Case Report

A Case Report of Primary Amoebic Meningoencephalitis in Thi-Qar Province, Southern of Iraq

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ABSTRACT

Naegleria fowleri is one of the most important free living amoeba can caused usually fatal disease, such as "primary amoebic meningoencephalitis" is a rare. We diagnosed of amoebic meningoencephalitis by Naegleria fowleri. This case was the first study in the province of Thi Qar to be recorded. This case was identified in Nassaryia City, where the patient was an 11-year-old boy. Thi-Qar province Naegleria fowleri was diagnosed in direct cerebral spinal fluid sample and cerebral spinal fluid cultured on non-nutrient agar medium, the flagellated and amoeboid trophozoite stages were observed, as well as the cystic form. The molecular detection of Naegleria fowleri showed PCR product was 183bp for 18srRNA gene. The cerebral spinal fluid must be microscopically examined in order to identify the amoeba stages and rule out the bacterial cause. Accurate and rapid diagnosis is important to treat the condition as quickly as possible Genetic identification can also be used to diagnose amoeba. Amoebic meningoencephalitis treatment may benefit from a prompt diagnosis. Preventing amoebic meningoencephalitis infection can be achieved in part by refraining from swimming or utilizing freshwater. Finally, clinical and laboratory personnel must be trained on the importance of prompt diagnosis and proper patient care for individuals with suspected primary amebic meningoencephalitis.

Key W: Primary amebic meningoencephalitis, Naegleria fowleri

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INTRODUCTION

The highly infectious free-spirited amoeba Naegleria fowleri is predominantly harvested from freshwater and soil during the sun-drenched summer months, as the sweltering temperatures forge a perfect environment for its proliferation and dissemination.[1] Naegleria fowleri, a self-sustaining amoeba, thrives in thermal freshwater, flourishing best in temperatures ranging from 35 to 46 degrees Celsius, and multiplies through the process of binary fission. This parasite undergoes a tripartite life cycle comprising a dormant cyst phase, a mobile flagellate phase, and a vigorous amoeboid trophozoite phase; notably, the amoebic form is consistently identified in brain biopsies, and tragically, the disease proves fatal regardless of timely and accurate diagnosis due to the challenge of delivering effective drug concentrations to the infection site.[2]

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The first recorded human infection case by N. fowleri took place in South Australia, documented by Fowler and Carter.[3] This remarkable microbial organism often thrives alongside various other microbial counterparts. Within the natural world, microorganisms frequently engage in interactions with eukaryotes.[4] Such interactions occur between humans and free-living amoebas; Primary amebic meningoencephalitis (PAM) is an uncommon and swiftly progressing illness that generally culminates in death within a mere 3 to 6 days. The condition is instigated by the bacterium Naegleria fowleri and has a dire impact on the central nervous system. The emergence of symptoms follows shortly thereafter.[5] Dubbed the "brain-eating" thermophilic flagellate amoeba, primary rhizopodan meningoencephalitis stands as a lethal and debilitating waterborne affliction.[6] Naegleria fowleri makes its journey to the brain, traversing the cribriform plate and infiltrating the body through the nasal canal.[7] Alternatively, it may navigate via the olfactory nerve and cribriform plate to reach the olfactory bulbs. Thus, it causes the central nervous system (CNS) to be destroyed, which ultimately results in primary amebic meningoencephalitis.[8] Globally, N. fowleri has been reported, notably in Thailand, Taiwan, and Hong Kong. America and Australia.[9] There has only been one instance reported in to yet in the north of Iraq in Mousal City.[10] In another study, it was widely diagnosed N. fowleri in Iraq by AL-Aboody[11] in clinical and environmental samples and founded widespread in

contaminated water and soil. Our case, this is first case in Thi-Qar and the second in Iraq, and none of them survived because of a severe infection in Brain, very late cases and increase of cerebrospinal fluid (CSF).In our study and othere studies we observed increased accuracies and prevalence of N. fowleri and other species of Naegleria such as N. polary in water and soil in huge numbers in all studies presented in Iraq.

CASE REPORT

In this case, cerebrospinal fluid was obtained from Dr. Muhannad Al-Assadi's private laboratory, Iraq vide letter No. 4545/QM/Approval/SJKDH379 dated March 9, 2024, belongs to an 11-year-old boy who lives in the city of Nasiriyah. On September 2022, patient was sever from headache, fever, fatigue, also a facial rash and myalgia. After that, he was transferred to a hospital to receive the necessary treatment. but he died before any treatment was given. He was sever from his vital signs such as temperature, 39.1°C; respiratory rate, 32 breaths/min; blood pressure, 122/56 mmHg. The blood test showed the leukocytes count of 11cells/mm3 with 60% neutrophils and C-reactive protein (CRP) of 285 mg/L and a low glucose of 15 mg/dL,. Then, lumbar puncture was dispensed, the CSF was turbid. Within twenty-four hours of collection, one milliliter of CSF sample was grown on non-nutrient agar (NN-agar) medium in duplicates in the lab. The samples were then incubated in 26 C0, and amoebic growth was monitored every day using a light microscope on a slide. and examined weekly with addition page amoebic slain (PAS) solution .The cultured of CSF show clearly movements of amoeboid trophozoites ,flagellate stages and cyst during examined on light microscope .Also, Nelson's growth medium with fetal calf serum can be used to culture Naegleria fowleri (Nuha,2022). N. fowleri trophozoite was observed with oval shape which measured 10–20 µm in size (Fig. 1). Naegleria amoeboid trophozoite. Figure 2 showed (A) cyst and(B) cyst with flagellated trophozoites form. Figure 3 emergence trophozoites excyst when trophozoite have suitable environment such as increase food.

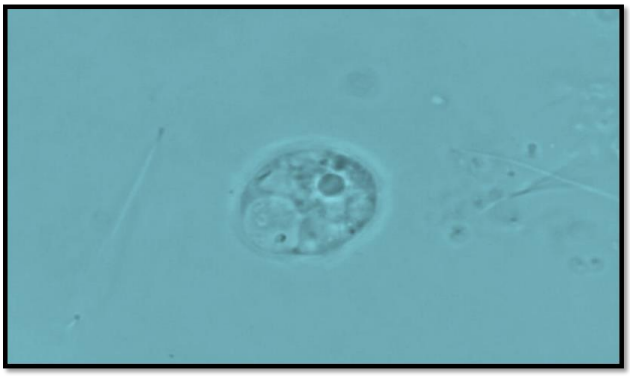
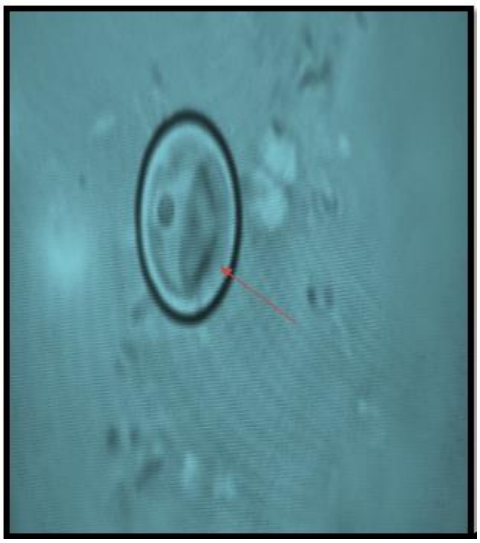


Figure No. 1: Naegleria fowleriamoeboid trophozoites (unstained)



A **B**

Figure No. 2: A Naegleria fowleri A-cyst stage. B} cyct and flagellated trophozoite and cyst from cultured CSF (unstained)

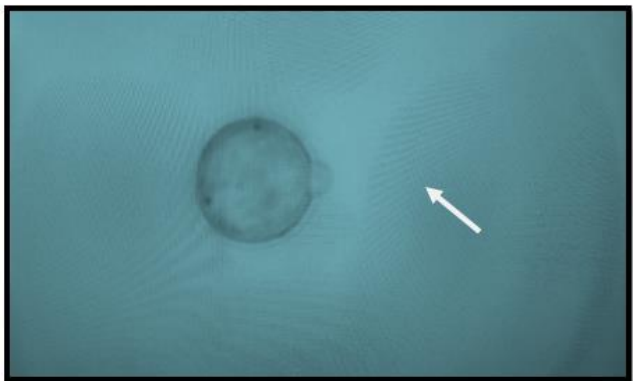


Fig. 3: Naegleria fowleri start emergence trophozoite from the cyst (unstained)

Analysis of Genetics: Genomic DNA was meticulously isolated from both direct CSF and CSF cultures, adhering to the company's guidelines using the innovative gSYNC TM Kit for DNA Extraction (Geneaid, Taiwan). Employing the 18S rRNA gene, PCR amplification was deftly utilized to detect the presence of N. fowleri. The PCR reaction was conducted within a total volume of 25 µl, encompassing 5 µl of each primer (10 mM), 12 µl of nuclease-free water, along with 5 µl of the Green Master Mix (Pre-Mix master mix Biolab/UK), and 5 µl of template DNA sourced from the CSF sample. The AccuPower® PCR PreMix Kit, which contains all essential components necessary for a successful PCR reaction, features tracking dye, dNTPs, KCl, MgCl2, stabilizer, and Tris-HCl at pH: 9.0.. The primers sequence applied (Bioneer/Korea) in the current study accord-ing to a previous study (Schild et al., 2007).Naegleria fowleri forward primer 3' -5' CAAACACCGTTATGACAGGG and Reveres primer 5' -3'

CTGGTTTCCCTCACCTACG (Schild et al., 2007). PCR step of thermo-cycling The conditions were: 1 minute of initial denaturation at 95 °C; 35 seconds of second denaturation at 95 °C for 35 cycles; 35 seconds of annealing at 58 °C for 35 cycles; and 40 seconds of extension at 72 °C for 35 cycles. However, the last extension was for ten minutes at 72°C. The PCR technique was performed for detection pathogenic Naegleria fowleri (FLA) based on subunit ribosomal rRNA gene (18SrDNA gene) from cell culture of N. fowleri, this study using using convential PCR. The PCR product was electrophoresed in 0.8% or 1.5% agarose gel powder, stained with Ethidium bromide, and visible under a UV lamp. The nucleotides from the DNA sequencing were sent to a Macrogene company in Korea, and the global isolates in Gen Bank were compared using BLAST.Ladder DNA (1517 bp) and the amplification band size of 183 bp were compared with primers to get the final results for PCR.

Figure No. 4: Image Agarose gel electrophoresis that show the PCR product (183bp) analysis of 18S r DNA gene from genomic DNA of Naegleria fowleri from CSF of patients with primary amebic meningoencephalitis. This image show (1,3 band direct from CSF and7,8 band from CSF cultured)

The sequencing and analysis of PCR products of Naegleria fowleri from CSF sample and CSF culture were showed 97. 96% homology identity to Naegleria fowleri. The 18S rRNA gene (accession numbers KT375442.1, OD958550.1, and MW033524.1) corresponds to Naegleria fowleri, which was found in a fatal case of primary amoebic meningoencephalitis in a Norwegian traveller who had just returned from Thailand.

DISCUSSION

The occurrence of Naegleria fowleri is surging among the youth and children in Iraq. This marks the second instance of reporting PAM in Iraq, with Thi-Qar being the location of the inaugural case. Typically linked to aquatic pursuits like diving and head immersion, the primary culprit behind this affliction is the inhalation of tainted water that finds its way into the nasal passages, potentially leading to the cribriform plate via the nasal cavity. Between 1962 and 2017, there were 143 documented cases of PAM in the USA.[12] Most of the patients in Chinese cases had prior encounters with fresh water.[13] The CSF chemistry of PAM mirrors that of bacterial meningitis, showcasing a decrease in glucose alongside an uptick in protein levels. N. fowleri fails to thrive on conventional culture media, as it necessitates bacteria-infused agar for growth. When "clearly motile" N. fowleri trophozoites make an appearance on the wet preparation of a fresh CSF sample, microscopy gains reliability.[14] Successful treatment of PAM cases hinges on swift and precise diagnosis.[15] A history of water exposure holds immense significance for prompt diagnosis. The clinical symptoms observed in this case study were remarkably similar to those in previously documented cases.[14] In other research efforts within Iraq, they validated the presence of highly contaminated samples with N. fowleri in both clinical and environmental contexts within Thi-Qar.[11] N. fowleri exhibits potent pathogenesis and produces regulatory surface proteins that shield the amoeba from the cytotoxic elements of the complement system, orchestrating lysis to deftly elude the host's immune defences.[7] Through the lens of molecular techniques, N. fowleri trophozoites, measuring 10–20 microns in diameter, can be seen to exhibit motility in freshly collected wet CSF samples under light microscopy. Nonetheless, the number of identifiable amoebae might be quite limited. These challenges can be effectively addressed via the molecular strategy of PCR testing.[16,17]

CONCLUSION

Primary amoebic meningoencephalitis is one of the most important and widespread diseases in Iraq, as evidenced by its spread in Iraqi water, frequent cases of meningitis after exposure to water swimming pools, rivers, as well as the water used for drinking that reaches homes, is also contaminated with this parasites, sterilizing drinking water in Iraq because it is also used to wash the body and accidentally inhaled by children and adults. CSF examined must be considered essential in identification PAM disease infection. Naegleria fowleri is considered one of the causes of meningitis throughout Iraq and Thi-Qar in particular. This is the first case recorded in Thi-Qar and the second case after case recorded in Mosul. Chlorinating heavily utilized swimming pools in Iraq - one part per million will halt the spread of N. fowleri. Local public health officials in high-risk areas ought to consider keeping an eye out for N. fowleri in recreational waterways, particularly in Iraq in the sweltering summer months.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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