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Case Report

Genetic Detection of Amoebic Meningoencephalitis Causing by *Naegleria Fowleri* in Iraq: A Case Report

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Abstract

We diagnosed a case report of amoebic meningoencephalitis by *Naegleria fowleri*. This case represented the first recording in Iraq where it was not recording previously. This case was diagnosed after the death of an 18-year-old girl patient who lived in a rural area of Mosul in Iraq. Genetics detection of *N. fowleri* showed PCR product was 183bp for *18S rRNA* gene. It was registered as the first recording of Iraqi isolate *N. fowleri* in GenBank with accession number OP380864.1. It is necessary to examine microscopically the cerebral spinal fluid (CSF) to observe the amoeba stages and exclude the bacterial causative. Rapid diagnosis may help in the treatment of amoebic meningoencephalitis. In addition, genetic identification can diagnose amoeba. Avoiding swimming or using freshwater contributes to prevent amoebic meningoencephalitis infection.

Introduction

Naegleria fowleri is a free-living amoeba; that causes primary amoebic meningoencephalitis (PAM). It is considered a rapidly fatal disease to the central nervous system. *N. fowleri* lives in warmer freshwater. This amoeba passes the body through the nasal passage, crosses the cribriform plate, and enters the brain (1). *Naegleria* species were isolated from ponds, freshwater lakes, domestic water supply, swimming pools, soil, hot pools, and dust. The first recording of human illness by *N. fowleri* was by Fowler and Carter in South Australia (2).

N. fowleri is recognized as a "brain-eating amoeba" due to its enhancement of intense encephalitis during infection, with a death rate above 95% (3). More than 40 species are from *Naegleria*; only *N. fowleri* causes PAM. PAM is a rapid, severe disease development, with an incubation period between 2-15 days and death occurring 3–7 days after the beginning of symptoms (4).

N. fowleri was recorded worldwide, including in America, Australia, Hong Kong, Taiwan, and Thailand (5). There are 300 statuses in total recorded in 50 years from the first status recorded in 1965 (3). *Naegleria* was diagnosed in drinking water and tanks, the hot seasons recorded a high number of cysts for the free-living amoeba (6). Furthermore, *Naegleria* has been diagnosed in the freshwater Euphrates River in Iraq (7). Other free-living amoeba in Iraq, such as *Acanthamoeba* spp. in one sample of CSF, one sample of eyes, and two samples of skin were diagnosed (8). It was diagnosed as ciliophora (Orchitophryidae) in the CSF sample from Thi-Qar Province/Iraq as the first recorded case (9).

Rapid diagnosis considers significant to initiate therapy immediately as possible. However, rapid diagnosis is not simple for those without expertise in the therapy infection of *N. fowleri*. PAM is mostly misidentified because there is no difference in diagnosis to recognize PAM from meningoencephalitis by bacteria (10). We present here a case of amoebic meningoencephalitis caused by *N. fowleri*.

Case report

We present here a case of an 18-year-old of the girl from Mosul Province in Iraq. She was living in a rural area. She used the river's water for sheep grazing, household uses, and personal cleanliness. In addition, she had loss-weight and malnutrition while not having a history of immune deficiency. She suffered from fever, severe headache, and stiff neck for two days; after then, she was admitted to the hospital (AL-Salam Hospital - Mosul City/March 2022), but her exposure to a coma and died before any therapy was given.

We obtained ethics approval from the Ministry of Health in Iraq Ethics Committee. Approval code (Ref: 6355 in 3/8/2022). Tests were done to find out the cause of death. CT scan was conducted. Neurologists collected cerebrospinal fluid (CSF) from the patient. The results appeared no viral or bacterial infection. CSF characterized the increasing turbidity as milky. It was the results of other examinations for CSF (Glucose: 10 mg /dl low; Protein: 90 mg/dl high; Lactate: 72 mg/dl high; White blood cells 11,100 cells/µL; RBCs 60 cells/µL; Differential count: Neutrophilia). The brain CT (computerized tomography) scan appeared non-specific oedema (Fig.1)

The trophozoite for *N. fowleri* was observed. There were various shapes of trophozoites, which measured 15–20 µm in size (Fig.2). *Naegleria* trophozoite appear encyst after freezing trophozoite and deficiency food. In addition, other smears were prepared by Leishman stain (Fig.3 A,B,C, D). Fig.4 shows the flagellated form of *Naegleria*.

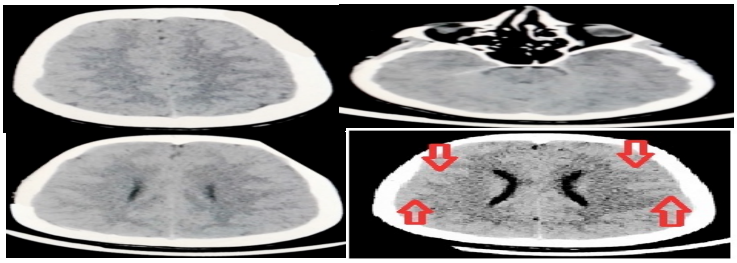

Fig. 1: Non contrast Computed Tomography(CT) scan appear non-specific oedema **(Red arrow)**

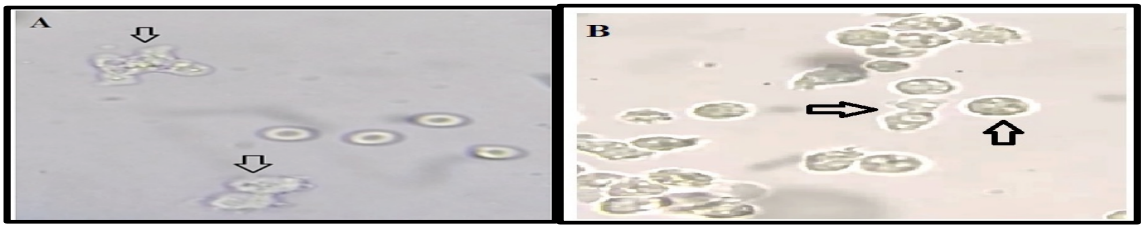

Fig. 2: A and B *Naegleria* trophozoites (round to pear-shape cells) in CSF direct smear unstained (Original)

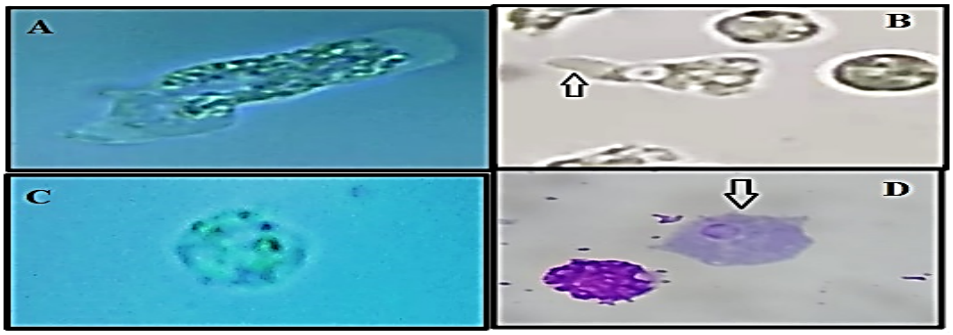

Fig. 3: (A and B) *Naegleria* trophozoite during movement with pseudopodia in CSF direct smear unstained ;(C)encyst after freezing trophozoite;(D) *Naegleria* trophozoite in CSF smear stained with Leishman stain (Original)

Fig. 4: The flagellated form of *Naegleria* (Original)

Genetic analysis

Genomic DNA was extracted from CSF (200 µl) using Zybio ™ RNA/DNA extraction kit (Zybio /China). PCR amplification was conducted to identify *N. fowleri* using *18SrRNA*. PCR reaction was carried out in a total volume of 25 µl, including 12 µl of free nuclease water, 1.5 µl of each primer (10 mM), 5 µl Green Master Mix (Pre-Mix master mix Biolab/UK), and 5 µl of template DNA from CSF specimen. The primers (Bioneer/Korea) sequence applied in the current study according to a previous study (11) which used a specific primer of *Naegleria* from 18S rRNA gene were Forward 3'-CAAACACCGT TATGACAGGG-5',and Reverse5'-CTGGTTTCCCTCACCTACG-3'. PCR of thermocycling conditions were initial denaturation for 1 min at 95 °C, after then the second denaturation for 35 sec at 95 °C for 35 cycles, annealing for 35 sec at 58 °C for 35 cycles and extension for 40 sec at 72 °C for 35 cycles. Whereas the final extension was at 72 °C for 10 min.

The PCR reaction was performed by PCR Thermocycler (T100Thermal cycler/Biorad/ USA). PCR product was electrophoresis in agarose gel (1.5%) stained by Ethidium Bromide and visualized by UV-Transilluminator. PCR amplicons were sequenced by sending them to a Macrogene company in Korea the sequence nucleotides result was edited by BioEdit software and its comparison by BLAST with global isolates in GenBank. The results of PCR were a band size of amplification 183 bp compared with Ladder DNA 1517 bp (Cat:N3231S/ Biolabs / UK) .The results evidenced the patient was infected by *N. fowleri* (Fig. 5). Furthermore, sequencing was performed by Macrogene/Korea. It was registered as the first Iraqi isolate *N. fowleri* recordingin GenBank with accession number OP380864.1. This local isolate, after conducting BLAST was identical at 96.4% with KT375442.1 (Norway), MT741533.1, MW033524.1, and AY376150.1 (USA).

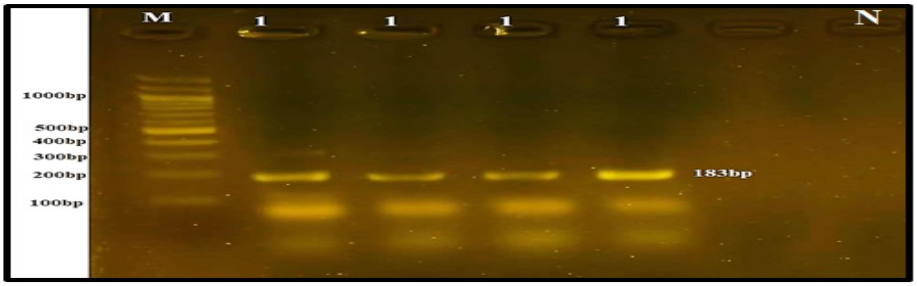

Fig. 5: PCR product with band size 183bp of 18S rRNA gene for genomic DNA of *Naegleria fowleri*, Lane M represents DNA Marker (1517bp), and the well number 1 represents the patient's sample, and other wells represent the same sample in three replicates, Lane N represent negative control

Discussion

This case considers the first reporting case of PAM in Iraq; and focuses on the importance of considering clinical doubt. The prevalence is increased in children and young adults. It is probably more associated with water activities such as diving and submerging the head, which can push water to the cribriform plate by a nasal cavity. PMA cases in many countries, such as the USA, recorded 143 PAM conditions from 1962 to 2017 (12).

PAM diagnosis considers a challenge due to the resemblance between the PMA symptoms and other cases, such as bacterial meningitis. PAM has a fast development period of 6-17 days after primary exposure, which causes death if not instantly diagnosed. Particular di-

agnostic devices and generic consciousness of PAM are wanted for treatment immediately (13). Few PAM cases were successfully treated due to accurate and rapid diagnosis (14, 15). The history of exposure to water considers significant in the diagnosis of PAM. The case in this study had clinical symptoms identical to previously recorded cases (2,16-18).

Our patient showed signs including fever, chills, confusion, photophobia, respiratory distress, and meningeal agitation. PAM symptoms are non-specific and involve acute headache, fever with high grade, confusion, and stiffness neck, occurring death, in most conditions, is result-increasing pressure in the skull and hernia (12). *N. fowleri* enhances pore-formation proteins during contact of cells, which leads to the lysis of mammalian cells and excretion of cytolytic molecules involving hydrolase, phospholipase, neuraminidase, and phosphorolytic enzymes. The organism also synthesizes regulatory surface proteins that protect amoeba against complement system-regulated lysis and cytotoxicity substances, consequently avoid the immunity of host (1).

Laboratory results of patients' PAM showed leukocytosis (polymorphonuclear cells). CSF is purulent with leukocytosis, increasing protein and decreasing glucose (2,16). In most conditions, neurological examinations in the early infection periods did not detect brain abnormalities (19). However, some cases in CT scan appear diffusion of cerebral edema (20). Besides, the diffusion sulcus appeared in the brain and encephalopathy due to hypoxia in one case infected with *N. fowleri* in USA (12). Meningitis cases suspicious need CSF specimens in their diagnosis; in addition to culture, gram stain, and diagnostic by molecular methods, also white blood cell count and biochemistry analysis (17). In PAM, the chemistry analysis of CSF mimics meningitis of bacteria with elevated protein and declined glucose. Thus, the amoeba is not diagnosed with gram stains.

N. fowleri did not grow on the standard cultures media, which demands agar enrichment with bacteria. Therefore, microscopic detection has a role in the detection of *N. fowleri* trophozoite, which can be seen as motile in wet fresh CSF specimens when without using molecular methods. Still, there may be few amoebae to diagnose. Therefore, those challenges can be controlled via the molecular method (PCR tests), which supplies a credible diagnosis (18). Consequently, the cerebral spinal fluid examination in cases of PAM similar to meningitis symptoms is essential, as a rapid diagnosis may help in treatment. Genetic identification can diagnose amoeba infection.

Conclusion

PCR technique appeared beneficial in quickly identifying the causative pathogen, especially for PAM, despite the patient's death. This technique should be used in diagnosis due to assist physicians in clinical diagnosis. In addition, it was registered as the first recording of Iraqi isolate *N. fowleri* in GenBank with accession number OP380864.1.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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