Short Communication

Retrospective Study of Parasites in CSF Samples in a Tertiary Care Hospital: A 10 Year Experience

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Received: 06 August 2016 Initial Review: 16 August 2016 Accepted: 29 August 2016 Published Online: 06 September 2016

ABSTRACT

Background: Free-living amoebae are widely distributed in mud, moist soil and fresh water worldwise, particularly members of the genera Acanthamoeba and Naegleria. Balamuthia mandillaris causes granulomatous amoebic encephalitis in human and animals. **Methods:** 2516 CSF samples were received in the mycology laboratory and processed for free living amoebae by microscopy and culture. **Results:** Out of 2516 samples, 32 were found to be positive for parasites. Out of 32 cases, 27 were identified as Acanthamoeba spp., one was diagnosed as Naegleria fowleri and the remaining 4 had presumptive diagnosis of Balamuthia mandillaris. Naegleria fowleri is the causal agent of a fulminant CNS condition, primary amebic meningoencephalitis (PAM). **Discussion:** PAM is a rare disease but almost always fatal. Therefore, early diagnosis is important in order to start treatment. Systemic amoebic diseases are difficult to diagnose and it is likely that many cases go unrecognized and are under-reported. Most of the diagnosis is made at post-mortem.

Keywords: Free-living amoebae , Acanthamoeba, Naegleria, Balamuthia mandillaris

Free-living amoebae are widely distributed in mud, moist soil and fresh water worldwise, particularly members of the genera *Acanthamoeba* and *Naegleri* [1-2]. Since 1960s, they have been recognized as opportunistic human pathogenic parasites, capable of causing fatal Central nervous system (CNS) infections like meningitis and encephalitis in both immunocompetent and immunocompromised hosts. *Naegleria fowleri* is the causal agent of a fulminant CNS infection, primary amebic meningoencephalitis (PAM) [1]. PAM is a rare disease but almost always fatal. Therefore, early diagnosis is very important in order to start treatment. The disease is often misdiagnosed because no distinctive differences in diagnosis exist to distinguish PAM from bacterial meningoencephalitis.

Direct microscopic examination of CSF as a wet mount is the method of choice for the diagnosis of PAM because CSF contains motile amoebae which can be recognized quite well by light microscopic observation [3-5]. Although Gram stain is used in clinical laboratories for detection of bacteria in CSF, Gram stain is not useful for diagnosis of amoebae because it does not depict the characteristic nuclear morphology of the amoebae. Amoebae can be mistaken as macrophages, but *N. fowleri* nucleus contains a large, central, round nucleolus which should distinguish it from host cells [4-7]. *Acanthamoeba* has two morphological forms in its life cycle, a trophozoite and a cyst stage. Both stages can be found in tissues of infected humans and in the environment. The trophozoite is the dividing form and is thought to be the infective

stage. The cysts are dormant and protect the amoebae from harmful environments. The cysts are resistant to biocides, chlorination, and antibiotics.

Acanthamoeba causes a more chronic and subacute infection termed granulomatous amoebic encephalitis and amoebic keratitis1. GAE, also known as *Acanthamoeba* Granulomatous Encephalitis (AGE), is a rare, chronic, progressive infection of the CNS that may involve the lungs [8]. GAE is usually associated with an underlying debilitating disease or immune suppressed individuals including HIV-AIDS patients, diabetics, and individuals undergoing organ transplants or cancer chemotherapy, and drug abusers [8-11].

Balamuthia mandillaris causes granulomatous amoebic encephalitis. Although *Balamuthia* is considered an opportunistic pathogen, it can cause disease in both immune compromised and immune competent individuals. Infections can occur in children and adults. Although the number of infections caused by them are low in comparison to other protozoal parasites, the difficulty in diagnosing them, the challenge of finding optimal antimicrobial treatment and high mortality rate have been a concern for clinicians and parasitologists. Mortality is very high and usually within a week of presentation, with the median time from symptom onset to death typically being 5 days [12]. Prevention is by chlorination of swimming pools [13].

Drugs used for treatment are Azithromycin, Pentamidine, Itraconazole and Flucytosine [14]. CDC is now investigating the use of miltefosine (also used to treat leishmaniasis) in combination with other drugs [15]. This drug has shown amoebicidal activity against several free-living species of amoeba (including *N. fowleri*) in the laboratory and has been used successfully to treat patients infected with *B. mandrillaris* and disseminated *Acanthamoeba* spp. [16]. Keeping these things in mind, our retrospective study aimed to see the distribution of free living amoebae in the CSF samples received in the lab.

MATERIALS AND METHODS

A total of 2516 CSF samples were received with full aseptic precautions in sterile screw capped universal containers from the year 2004 to 2013 in the Department of Microbiology of the institute from various departments. On macroscopic examination of CSF, it was found to be clear in most cases. There was no evidence of haemorrhage. After centrifugation at 3000 rpm for 15 minutes, the supernatant was discarded and the deposit was used for microscopy and culture. For microscopy, India ink preparation and wet mount were carried out. Culture was carried out from the deposit on Sabouraud's Dextrose Agar (SDA) slant, Brain Heart Infusion agar (BHI) and non-nutrient agar (NNA) layered with *Escherichia coli* ATCC 25922 available in the department. All the culture plates and tubes were incubated at 370C aerobically for 3 weeks before ruling out as negative. Identification was mainly based on typical microscopic morphological features and the motility of trophozoites. On non-nutrient agar (NNA) layered with *Escherichia coli*, cystic form was revealed and observed from microscopic examination of growth obtained on it.

RESULTS

A total of 2516 CSF samples were received and processed in the mycology laboratory of the institute in this period. Out of these 2516 CSF samples, 32 (around 1.3 %) were found to be positive for protozoa; out of these 32, 27 (around 85%) were identified as *Acanthamoeba* spp., one was diagnosed as *Naegleria fowleri* and the remaining 4 were presumptively diagnosed to be *Balamuthia mandillaris*. All were identified based on microscopic morphology.

DISCUSSION

Amoebic meningitis and encephalitis are very important clinical entities which can confuse clinicians due to their non-specific clinical features. Signs and symptoms of PAM, which occurs commonly after swiming in lakes, swamps and hot water springs, are very similar to those of bacterial or viral meningitis and typically include headache, fever, stiff neck, anorexia, vomiting, altered mental status, seizures, and coma [12]. Being thermophiic, *Naeglria fowleri* can easily be found in hot springs and tropical lakes [12]. In fact, it tolerates temperatures of up to 45°C and survives well during warmer months of the year when the ambient temperature increases [12].

In the U.S., most infections are reported from freshwater lakes, rivers, and hot springs located in the Southern states; recently, people have become infected in the northern states also following periods of very warm temperatures [13]. Hence they are difficult to pick up, and

although rare as found out in our study, key to diagnosis is accurate microscopy and culture on specific media. Primary amoebic meningoencephlaitis is more fatal than Granulomatous amoebic encephalitis. Since detection rests of microscopic morphology, smears and mounts of CSF should be meticulously observed before ruling as negative.

CONCLUSION

Systemic amoebic diseases are difficult to diagnose and it is likely that many cases go unrecognised and are under-reported. Most of the diagnoses are made post-mortem. They should be looked for quite meticulously for they are likely to be missed or misidentified, and development of modern techniques to improve their isolation and cultivation will help in obtaining a more accurate assessment of this fatal infection.

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How to cite this article: Priyadarshini, Kumar D, Kumar D, Tilak R. Retrospective Study of Parasites in CSF Samples in a Tertiary Care Hospital: A 10 Year Experience. Eastern J Med Sci. 2016; 1(1): 34-36.

Conflict of interest: None stated, Funding: Nil