CENTRAL NERVOUS SYSTEM INFECTION DUE TO
Balamuthia spp.: CLINICAL DIAGNOSIS AND
MANAGEMENT

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ABSTRACT

Balamuthia amoebic encephalitis is a rare infection but it almost always proves fatal with the mortality rate of more than 95%. Thus it is not surprising that the majority of cases are identified at the post-mortem stage. Early diagnosis followed by aggressive treatment may lead to successful prognosis for the patient. The clinical diagnosis requires a high level of suspicion, awareness of the clinical signs, and a familiarity of the causative agent, Balamuthia mandrillaris. A complete understanding of the disease, clinical symptoms, available diagnostic methods, possible therapeutic interventions and knowledge of the causative agent would undoubtedly augment our ability to counter this deadly infection.

Key words: Balamuthia mandrillaris, encephalitis, protozoa, central nervous system, blood-brain barrier.

1. INTRODUCTION

Amoebic encephalitis due to Balamuthia mandrillaris is a serious disease of humans and animals that almost always leads to death. First discovered in 1986 from the brain necropsy of a mandrill baboon (Papio sphinx) that died of a neurological disease at the San Diego Zoo Wild Animal Park, California, USA (Visvesvara et al., 1990), this protozoan pathogen produces necrotising haemorrhagic encephalitis. Being a free-living amoeba that is widely distributed in the environment, B. mandrillaris is a significant threat to human and animal health and has gained particular attention in recent years. Although the predisposing factors for Balamuthia amoebic encephalitis (BAE) remain incompletely understood, it can produce encephalitis in relatively immunocompetent individuals leading to fatal consequences (Jayasekera et al., 2004; White et al., 2004). This is of particular concern in view of i) the increasing numbers of immunocompromised patients ii) the excessive use of antibiotics, iii) global warming with increased outdoor activities adding to the ubiquity of these pathogens, thus increased exposure to the susceptible hosts. Perhaps, the most distressing aspect is the limited availability of effective and/or recommended treatment against BAE (Matin et al., 2008). The purpose of this review is to provide briefly our current understanding of the disease and the infectious agent, diagnostic methods and possible therapeutic interventions.

2. DISEASE

Balamuthia amoebic encephalitis is a rare, subacute to chronic disease that is characterized by hemorrhagic necrotizing lesions or brain abscess (detected by neuroimaging scans) with severe meningeal irritation and encephalitis. The lesions are most numerous in the basal ganglia, midbrain, brainstem and cerebral hemiparesis with characteristic lesions in the CNS parenchyma. Typically, encephalitis is of the granulomatous type composed of the parasite, CD4 and CD8 T cells, B lymphocytes, few plasma cells, macrophages and multinucleate giant cells (Martinez and Visvesvara, 1997; 2001). However, in immunocompromised patients with an impaired cellular immune response, granuloma formation may be minimal or absent (Martinez and Visvesvara, 1997; 2001). Post mortem examination often shows severe edema and hemorrhagic necrosis. The parasite attacks the brain tissue and
produce subacute necrotising hemorrhagic encephalitis leading to brain dysfunction. *Balamuthia mandrillaris* trophozoites and cysts are present within the perivascular spaces and within the necrotic CNS parenchyma (Martinez and Visvesvara, 1997). The disease is likely to take a cutaneous route before secondarily attacking the CNS. The time period of transition from the cutaneous form to the CNS ranges from 30 days to 2 years, with an average of 5 – 8 months (Bravo and Sanchez, 2003). The skin lesions may appear at the site of an abrasion of the skin surface of the patient, or lesions can appear as single or multiple plaques or nodules (Gordon et al., 1992; Deetz et al., 2003). These plaques may appear on the face, the trunk or the limbs, with a rubbery to hard consistency (Bravo and Sanchez, 2003). Skin lesions indicate a site of entry and are frequently observed in BAE patients.

3. Clinical Diagnosis

Due to the rarity of the disease, the diagnosis of BAE is problematical. The clinical symptoms are similar to other CNS pathogens including viruses, bacteria, fungi and other protozoa. This makes the diagnosis of BAE challenging and requires prior experience of symptomatic indications. Patients with respiratory infections, skin ulcerations with the CNS involvement and brain abscesses should be suspected for BAE infection. The brain image analyses using computed tomography (CT) or magnetic resonance imaging (MRI) scan of the head may reveal multiple space-occupying lesions indicating brain abscess or tumours, suggestive of the CNS defects (Martinez and Visvesvara, 1997; 2001). The cerebrospinal fluid (CSF) obtained by lumbar puncture rarely shows the presence of *B. mandrillaris* and may resemble that of aseptic meningitis. The CSF findings, although not confirmatory of BAE, are of value in diagnosing the CNS involvement and may show pleocytosis with lymphocytic predominance with elevated polymorphonuclear leukocytes, normal or slightly low glucose level, increased levels of proteins (>1000mg/dL), and minimal cloudiness (Jayasekera et al., 2004; White et al., 2004).

Both immunofluorescence-based and PCR-based assays have been developed for the rapid identification of BAE (Booton et al., 2003; 2003a; Huang et al., 1999; Yagi et al., 2005; Qvarnstrom et al., 2006; Tavares et al., 2006). The demonstration of high levels of *B. mandrillaris*-specific antibodies in a patient’s serum is particularly useful and a straightforward method to further suspect BAE infection (Fig. 1).

**Figure. 1**

*Balamuthia mandrillaris* trophozoites at 1: 10000  
*Balamuthia mandrillaris* trophozoites 1: 640

This is performed using indirect immunofluorescence assays (IIF). The serial dilutions of the patient’s serum are incubated with fixed amoebae-coated slides, followed by incubation with fluorescein isothiocyanate (FITC)-labelled anti-human antibody and visualized under fluorescent microscope. It is important to remember that the levels of anti-*B. mandrillaris* antibodies in normal populations may be in the range of 1: 64 – 1: 256 (Huang et al., 1999). However, a titre of 1:128 was observed in some BAE cases; which proved fatal (Schuster and Visvesvara, 2004). Despite the prevalence of *B. mandrillaris* antibodies in normal populations (Huang et al., 1999) and that the levels of anti-*B. mandrillaris* antibodies in BAE patients have been observed in the range of 1: 10,000 (Jayasekera et al.,
2004; White et al., 2004), it is not clear why antibodies do not provide protection against *B. mandrillaris*. Similarly, patients with severely impaired immune system may not develop a high titre. This suggests that interpretation of IIF findings should be dealt with care and other clinical findings should be taken into account for the correct BAE diagnosis. The confirmatory evidence comes from direct microscopic demonstration of *B. mandrillaris* in the tissues but requires familiarity with morphological characters. In addition, it is helpful to inoculate a portion of CSF and/or biopsy onto mammalian cell cultures to isolate *B. mandrillaris*, but this may take up to several weeks (Jayasekera et al., 2004). Polymerase chain reaction methods using *Balamuthia*-specific primers have been developed for the rapid and sensitive detection of *B. mandrillaris* and may aid in the clinical diagnosis of BAE (Fig. 2) (Booton et al., 2003; 2003a; Jayasekera et al., 2004).

4. Host Susceptibility

So far, over 150 cases of BAE have been reported (Matin et al., 2008; Siddiqui and Khan, 2008). The majority of these cases have been reported from Latin America. Approximately 30 cases have been reported in the United States. The majority of these have been in the Southwest with California, Texas, and Arizona recording the largest numbers of cases. However, the exact number of BAE cases worldwide will never be known; this is due to a lack of awareness, poor public health systems especially in the less developed countries. The data currently available shows that BAE can occur in healthy people of any age, though there is a predominance of cases in the young (under 15 years of age) and the elderly (over 60 years of age), probably due to their weaker immune systems (Maciver, 2007). However, as the disease is rare, this suggests the presence of predisposing factors. Interestingly, BAE infections have been predominantly reported in Hispanic people (Siddiqui and Khan, 2008). This is difficult to understand, since *B. mandrillaris* infects such a broad range of mammals in addition to humans. Notably, it has been reported that Hispanic people are less able to make antibodies against certain *Acanthamoeba* species (Chappell et al., 2001) and this may be the case for *Balamuthia*. Another possibility is that Hispanics in Southern California are more likely than other groups to be exposed to infected soils during agricultural activities (Schuster and Visvesvara, 2004). Notable, BAE has been reported in patients suffering from cancer or diabetes, human immunodeficiency virus (HIV)-infected patients, or drug and alcohol abusers. Temperature seems to be an important factor in the occurrence of BAE, as the disease seems to be more common in warmer regions, such as Southern California and South America. Soil has also been a factor in two cases of BAE that occurred in immunocompetent individuals. In one case, a Californian man working in his backyard developed an infection soon after sustaining a puncture wound that was probably contaminated by soil (Deetz et al., 2003). In the second case, a woman from New York was reported to have worked in her garden with compost soil, prior to developing an infection (Jung et al., 2004). Based on these two cases and others that have developed around the world, it appears that *Balamuthia* are not confined to enriched soils, but may be dispersed widely in a variety of soil ecosystems. Overall, it is shown that although BAE can occur in healthy individuals, immunocompromised or debilitated patients due to HIV infection, diabetes, immunosuppressive therapy, malignancies, malnutrition, and alcoholism are particularly at risk (Schuster and Visvesvara, 2004; Visvesvara et al., 2007; Siddiqui and Khan, 2008). The risk factors for patients suffering from the
above diseases include exposure to contaminated water such as swimming pools, on beaches, or working with garden soil.

5. TREATMENT

One of the most distressing aspects is the limited availability of effective and/or recommended treatment against BAE. The current treatment regimen involves a mixture of limited antimicrobial drugs to provide additive/synergistic effects as well as dexamethasone for cerebral edema. Even then mortality remains very high (~98% mortality) and the majority of cases due to BAE are identified at autopsy stage (Kodet et al., 1998; Galarza et al., 2002). Early diagnosis, followed by aggressive treatment may offer potential for antimicrobial therapeutic intervention. To date, there have only been three reported cases of BAE with successful antimicrobial chemotherapy, a 64-year old male, a 5-year old female and a 72-year old female (Deetz et al., 2003). The 64-year old male was treated with 5-fluorocytosine (flucytosine), fluconazole, sulfadiazine, clarithromycin and trifluoperazine, while the 5-year old female survived after treatment with a similar regimen but without sulfadiazine. In both cases, pentamidine isethionate was also administered initially; however its use was discontinued due to side effects (Deetz et al., 2003). The 72-year old female was treated with sulfadiazine, fluconazole and clarithromycin as well as pentamidine isethionate. Despite the limited success, the prognosis for BAE remains extremely poor. This may be due to variability in the virulence and antimicrobial sensitivity of *B. mandrillaris* isolates causing the infection, time at which drug treatment is initiated, infectious dose of amoebae, and the limited ability of antimicrobial compounds to cross the blood-brain barrier (Schuster and Visvesvara, 2004). Of interest, miltefosine (hexadecylphosphocholine) has shown promise against the clinical isolates of *B. mandrillaris*; it can cross the blood-brain barrier and has its potential in the BAE treatment (Schuster and Visvesvara, 2004). Overall, these studies indicate that there is a clear and urgent need for the development/identification of novel compounds against BAE.

6. The infectious agent

*B. mandrillaris* is a protozoa pathogen that is widely distributed in the environment. The life cycle of *B. mandrillaris* undergoes two stages, a vegetative trophozoite stage and a resistant cyst stage (Fig. 3) (Martinez and Visvesvara, 2001; Visvesvara et al., 1990). The trophozoites are normally in the range of 15 – 60 µm in diameter; however the size may vary between different isolate and stage of the life cycle. The cell division is asexual and occurs by binary fission. Under harsh conditions (lack of food, extremes in osmolarity, pH and temperatures), amoebae switch into resistant cyst stage. In simple terms, trophozoite becomes metabolically inactive (minimal metabolic activity), excess food, water and particulate matter is expelled and the trophozoite encloses itself within a resistant shell to survive harsh periods (Fig. 3). The cellular levels of RNA, proteins, triacylglycerides and glycogen declines substantially during the encystment process resulting in decreased cellular volume and dry weight. The cyst stage is approximately 10 – 20 µm. The trophozoites emerge from the cysts under favourable conditions leaving behind the outer shell and actively reproduce, thus completing the cycle.
Recently it has been shown that *B. mandrillaris* can act as host for the intracellular survival of bacteria, including the causative agent of Legionnaires’ disease, *Legionella pneumophila*. The ability of these amoebae to host bacteria may enhance bacterial infectivity for mammalian cells, thus increasing their transmission to susceptible hosts, as well as enhancing the pathogenicity of the host amoebae.

Light microscopic observations of co-cultures of *B. mandrillaris* and *L. pneumophila* revealed that, *L. pneumophila* remained and multiplied within large vacuoles inside the amoebae. Subsequently, the amoeba became round; this eventually lead to the lysis of the amoebae (Shadrach et al., 2005). The discovery that *L. pneumophila* bacteria are able to infect and multiply within *B. mandrillaris* is surprising, as it has been shown clearly that *B. mandrillaris*, in contrast to most free-living amoebae (e.g., *Acanthamoeba*), cannot be grown on bacterial-coated non-nutrient agar plates. Instead, *B. mandrillaris* readily thrive on a large variety of eukaryotic cells. These studies show that *B. mandrillaris* can harbour viable *L. pneumophila* and may serve as hosts for other pathogenic bacteria.

**7. Balamuthia mandrillaris as a host for pathogenic bacteria**

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However, whether this renders *B. mandrillaris* more pathogenic or whether these amoebae may also contribute to the dissemination of *L. pneumophila* remains to be investigated.

7. CONCLUSIONS

Although the number of infections (150 cases) due to *B. mandrillaris* is few, the difficulty in diagnosis, lack of awareness, problematical treatment of BAE and the resulting fatal consequences are all factors that highlight the conclusion that this infection is of concern. Furthermore, it suggests that a vast number of BAE infections have most likely been undetected, and the actual number of BAE cases is significantly higher. For example, there is no single report of BAE in Africa, despite the presence there of millions of HIV-infected individuals, who are susceptible hosts to opportunistic pathogens, as well as warm climate, probable frequent environmental exposure and subordinate sanitation. More worryingly, *B. mandrillaris* have been shown to produce BAE in immunocompetent individuals and thus present a real threat to human health. Current methods of treatment require increased awareness of physicians and pathologists of BAE and strong indications based on clinical findings. Early diagnosis followed by aggressive treatment using a mixture of drugs is crucial, and even then the prognosis remains extremely poor. Thus there is an urgent need for the understanding of the pathogenesis and pathophysiology of BAE both at the molecular- and cellular-level. Also the ability of *B. mandrillaris* to transmit to the susceptible hosts, adapt to diverse host and environmental conditions, as well as their ability to overcome host defence barriers and emerge as infective trophozoites to produce CNS infection will provide targets for therapeutic interventions and to design novel strategies for preventative measures.

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