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Case Reports

Free-Living Amoebae and Central Nervous System Infection: Report of Seven Cases

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Abstract: The Free-Living Amoeba (FLA) is an opportunistic protozoan with a cosmopolitan distribution that can cause Central Nervous System (CNS) infection. It develops in relatively stagnant waters such as swimming pools, lagoons and ponds but only species belonging to the genera *Hartmannella*, *Naegleria*, *Acanthamoeba* and *Balamuthia* have been found in humans. The resulting pathology is highly lethal due to the lack of effective treatment. The aim of this study is to describe a series of neuroinfection cases treated at the *Hospital de Infectología* in Mexico City. This is a descriptive study conducted between July 2008 and June 2016. It includes all patients admitted with signs and symptoms of meningoencephalitis and a laboratory work-up confirming the presence of FLA trophozoites in Cerebrospinal Fluid (CSF). Statistical Analysis. Nominal variables are reported as relative frequencies and quantitative variables, as medians, maximum and minimum. Seven cases were identified, 43% of which were male. The median number of days between exposure and symptom development was nine days. The most frequent symptoms were: Headache 57%, vomiting 29%, fever 57%, meningeal irritation 43% and altered consciousness 86%. Three of the seven analyzed cases died and one case was also HIV positive. It is important to consider this presumptive diagnosis in order to search for FLA in tissues or CSF and obtain cultures in selected media. Mortality is high, particularly when the brain is compromised.

Keywords: Free-Living Amoebae, (*Hartmannella, Naegleria, Acanthamoeba, Balamuthia*), Meningoencephalitis, Amphizoic

Introduction

Infectious disease due to Free-Living Amoebae (FLA) are now evidently present and recognized as a result of the communication of a case in 1948; in 1965, Fowler and Carter reported the first case of primary amoebic meningoencephalitis due to *Naegleria fowleri* in Australia (Fowler and Carter, 1965). FLA have particular morphologic, biochemical and molecular characteristics (Adi *et al*., 2005) and based on the classification established by the International Society of Protozoologists, they are considered amphizoic amoebae (Page, 1967), that usually develop as parasites in relatively stagnant waters such as swimming pools, lagoons and ponds. They are opportunistic microorganisms with a cosmopolitan distribution that can cause Central Nervous System (CNS) infections. They can survive freely and as endoparasites they proliferate at temperatures between 4 and 45 degrees Celsius. There are numerous FLA in the soil and in water, but only species of the genera *Hartmannella*, *Naegleria*, *Acanthamoeba* and *Balamuthia* have been detected in humans. Free-living amoebae can lead to four clinical syndromes: Primary Amoebic Meningoencephalitis (PAM), Granulomatous Amoebic Encephalitis (GAE), disseminated granulomatous amoebic disease (skin, lung and paranasal sinus infection) and amoebic keratitis (Visvesvara *et al*., 2007; Chong and Dana, 2007).

Primary amoebic meningoencephalitis develops throughout the world and to date, most cases have been reported during the summer in the southeastern United States, Australia and some European countries. Few cases have been reported: Approximately 250 cases of *N*

fowleri and 156 of *Acanthamoeba sp* in the CNS and 300 cases of keratitis. These are not infections strictly requiring epidemiological reporting so their true frequency is unknown. Infections due to FLA are associated to a 95% mortality, compounded perhaps by untimely diagnoses and the lack of appropriate and effective treatment (Peralta-Rodríguez and Ayala-Oviedo, 2009; Oddó, 2006; Petit *et al*., 2006).

Free- living amoebae that are pathogenic to man have been identified in the water of swimming pools as well as natural recreation waters in several parts of the world, that are now considered as dangerous contact sources (Ávila *et al*., 2006; Petit *et al*., 2006; Muñoz *et al*., 2003; Sánchez-Lihon *et al*., 2004).

Further, there are case reports unrelated to contact with stagnant waters, particularly due to *Balamuthia mandrallaris* (Visvesvara *et al*., 2007; Matin *et al*., 2008).

The clinical manifestations of FLA infections are mainly in the CNS, although infection can also be manifested as keratitis or as indurated skin lesions that can subsequently lead to neuroinfection (Sánchez-Lihon *et al*., 2004). *Naegleria fowlerii* (N fowleri) causes PAM; Acanthamoeba *sp* and *Balamuthia mandallarias (B mandrallaris)* are the etiologic agents of GAE. Clinically, infection by *N. fowleri* has an acute fulminant presentation in previously healthy individuals that have recently submerged in warm swimming pools, ponds or rivers in the summer months, 3 to 7 days before symptoms first appear. *Acanthamoeba sp* requires an immunosuppressed host or one with immune deficiency that will promote its transformation into a facultative parasite, whereas *B mandrallaris* affects both healthy and immunosuppressed individuals, following a more indolent but no less lethal course (Oddó, 2006).

The aim of this study is to describe a case series of neuroinfections due to free-living amoebae and treated at the Hospital de Infectología, IMSS, in Mexico City.

Material and Methods

Design. We conducted a retrospective descriptive study between July 2008 and June 2016, at the Hospital de Infectología del Centro Médico la Raza, IMSS. We included all patients admitted with signs and/or symptoms of meningoencephalitis and with laboratory results reporting the presence of free-living amoebic trophozoites in Cerebrospinal Fluid (CSF). An epidemiologic analysis was obtained in all cases and patients were followed until the final outcome.

Definitions

A confirmed case was defined as any individual with clinical manifestations compatible with central nervous system infection and in whom FLA were identified by laboratory testing (direct observation of CSF). FLA meningoencephalitis was clinically considered in cases of abrupt or insidious onset severe frontal and/or bitemporal headache, fever, nausea, meningeal irritation, nuchal rigidity and positive Kernig and Brudzinski signs, encephalitis, photophobia, projectile vomiting, cerebral edema, seizures, intracranial hypertension, lethargy, confusion and coma, with subsequent laboratory confirmation of trophozoites in CSF.

Microbiological Analysis

Cerebrospinal fluid was obtained by lumbar puncture for cytochemical and cytological analysis, as well as bacterial and mycobacterial culture. Direct microscopic examination revealed the presence of amoebic trophozoites (FLA).

Statistical Analysis

Relative frequencies were calculated for nominal variables and central tendency and dispersion measurements were used for qualitative variables; population distribution was determined with the Shapiro-Wilk test.

Results

During the study period, seven cases were identified; their average age was 38, 6 were residents of the State of Mexico and one was from Mexico City. Three of the 7 cases had a recently bathed in a river or with water from a well and a risk factor was not established in the remaining patients. Forty-three percent (43%) of them were male. The mean number of days between exposure and symptom onset was nine days, with a minimum of seven and a maximum of 11 days; the median number of days until the diagnosis was established was nine days.

The Shapiro-Wilk test is a powerful test used in small samples to identify normality contrast in a population; in this study, we considered a p value of 0.64 when analyzing the number of days needed to establish the diagnosis: No significant differences were found in the number of days until diagnosis and also in the age analysis (Table 1). FLA meningoencephalitis was clinically suspected in 43%, FLA neuroinfection diagnosis was established in 14 % and bacterial meningoencephalitis was suspected in 29%; although the initial diagnosis in this case was bacterial meningoencephalitis, subsequent laboratory results established the diagnosis of FLA meningitis.

The final diagnoses were: FLA meningoencephalitis in 57% of cases, bacterial meningoencephalitis in 14% and FLA neuroinfection in 29%. The most frequently identified symptoms were headache 57%, vomiting 29%, fever 57%, meningeal irritation 43% and altered consciousness 86%.

Three of the 7 cases died (43%) and one case was HIV positive (Table 2).

Table 1: General characteristics of the patients

	Min	Max	Median	P*
Age	14	86	38	0.27
Days until Diagnosis	3	19	9	0.64

*Shapiro-Wilk

Table 2: Clinical and epidemiological characteristics of the patients

Characteristics	Yes N (%)	No N (%)	Unknown N (%)
Exposure to non-potable water (submersion in ponds, rivers)	3 (42.9)	0	4 (57.1)
Symptoms and signs			
Headache	4 (57.1)	3 (42.9)	0
Vomiting	2 (28.6)	5 (71.4)	0
Fever	4 (57.1)	3 (42.9)	0
Gait abnormalities	5 (71.4)	2 (28.6)	0
Altered consciousness	6 (85.7)	1 (14.3)	0
Meningeal irritation	4 (57.1)	3 (42.9)	0
Associated factors			
Excess weight	1 (14.3)	2 (28.6)	4 (57.1)
Diabetes	1 (14.3)	3 (42.9)	3 (42.9)
HIV	1 (14.3)	3 (42.9)	3 (42.9)
Treatment			
Amphotericin B	7 (100)	0	0
Evolution			
Death	3 (42.9)	4 (57.1)	0

Discussion

Infections by free-living amoebae are infrequent, or perhaps underdiagnosed. Their course tends to be fatal regardless of the patient´s immune status. The relationship between poor sanitation and the transmission of FLA to humans and the subsequent development of fatal CNS involvement, remains to be determined (Koshy, 2012). A history of recent exposure to apparently stagnant waters in river ponds or warm swimming pools is frequent, although to date, the factors contributing to the development of CNS infection remain unknown. The disease has been reported in patients with AIDS, diabetes mellitus and in the post transplant period, as well as in patients on steroid therapy. Apparently, a risk factor for the development of FLA keratitis is the use of contact lenses. FLA infection has also been reported in immunocompetent individuals with no evident associated risk factors (Schustr and Visvesvara, 2004). As in the case of other microorganisms, the pathogenicity of FLA is closely linked to the characteristics of the host and of the organism; FLA probably reach the CNS by hematogenous dissemination (Siddiqui *et al*., 2011). In this case series, as in those reported by other authors, the need for the clinician to consider these organisms as possible etiologic factors of CNS infection, cannot be overemphasized; in general, exposure to stagnant waters, recent swimming or playing in sweet water or natural or artificial thermal waters, can be readily identified, so considering FLA neuroinfection in the differential diagnosis with bacterial meningitis in children and adolescents engaging in aquatic activities, is mandatory.

This retrospective study revealed that in over 50% of cases, the risk factor associated to FLA infection, was not recorded in the patient´s chart and in one case, the initial diagnosis was bacterial infection.

It is important to consider that cultures are necessary to establish the diagnosis and identify the genus and species of the FLA, as well as to further understand and explain the infectious disease and its clinical course, as in the cases in which exposure to risky waters was not determined; moreover, they are relevant in infections with *B mandrillaris* since the infection tends to evolve slowly (Oddó, 2006).

Among free-living amoebae, *Naegleria fowleri*, *Acanthamoeba spp* and *Balamuthia mandillaris* are considered infectious agents affecting the CNS. In 2001, a case of encephalitis caused by another amoeba species, *Sappinia diploidea*, was reported; it was subsequently identified as *S pedata* by polymerase chain reaction, a tool that is not readily available in all institutions; however, other FLA can be potentially identified (Qvarnstrom *et al*., 2009). The diagnosis can be established by direct examination of the CSF as well as in tissue biopsies, in which the trophozoite (vegetative phase) or cyst (resistance phase) can be identified; however, each amoebic agent has different trophozoite morphologies (Uribarren, 2016; De Jonckheere, 2011; Galarza *et al*., 2006).

Other diagnostic tools are culture in non-nutritive agar and *E. coli*. Immunofluorescence and real-time PCR can also simultaneously detect *N fowleri*, *Acathamoeba spp* and *Balamuthia*, as well as *Sappinia pedata* (Qvarnstrom *et al*., 2009; CDC, 2016; Qvarnstrom *et al*., 2006).

The limitations of this study include its retrospective nature and a lack of complete data, but we believe that groups with more available diagnostic tools will be able to benefit from our report by reaching a timely diagnosis and initiating early appropriate therapy.

Conclusion

In the differential diagnosis of bacterial meningoencephalitis, it is important to consider the possibility of free-living amoebae infection.

The diagnosis of FLA infection can be established in CSF or tissue biopsy, by usually identifying the trophozoites.

Molecular diagnosis can be established by real-time PCR.

As in all infectious processes, a timely diagnosis is imperative so as to initiate early treatment; the lethality of infections by FLA is related with the disease´s time course, its diagnosis and treatment.

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Author's Contributions

Alcala-Martinez: Idea, design and statistical analysis.
Gaona-Flores: Preparation and writing, translation.
Paz-Ayar: Data compilation.
Gonzalez-Guerra: Data compilation.

Ethics

There is no ethical commitment. the information was obtained from files and the confidentiality of personal data is respected.

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