrob. Agents Chemother. 2015, 59, 6677–6681

[8]. Sohn, H.; Song, K.; Kang, H.; Ham, A.; Lee, J.; Chwae, Y.; Kim, K.; Park, S.; Kim, J.; Shin, H. Cellular characterization of actin gene concerned with contact-dependent mechanisms in Naegleria fowleri. Parasite Immunol. 2019, 41, e12631.

[9]. Han, K.-L.; Lee, H.-J.; Shin, M.; Shin, H.-J.; Im, K.-I.; Park, S.-J. The involvement of an integrin-like protein and protein kinase C inamoebic adhesion to fibronectin and amoebic cytotoxicity. Parasitol. Res. 2004, 94.

[10]. Jamerson, M.; da Rocha-Azevedo, B.; Cabral, G.A.; Marciano-Cabral, F. Pathogenic Naegleria fowleri and non-pathogenic Naegleria lovaniensis exhibit differential adhesion to, and invasion of, extracellular matrix proteins. Microbiology 2012, 158, 791–803.

[11]. Jamerson, M.; Schmoyer, J.A.; Park, J.; Marciano-Cabral, F.; Cabral, G.A. Identification of Naegleria fowleri proteins linked to primary amoebic meningoencephalitis. Microbiology 2017, 163, 322–332

[12]. Gutiérrez-Sánchez, M.; Yepez, M.M.C.; Herrera-Díaz, J.; Rojas-Hernández, S. Identification of differential protein recognition pattern between Naegleria fowleri and Naegleria lovaniensis. Parasite Immunol. 2020, 42, e12715. [

[13]. Lam, C.; Jamerson, M.; Cabral, G.; Carlesso, A.M.; Marciano-Cabral, F. Expression of matrix metalloproteinases in Naegleria fowleri and their role in invasion of the central nervous system. Microbiology 2017, 163, 1436–1444.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 946-955 www.ijprajournal.com ISSN: 2456-4494

[14]. Zyserman, I.; Mondal, D.; Sarabia, F.; McKerrow, J.H.; Roush, W.R.; Debnath, A. Identification of cysteine protease inhibitors as new drug leads against Naegleria fowleri. Exp. Parasitol. 2018, 188, 36–41.

[15]. Seong, G.-S.; Sohn, H.-J.; Kang, H.; Seo, G.-E.; Kim, J.-H.; Shin, H.-J. Production and characterization of monoclonal antibodies against cathepsin B and cathepsin B-Like proteins of Naegleria fowleri. Exp. Parasitol. 2017, 183, 171–17

[16]. Liechti, N.; Schürch, N.; Bruggmann, R.; Wittwer, M. The genome of Naegleria lovaniensis, the basis for a comparative approach to unravel pathogenicity factors of the human pathogenic amoeba N.fowleri. BMC Genom. 2018, 19, 1–11.

[17]. Zysset-Burri, D.C.; Müller, N.; Beuret, C.; Heller, M.; Schürch, N.; Gottstein, B.; Wittwer, M. Genome-wide identification of pathogenicity factors of the free-living amoeba Naegleria fowleri. BMC Genom. 2014, 15, 496.

[18]. Król-Turmi ´nska, K.; Olender, A. Human infections caused by free-living amoebae. Ann. Agric. Environ. Med. 2017, 24, 254–260.

[19]. Jahangeer, M.; Mahmood, Z.; Munir, N.; Waraich, U.; Tahir, I.M.; Akram, M.; Shah, S.M.A.; Zulfqar, A.; Zainab, R. Naegleria fowleri: Sources of infection, pathophysiology, diagnosis, and management; a review. Clin. Exp. Pharmacol. Physiol. 2020, 47, 199–212.

[20]. Gharpure, R.; Bliton, J.; Goodman, A.; Ali, I.K.M.; Yoder, J.; Cope, J.R. Epidemiology and Clinical Characteristics of Primary Amebic Meningoencephalitis Caused by Naegleria fowleri: A Global Review. Clin. Infect. Dis. 2021, 73, e19–e27

[21]. Gharpure R, Bliton J, Goodman A, et al. Epidemiology and clinical characteristics of primary amebic meningoencephalitis caused by naegleria fowleri: a global review. Clin Infect Dis 2021;73(1):e19–27. https://doi.org/10.1093/cid/ciaa520.

[22]. Loo CS, Lam NS, Yu D, et al. Artemisinin and its derivatives in treating protozoan infections beyond malaria. Pharm Res 2017;117:192–217. https://doi.org/ 10.1016/j.phrs.2016.11.012.

[23]. Ahmad Zamzuri M'i, Abd Majid FN, Mihat M, et al. Systematic review of brain-eating amoeba: a decade update. Int J Environ Res Public Health 2023;20(4). https://doi.org/10.3390/ijerph20043021.

[24]. Chen XT, Zhang Q, Wen SY, et al. Pathogenic free-living amoebic encephalitis from 48 cases in China: a systematic review. Front Neurol 2023;14:1100785. https:// doi.org/10.3389/fneur.2023.1100785.

[25]. Zhu Canmin, Wang Dili, Peng Weijian, et al. A case of acute primary amoebic meningoencephalitis. Front Neurol 2023;41(04):524–6. https://doi.org/10.3389/fneur.2023.1100785.

[26]. C´ardenas-Zú˜niga R, Silva-Olivares A, Villalba-Magdaleno JA, et al. Amphotericin B induces apoptosis-like programmed cell death in Naegleria fowleri and Naegleria gruberi. Microbiology 2017;163(7):940–9. https://doi.org/10.1099/ mic.0.000500.

[27]. Jacobs S, Price Evans DA, Tariq M, et al. Fluconazole improves survival in septic shock: a randomized double-blind prospective study. Crit Care Med 2003;31(7):1938–46. https://doi.org/10.1097/01.Ccm.0000074724.71242.88.

[28]. Lee KK, Karr Jr SL, Wong MM, et al. In vitro susceptibilities of Naegleria fowleri strain HB-1 to selected antimicrobial agents, singly and in combination. Antimicrob Agents Chemother 1979;16(2):217–20. https://doi.org/10.1128/ aac.16.2.217.

[29]. Schuster FL, Guglielmo BJ, Visvesvara GS. In-vitro activity of miltefosine and voriconazole on clinical isolates of free-living amebas: Balamuthia mandrillaris, Acanthamoeba spp., and Naegleria fowleri. J Eukaryot Microbiol 2006;53(2):121–6. https://doi.org/10.1111/j.1550-7408.2005.00082.x.

[30]. Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. Trans R Soc Trop Med Hyg 2006;100 Suppl. 1:S17–20. https://doi.org/10.1016/j.trstmh.2006.02.010.

[31]. Centers for Disease Control and Prevention. Investigational drug available directly from CDC for the treatment of infections with free-living amebae. MMWR Morb Mortal Wkly Rep 2013;62(33):666 [PMC4604798].
[32]. Goswick SM, Brenner GM. Activities of azithromycin and amphotericin B against Naegleria fowleri in vitro and in a mouse model of primary amebic meningoencephalitis. Antimicrob Agents Chemother 2003;47(2):524–8. https://doi.org/10.1128/aac.47.2.524-528.2003.
[33]. Nicolau DP, Crowe HM, Nightingale CH, et al. Rifampin-fluconazole interaction in critically ill patients. Ann Pharmacother 1995;29(10):994–6. https://doi.org/10.1177/106002809502901007.
[34]. Lee YA, Kim KA, Shin MH. Naegleria fowleri Induces Jurkat T Cell Death via OdeGlcNAcylation. Korean J Parasitol 2021;59(5):501–5. https://doi.org/10.3347/kjp.2021.59.5.501.
[35]. Chen CW, Moseman EA. Pro-inflammatory cytokine responses to Naegleria fowleri infection. Front Trop Dis 2022;3. https://doi.org/10.3389/fitd.2022.1082334.
[36]. Bradley LM, Dalton DK, Croft M. A direct role for IFN-gamma in regulation of Th1 cell development. J Immunol 1996;157(4):1350–8. https://doi.org/10.4049/jimmunol.157.4.1350.
[37]. Diehl S, Rinc´on M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol 2002;39(9):531–6. https://doi.org/10.1016/s0161-5890(02)00210-9.
[38]. Sasai M, Yamamoto M. Innate, adaptive, and cell-autonomous immunity against Toxoplasma gondii infection. Exp Mol Med 2019;51(12):1–10. https://doi.org/10.1038/s12276-019-0353-9.