Review Paper

Clinical Features, Diagnosis, and Treatment Plans of *Balamuthia mandrillaris* Encephalitis in Pediatrics: A Systematic Review

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ABSTRACT

Background: *Balamuthia mandrillaris* is a free-living amoeba responsible for a rare but fatal microbial encephalitis in pediatric patients. Pediatric cases are extremely rare and typically present with nonspecific symptoms, leading to delayed diagnosis and poor outcomes.

Objectives: The aim of this study was to systematically review reported pediatric cases of *Balamuthia*-induced encephalitis to better characterize clinical manifestations, diagnostic approaches, treatment strategies, and patient outcomes.

Methods: This study was conducted based on the PRISMA flow diagram. Papers reporting *B. mandrillaris* encephalitis in pediatrics were identified through searching in international databases, including MEDLINE/PubMed, Scopus, Web of Science, and Science Direct, and the Google Scholar search engine up to July 2023. Inclusion criteria were English-language case reports or case series involving patients under 18 years of age with confirmed Balamuthia encephalitis. The collected studies were screened based on the inclusion and exclusion criteria. The review protocol was registered in PROSPERO. The quality of included articles was assessed using JBI critical appraisal tools for case reports.

Results: Thirty-four articles met the inclusion criteria, reporting 57 infected children. Patients ranged in age from 8 months to 18 years, with a mean age of 7.8 years. Only four children could survive (7%). The most common clinical manifestations were fever, headache, and loss of consciousness. There was a lack of specificity in cerebrospinal fluid, computed tomography (CT) scan, and magnetic resonance imaging (MRI) findings, which made it hard to diagnose. MRI was performed in 45 patients, and all of them showed abnormalities, such as multiple lesions. The most common diagnostic method was polymerase chain reaction (PCR) on cerebrospinal fluid, brain tissue, or skin lesion; however, next-generation sequencing (NGS) appeared to be a more efficient and faster alternative. Multiple-drug therapy was used in the surviving cases.

Conclusions: Although *B. mandrillaris* encephalitis is rare, it might become a health concern in the future due to its extremely high mortality rate. Healthcare providers should be informed about the clinical manifestations, appropriate diagnostic tools, and possible treatments of this potentially life-threatening microbial encephalitis. Early diagnosis and rapid intervention may improve the chance of survival.

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Introduction

Encephalitis is an acute and progressive infection of the brain parenchyma, which can lead to neurological deficits and even death. This infection occurs in both adults and children, and a wide range of etiologies cause encephalitis, including viral, bacterial, autoimmune agents, and parasites. Encephalitis usually manifests as altered consciousness, abnormal behaviors, seizures, fever, and headaches. Diagnosis is typically made by neuroimaging, laboratory analysis of cerebrospinal fluid (CSF) (protein levels, glucose levels, blood cell counts), and electroencephalography (EEG). Treatment differs in each case based on the etiology of encephalitis. Antiviral agents, antibiotics, and corticosteroid therapy are mostly used [1].

Balamuthia mandrillaris is a free-living amoeba and an opportunistic protozoan pathogen. This pathogen can lead to a severe encephalitis with an extremely high mortality rate (>98%) [2]. The organism's life cycle includes dormant cysts and vegetative trophic stages. Its trophozoites measure 15 to 50 µm and have round nuclei with a dense nucleolus and a cytoplasm containing empty vacuoles [3].

Some studies have shown that *B. mandrillaris* can be isolated from soil and water; however, this amoeba is very difficult to isolate and culture [3-5]. Studies have suggested that contact with contaminated soil is a major risk factor for *Balamuthia* amoebic encephalitis (BAE) [5]. Visvesvara et al. reported the first human *B. mandrillaris* infection in 1993 [6], and to date, the number of BAE reported cases is more than 300 globally, which also includes pediatrics [7, 8]. It does not matter whether the host is immunocompromised or immunocompetent; this amoeba can infect both groups [9]. Pediatric patients may present differently compared to adults, and also their immune response and treatment tolerability can be significantly different, which highlights the need for a child-specific analysis. Based on case reports, only less than ten infected children have survived worldwide. Diagnosis is also challenging due to insufficient awareness and inefficient health systems [10]. Despite the severity of this infection, especially in pediatrics, there is no comprehensive summary of published case reports in the literature. Therefore, it is important to be aware of manifestations, paraclinical findings, and possible treatments for BAE, especially in early stages. This systematic review evaluated pediatric patients (0–18 years) diagnosed with BAE, examining clinical presentations, diagnostic methods (including polymerase chain reaction (PCR), imaging, and CSF analysis), and pharmacological treatments (miltefosine, pentamidine, fluconazole). Only case reports and cross-sectional studies in English were included.

Methods

Search strategy and data sources

This study is structured as a systematic review that followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) checklist. The review protocol was registered in the PROSPERO database under registration number 1034394. On July 23 and 24, 2023, PubMed, Web of Science, Scopus, and ScienceDirect databases were searched using keywords "*Balamuthia mandrillaris*", "encephalitis", "*Balamuthia mandrillaris* encephalitis", "pediatrics", "neonatal", "infant", and "children". To find more articles, Google Scholar was also searched, and the first 250 results were screened. The search in all databases was finally updated on July 26, 2023. A systematic search in PubMed was conducted using a combination of Medical Subject Headings (MeSH) terms and free-text keywords related to Balamuthia encephalitis in pediatric patients.

The search terms used included ("*Balamuthia*"[MeSH Terms] OR "*Balamuthia*"[All Fields]) AND ("encephalitis"[MeSH Terms] OR "encephalitis"[All Fields]) AND ("child"[MeSH Terms] OR "pediatrics"[MeSH Terms] OR "child"[All Fields] OR "pediatric"[All Fields]).

Only peer-reviewed, published papers were included in this review. Grey literature, preprints, and unpublished data were not included in this study.

Data extraction

All the retrieved articles were entered into EndNote software, version 20.2.1 and screening of all articles based on title and abstract was carried out considering the inclusion and exclusion criteria. The remaining studies were then evaluated on a full-text basis. Additionally, reference lists of the included literature were also reviewed, and if they met the inclusion criteria, they were included in our study. The first author (Hoda Mehrabi) independently was responsible for checking search terms and strategy, and it was confirmed and supervised by the second author (Reza Ghasemikhah).

Inclusion and exclusion criteria

Inclusion criteria consisted of English-language case reports and cross-sectional articles reporting patients with encephalitis due to *B. mandrillaris* in the age range of neonates up to 18 years. There were no restrictions regarding nationality, race, ethnicity, number of individuals, or gender.

Exclusion criteria included irrelevant and duplicate reports, quasi-experimental articles, articles whose full text was not available or published, and articles in which cases were older than 18 years.

Quality assessment

The quality of the included case reports and case series was independently assessed by two authors using the Joanna Briggs Institute (JBI) critical appraisal checklists. Discrepancies were resolved through discussion or consultation with another reviewer.

General considerations

Based on the paper selection stages (Figure 1), 880 articles were identified after searching Scopus, Web of Science, PubMed, ScienceDirect, and Google Scholar. Some unrelated papers (717) and related articles (163) were identified, among which 111 were duplicates. In the end, 34 papers out of 52 articles met the inclusion criteria and entered into our study.

Assessment of publication bias

Due to the descriptive nature of the included articles (case reports and case series) and the absence of quantitative data, which were suitable for meta-analysis, formal assessment of publication bias was not feasible.

Data synthesis

Because of the heterogeneity of the included articles in terms of patient characteristics, clinical presentations, diagnostic methods, and treatment plans, no meta-analysis was conducted. Instead, a narrative synthesis was performed. Relevant data were summarized in tables, which highlight clinical presentations, diagnostic findings, treatment approaches, and outcomes across pediatric cases.

Results

Study selection

A total of 163 records were identified through database search, and after removing 111 duplicates, 52 articles remained for screening of title and abstract, and then full text. Of these, 34 studies were included in this systematic review. The study selection process is illustrated in Figure 1.

Due to methodological and clinical heterogeneity across the included case reports, a meta-analysis was not feasible. Instead, a narrative descriptive synthesis was evaluated, summarizing the demographic, clini-

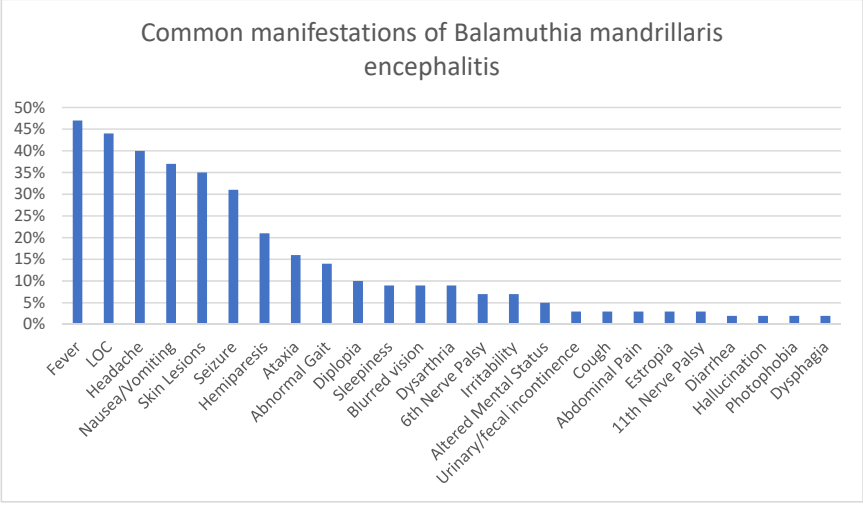

Diagram 1. Common manifestations of BAE in pediatrics

LOC: Loss of consciousness.

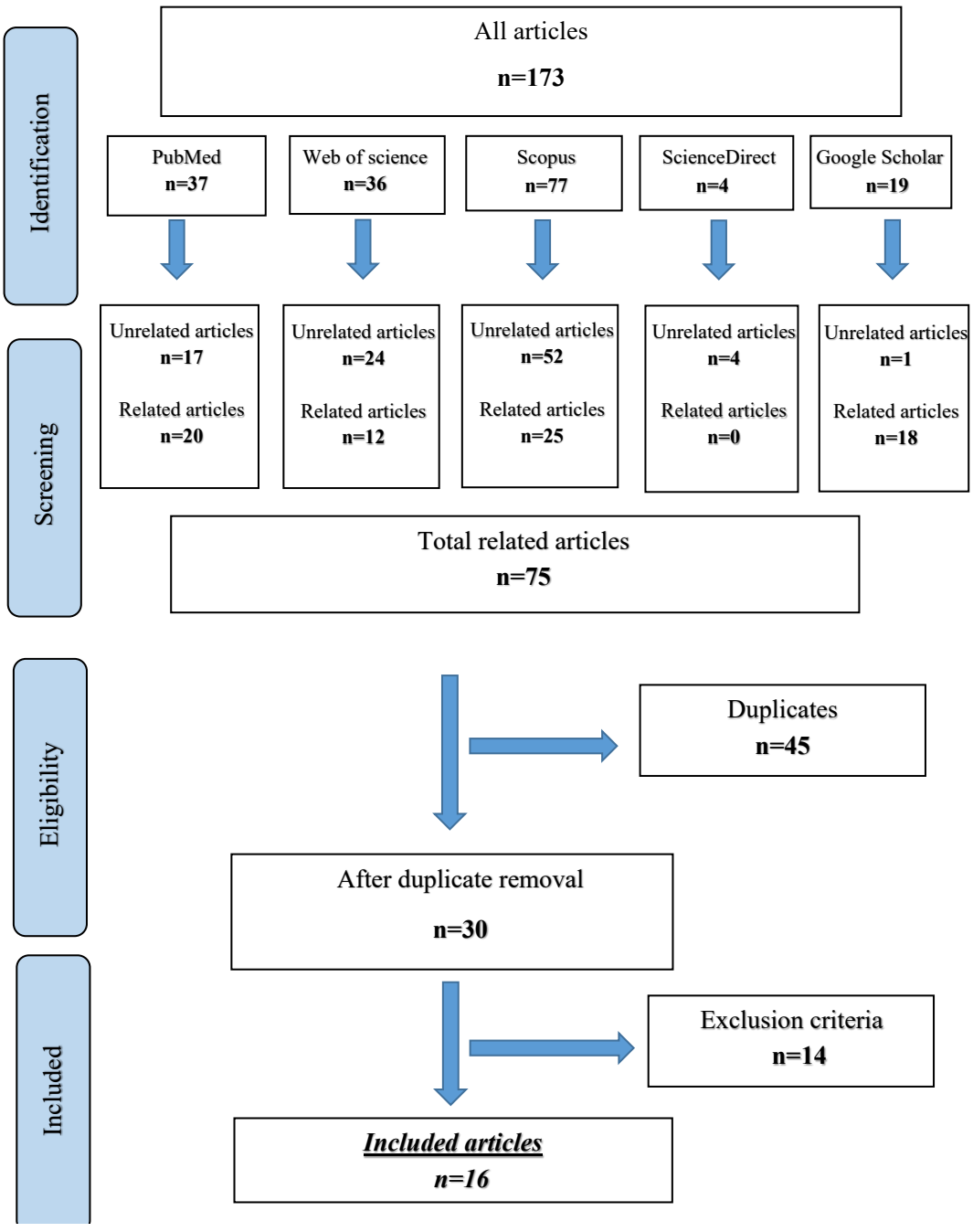

Figure 1. Study selection process

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cal, diagnostic, and therapeutic data across articles. Because of the limited number of included studies and variability in reported data, no pooled effect estimates or confidence intervals were calculated.

Risk of bias assessment

The methodological quality of the included case reports and case series was evaluated using the JBI critical appraisal tool. Overall, the studies demonstrated high methodological rigor. All included literature met the minimum quality standards required for inclusion in this systematic review.

Demographic information

A total of 57 pediatric patients were included in this study, with 5 missing data points regarding the sex of the patients. In the 52 cases with known sex, 36 patients were male (69%) and 16 patients were female (32%) (Table 1). The mean age of the patients was 7.8 years (pediatric age range: 0–18 years). There is a variety of

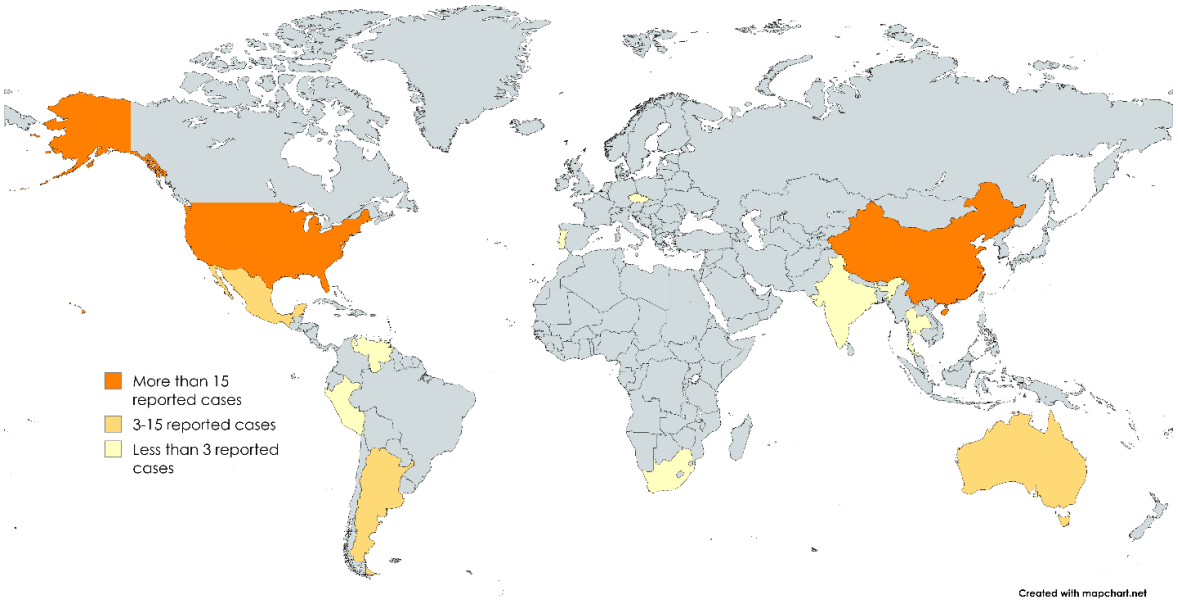

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Figure 2. Number of cases of reported BAE in children in different countries around the world: USA, 23; China, 16; Argentina, 4; Mexico, 3; Australia, 3; India, 2; Peru, 1; Thailand, 1; Czech, 1; Portugal, 1; South Africa, 1; Venezuela, 1.

countries represented, and this infection has been reported on all continents (Figure 2).

Manifestations

Articles have mentioned different manifestations in infected children, with the most common ones reported as follows:

Fever (in 27 cases), headache (in 23 cases), loss of consciousness (in 25 cases), seizures (in 18 cases), hemiparesis (in 12 cases), and others (Figure 1). These symptoms are usually due to increased intracranial pressure (ICP), which is caused by *B. mandrillaris* infection in the central nervous system.

Research has suggested two patterns of manifestations for this encephalitis. In the first pattern, patients developed specific skin lesions mainly on their faces months or years before the onset of encephalitis; this type has been reported mostly in China and Peru [10, 11]. In our study, we observed that 35% of cases experienced skin lesions before or during the occurrence of encephalitis. In the second pattern, patients presented with encephalitis without any signs of cutaneous lesions, which has usually been reported in the USA [12].

Diagnosis

Confirmatory method

Depending on the date of each case report, various diagnostic methods have been used to confirm the infection. Confirmatory techniques are reported as follows: next-generation sequencing (NGS) on CSF (in 5 cases), indirect immunofluorescence test on brain tissue (in 24 cases), serum or CSF antibody titer (in 3 cases), PCR (on CSF, brain tissue, or skin lesion) (in 25 cases), and immunohistochemical staining (in 3 cases). It is noteworthy that in some reports, more than one confirmatory test has been performed (Table 2).

CSF analysis

CSF analysis was reported in detail for 25 cases. Leukocytosis was observed in all cases. The maximum and minimum counts of CSF leukocytes were 555 and 14 cells/mm^3, which belonged to a 27-month-old boy and a 2.5-year-old boy, respectively. Lymphocyte dominance was detected in almost all the tests performed. The CSF protein level was high (more than 45 mg/dL) in all patients except for four, who had normal protein levels. The glucose level was generally low, except in four tests in which normal levels were reported (Table 3).

Table 1. Demographic data, brain imaging findings, treatment plans, and prognosis of BAE

Author(s), Year	Country	Age/Sex	Brain Imaging		Main Hospital Treatment	Prognosis
			CT scan	MRI		
Tootla et al. 2022 [27]	South Africa	17 Y/ NA	Sinusitis, opacification of sinuses, destruction of the right bony orbit, multiple lesions	Multiple lesions	Fluconazole, albendazole, flucytosine, trimethoprim sulfamethoxazole, clarithromycin, Miltefosine, and prednisone	Died
Tao et al. 2022 [28]	China	15 Y/ M	Multiple abnormal low-density foci	NA	Itraconazole and Linezolid	Died
Zhang et al. 2021 [8]	China	7 Y/ M	NA	Multiple lesions with patchy or nodular enhanced areas	Subcutaneous recombinant human interferon α2b (for skin lesion)	Died
Ai et al. 2021 [29]	China	15 Y/ M	NA	Multiple lesions	Amphotericin B, fluconazole, sulfamethoxazole, azithromycin, and flucytosine	Died
Cuoco et al. 2021 [24]	USA	4 Y/ M	Focal subcortical vasogenic edema	A ring enhancement with surrounding vasogenic edema	Flucytosine, miltefosine, trimethoprim-sulfamethoxazole, azithromycin, and fluconazole	Survived
Yi et al. 2020 [19]	China	9 Y/ F	NA	Multiple lesions	Aggressive use of mannitol, 3% hypertonic saline, and Furosemide	Died
Wu et al. 2020 [20]	China	13 Y/ F	An area of hypodensity	Multiple enhanced lesions and patchy lesions	Amphotericin B liposome, 5-fluorocytosine, and fluconazole	Died
Yang et al. 2020 [30]	China	2 Y/ M	Multiple patchy, low-density shadows	Multiple lesions	Antituberculosis treatment, methylprednisolone, and human immunoglobulin	Died
Wang et al. 2020 [10]	China	4 Y / M	NA	Not performed before death	Lincomycin and interferon-γ	Died
		5 Y/ M	NA	Not performed before death	Lincomycin, interferon-γ, doxycycline, rifampin, and azithromycin	Died
		5 Y/ M	NA	Multiple infections	Died before treatment	Died
		6 Y/ M	NA	Infection of the brain stem	Died before treatment	Died
		7 Y/ M	NA	Not performed before death	Lincomycin, interferon γ, and azithromycin	Died
		7 Y/ F	NA	Focal infection	Lincomycin and interferon γ	Died
		8 Y/ M	NA	Not performed before death	Lincomycin, azithromycin, interferon-α, and interleukin-2	Died
		13 Y/ F	NA	Multiple Infections	Lincomycin and azithromycin	Died
		13 Y/ M	NA	Multiple infections	Lincomycin, azithromycin, and interferon-γ	Died
		18 Y/ F	NA	Not performed before death	Lincomycin and interferon-γ	Died
Joo et al. 2018 [26]	USA	6 Y/ M	Communicating hydrocephalus	Hydrocephalus, cerebellar tonsillar herniation, and ventriculitis	Miltefosine, sulfadiazine, flucytosine, fluconazole, pentamidine, and azithromycin	Died

Author(s), Year	Country	Age/Sex	Brain Imaging		Main Hospital Treatment	Prognosis
			CT scan	MRI		
Cope et al. 2018 [12]	USA	16 Y/ NA	NA	NA	Acyclovir, ceftriaxone, clarithromycin, fluconazole, flucytosine, miltefosine, pentamidine, rifampin, sulfadiazine, and meropenem	Died
		15 Y/ NA	NA	NA	Acyclovir, amphotericin B liposomal, azithromycin, flucytosine, metronidazole, pentamidine, methylprednisolone, sulfadiazine, voriconazole, cefotaxime, and vancomycin	Died
		13 Y/ NA	NA	NA	Amphotericin B liposomal, azithromycin, fluconazole, rifampin, dexamethasone, sulfadiazine, caspofungin, and meropenem	Died
		11 Y/ NA	NA	NA	Amphotericin B liposomal, azithromycin, fluconazole, flucytosine, metronidazole, miltefosine, pentamidine, dexamethasone, thioridazine, and meropenem	Died
Shehab et al. 2017 [31]	USA	13 Y/ F	A small heterogeneous mass with marked surrounding vasogenic edema	Multiple ring-enhancing and non-enhancing lesions and central microhemorrhage	Miltefosine, fluconazole, flucytosine, azithromycin, and sulfadiazine	Died
Khurana et al. 2015 [32]	India	18 Y/ M	Focal cerebritis, leptomeningitis	Multiple mass lesions	Isoniazid, rifampicin, ethionamide, pyrazinamide, ceftriaxone, and vancomycin	Died
		18 Y/ M	Edema	Ill-defined lesions	Albendazole, amphotericin, and clarithromycin	Died
Krasaelap et al. 2013 [33]	Thailand	4 Y/ F	Iso-density mass, obstructive hydrocephalus	An enhanced mass, edema, right tonsillar herniation, and obstructive hydrocephalus	Pentamidine, sulfasalazine, fluconazole, clarithromycin, and amphotericin B	Died
Moriarty et al. 2013 [34]	Australia	4 Y/ F	NA	Multiple well-defined focal lesions	Flucytosine, fluconazole, azithromycin, pentamidine, and sulfadiazine	Survived
Stidd et al. 2012 [35]	USA	2 Y/ F	Multiple areas of decreased attenuation	Multiple bilateral heterogeneously enhancing lesions	Cefuroxime and micafungin	Died
Ghosh et al. 2011 [36]	USA	4 Y/ M	NA	A partially calcified lesion	Vancomycin, ceftazidime, metronidazole, amphotericin, and fluconazole	Died
Orozco et al. 2011 [37]	USA	4 Y/ M	Tonsillar herniation, focal subarachnoid hemorrhage, and effacement of the basilar cisterns	Multiple lesions	Treatment for a presumptive diagnosis of acute disseminated encephalomyelitis	Died
Hill et al. 2011 [38]	Australia	8 months/ F	NA	Edema, cortical hemorrhage, and one aneurysmal dilatation of the right middle cerebral artery	Liposomal amphotericin B, isoniazid, rifampicin, amikacin, moxifloxacin, and low-dose corticosteroids	Died
Combs et al. 2010 [39]	USA	3 Y/ F	Multiple hypoattenuating areas, edema	Multiple masses	Antimicrobial therapy	Died

Author(s), Year	Country	Age/Sex	Brain Imaging		Main Hospital Treatment	Prognosis
			CT scan	MRI		
Cary et al. 2010 [40]	USA	2 Y/ M	An area of hypodensity with surrounding edema	Multiple lesions with surrounding edema, ventricular enlargement, and obstructive hydrocephalus	Pentamidine, flucytosine, sulfadiazine, clarithromycin, and thioridazine	Survived
Schuster et al. 2009 [22]	USA	1.5 Y/ M	NA	Multiple ring-enhancing lesions	NA	Died
		7 Y/ M	NA	White matter lesions and minimal enhancement	NA	Died
		12 Y/ M	NA	Multiple ring-enhancing lesions	NA	Died
Valverde et al. 2006 [41]	Peru	7 Y/ M	NA	NA	Itraconazole and albendazole for skin lesions. No treatment was mentioned for encephalitis	Died
Tavares et al. 2006 [42]	Portugal	8 Y/ M	A hypodense lesion, distortion of the midline, and ventricular thinning	Disseminated heterogeneous hypointense lesions and edema	Fluconazole, trimethoprim sulfamethoxazole, and rifampin	Died
Bakardjiev et al. 2003 [43]	USA	2 Y/ F	NA	Hydrocephalus and enhancing lesions	Isoniazid, rifampin, pyrazinamide, ethambutol and fluconazole, dexamethasone, and ceftriaxone	Died
		2.5 Y / M	Multiple discrete lesions	Hydrocephalus and multiple lesions	Intravenous pentamidine, metronidazole, fluconazole, amphotericin B, itraconazole, flucytosine, azithromycin, and sulfadiazine	Died
		3 Y/ F	NA	Hydrocephalus and two lesions	Isoniazid, rifampin, ethambutol, pyrazinamide and methylprednisolone, ceftriaxone, and acyclovir	Died
		7 Y/ M	An enhancing lesion	Edema and multiple lesions	Isoniazid, rifampin, pyrazinamide, ethambutol, meropenem, amphotericin B, Flucytosine	Died
Deetz et al. 2003 [44]	USA	5 Y/ F	Edema, two large lesions with partial calcification	Edema and two large lesions with partial calcification	Clarithromycin, fluconazole, flucytosine, and thioridazine	Survived
Healy et al. 2002 [45]	USA	5 Y/ F	Internal calcifications and calcified rims in both lesions	Two lesions	Multiple antiamebic	Survived
	USA	32/ M	NA	3-cm enhancing left temporal lobe lesion	NA	Died

Author(s), Year	Country	Age/Sex	Brain Imaging		Main Hospital Treatment	Prognosis
			CT scan	MRI		
Galarza et al. 2001 [46]	Argentina	3 Y/ F	A hyperdense lesion	Multiple lesions	Pentamidine, clarithromycin, fluconazole, and 5-fluorocytosine	Died
		5 Y/ M	Multiple hypodense lesions	NA	Pentamidine, clarithromycin, fluconazole, and 5-fluorocytosine	Died
		6 Y/ M	Hypodense without enhancement lesion	One lesion	Pentamidine, clarithromycin, fluconazole and 5-fluorocytosine	Died
		12 Y/ M	Multiple hypodense lesions	Multiple lesions	Pentamidine, fluconazole and 5-fluorocytosine	Died
Kodet et al. 1998 [47]	Czech	3 Y/ M	Multiple necrotic foci, progressive supratentorial hydrocephalus	Two lesions	Trimethoprim-sulfamethoxazole, amphotericin B, azithromycin, and pentamidine diisethionate	Died
Reed et al. 1997 [48]	Australia	5 Y/ M	NA	Multiple lesions	Amphotericin B, cefotaxime, and acyclovir	Died
Duke et al. 1997 [49]	USA	3 Y/ M	Ventricle dilatation, periventricular hypodensity	Cerebellar tonsillar herniation and ventricular enlargement	Isoniazid, rifampin, pyrazinamide, streptomycin, amphotericin B, and steroids	Died
Riestra- Castaneda et al. 1997 [50]	Mexico	3 Y/ M	NA	A hypodense lesion	NA	Died
		9 Y/ M	NA	High-density lesions	NA	Died
		14 Y/ M	NA	A hypodense lesion	NA	Died
Griesemer et al. 1994 [51]	USA	27 months/ M	Left middle cerebral artery distribution infarct, ventricles dilation	Occlusion of the left internal carotid artery	Praziquantel, fluconazole, ampicillin, cefotaxime, trimethoprim sulfamethoxazole, vancomycin, isoniazid, rifampin, and pyrazinamide	Died
		13 Y/ F	A large mass with multiple cystic lesions	Cystic lesions	Dexamethasone, praziquantel, and mannitol	Died
Martínez et al. 1993 [23]	Venezuela	14 Y/ M	Hypodense lesions	Hypodense lesions	Albendazole and steroids	Died

Abbreviations: Y: Year; NA: Not available; M: Male; F: Female.

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Computed tomography (CT) scan and magnetic resonance imaging (MRI) findings

In 27 cases, CT scan findings were mentioned. The most common findings were hyperdense or hypodense lesions (s) (13 cases), brain edema (6 cases), and hydrocephalus (3 cases). In 45 patients, the MRI findings are mentioned. The most common MRI finding was lesions (in 34 patients) (Table 1).

Therapy

Treatment details were available for 49 cases (Table 1). Among the surviving patients, the treatment included multiple drugs, such as flucytosine, fluconazole, pentamidine, sulfadiazine, clarithromycin, thioridazine, miltefosine, trimethoprim-sulfamethoxazole, and azithromycin.

In other cases, based on an unknown diagnosis in early stages, different empirical treatments were administered. For instance, anti-tuberculosis therapy was used in 7 cases, but none of them led to survival. Amphotericin B was also used in 12 patients, all of whom experienced fatal outcomes (Table 1). Due to the limited number and variability of cases, statistical comparison of treatment results could not be conducted, and a descriptive overview of the therapies and their associated outcomes is provided.

Discussion

Balamuthia mandrillaris is a free-living amoeba responsible for a rare but fatal encephalitis in healthy and immunosuppressed hosts. Its cysts exist in soil and water, and this amoeba can infect humans via the skin, olfactory nerve, or pulmonary system [13, 14].

Table 2. The diagnostic methods used to confirm *BAE*

Author(s), Year		NGS	Indirect Immunofluorescence Test	PCR	Balamuthia Antibody Titer	Immunohistochemical Staining
Yi et al. 2021 [19]		+				
Wu et al. 2020[20]		+				
Bakardjiev et al. 2003 [43]	Case 1		+			
	Case 2		+			
	Case 3				+	
	Case 4				+	
Yang et al. 2020 [30]		+				
Reed et al. 1997 [48]			+			
Kodet et al. 1998 [47]			+			
Wang et al. 2020 [10] (all 10 cases)				+		+
Moriarty et al. 2014 [34]			+	+		
Deetz et al. 2003 [44]			+		+	
Ai et al. 2022 [29]		+		+		
Stidd et al. 2012 [35]			+	+		
Tavares et al. 2006 [42]			+	+		
Tootla et al. 2022 [27]				+		
Krasaelap et al. 2013 [33]				+		
Zhang et al. 2022 [8]				+		+
Cary et al. 2010 [40]			+			
Khurana et al. 2015 [32]				+		
Martínez et al. 1994 [23]			+	+		
Griesemer et al. 1994 [51]			+			
Hill et al. 2011 [38]			+			
Combs et al. 2011 [39]			+	+		
Joo et al. 2018 [26]				+		
Galarza et al. 2002 [46] (all 4 cases)			+			
Tao et al. 2022 [28]		+				
Orozco et al. 2011 [37]			+			+
Schuster et al. 2009 [22] (all 3 cases)			+	+		
Cuoco et al. 2022 [24]				+		
RiestraCastaneda et al. 1997 [50] (all 3 cases)			+			

PCR: Polymerase chain reaction.

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Table 3. CSF analysis of the reported cases

Ref.	1st CSF								2nd CSF							
	Hematological Factors				Biochemical Factors		Pathological Factors		Hematological Factors				Biochemical Factors		Pathological Factors	
	WBC			RBC (Cell/mm³)	PR (mg/dL)	Glucose (mg/dL)	PCR	Amoebic Culture	WBC			RBC (Cell/mm³)	PR (mg/dL)	Glucose (mg/dL)	PCR	Amoebic Culture
	Total (Cell/mm³)	N (%)	L (%)						Total (Cell/mm³)	N (%)	L (%)					
[19]	331	22			>300	25.5	Neg	Neg								
[20]	100	20	80		146.7	28.8	NA	Neg								
[43]	540	90	10		122	47	+	Neg	105	15	79		1918	4	NA	NA
	124	1	72		84	58	NA	NA								
	230	12	62		308	<20	NA	NA	102	1	91		1400	62	NA	NA
	14		100		116	39	NA	NA	83				1600	47	NA	NA
[30]	130	3	97	200	72	46.8	NA	NA	70	3	97	5	107	36	NA	NA
[48]	34		100		50	81	NA	Neg								
[34]	59			1	56	41.4	NA	NA								
[44]	162	65	27	2	41	73	NA	NA								
[29]	488			216	470.5	37.8	NA	Neg								
[35]	High				Normal	Normal	NA	Neg								
[33]	255			157	148	27	+	Neg								
[40]	178		93		251	Normal	NA	Neg	143		98		239	20	NA	NA
[32]	20		100		132	71	NA	NA	20		100		132	71	NA	NA
[23]	266		98		Slightly high	Normal	NA	NA								
[51]	90			2	64	34	NA	NA	555			2920	69	48	NA	NA
	470	1		2	50	70	NA	NA								
[38]	44			9	56	43.2	NA	NA								
[49]	136		52	340	67	56	NA	Neg								
[26]	286				65	24	NA	Neg	366	34.7	59.2		180	4	NA	NA
[37]	170		76		29	49	+	+	150		85		Normal	Normal	NA	NA
[22]	153				122	23	NA	NA	160				127	24	NA	NA
	78				Normal	74	NA	NA	287				69	40	NA	NA
							+	NA								

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Abbreviations: CSF: Cerebrospinal fluid; WBC: White blood cell; N: neutrophils; L: lymphocyte; RBC: Red blood cell; PR: Protein; Neg: Negative; NA: Not available.

Encephalitis manifestations caused by *B. mandrillaris* are similar to other causes, and fever, headache, and seizures may be observed. These similar symptoms may lead specialists to misdiagnosis, and they might consider this infection tuberculoid, viral, or bacterial encephalitis.

We compared the demographic data, clinical symptoms, diagnostic tools, and treatment plans of pediatric patients with encephalitis caused by *B. mandrillaris*. This is the first systematic review globally intended to explore BAE in children. The age range of reported cases was from an 8-month-old female infant to an 18-year-old female teenager. The majority of cases were male children. The infection was mostly reported in the USA and China, and Africa had the fewest reported cases. It seems that the less-diagnosed cases in every area are related to the lack of awareness and inappropriate diagnostic tools. It is known that *B. mandrillaris* spreads and transmits via inhalation or injection from injured skin. It can reach the patient's CNS through blood flow. Another theory for the transmission of this amoeba is the inhalation of airborne cysts [15]. In this study, probable risk factors were reported in 22 children, of which the most common are traumas leading to skin injury (12 cases) and contact with infected water (3 cases – swimming or flood).

Diagnosing this amoeba is still challenging for healthcare systems because of the lack of knowledge about this infection. Due to this condition, there was a delay and misdiagnosis in most cases, and some patients presented with severe manifestations.

Different diagnostic methods, such as NGS, PCR, serological tests, biological tests, immunohistochemical assays, biopsy, and special staining methods for brain or skin lesions, have been used to confirm BAE. However, many of these tests do not need a specialty and are unavailable in some areas [8].

In our study, the most common confirming diagnostic tool was PCR on the involved body tissues. PCR on CSF, brain tissue, or skin lesions is a rapid and sensitive diagnostic tool to detect *B. mandrillaris*. It can be useful in clinical diagnosis and has been developed recently [16].

ELISA and indirect immunofluorescence assay are two serological methods that detect *B. mandrillaris*. Based on some studies, positive serology is a good way to diagnose BAE, but it cannot always be a certain tool to indicate a diagnosis [17].

Another agent for detecting this amoeba is indirect immunofluorescence using rabbit anti-*Balamuthia* spp. serum or a rabbit antiserum-based immunohistochemical assay on brain or skin tissue samples [18].

NGS is a new, noninvasive, and rapid method for diagnosing BAE (used in 5 patients) [19]. Wu et al. could diagnose BAE in a 13-year-old Chinese girl via NGS of CSF [20]. Yi et al. confirmed the presence of this amoeba using metagenomic NGS of CSF [19].

Neuroimaging studies, such as CT scans and MRIs, have shown some abnormalities, such as multiple hypodense and hyperdense lesions and edema. This is consistent with findings from Piper et al. [21].

In the CSF findings, lymphocytic pleocytosis, elevated protein levels, and low glucose have been predominantly reported. Overall, no significant signs in CSF analysis distinguish BAE from other encephalitis causes [8].

Until now, a definitive treatment method for this infection has not been established. Based on a study, the average survival time for patients admitted to the hospital is about 16 days, which indicates the poor prognosis of BAE [22]. Combination therapy is suggested, and in surviving cases, it includes multiple drugs, such as flucytosine, fluconazole, pentamidine, sulfadiazine, Clarithromycin, thioridazine, miltefosine, trimethoprim-sulfamethoxazole, and azithromycin. Miltefosine is a drug approved by the US Food and Drug Administration (FDA) for leishmaniasis treatment in 2013. It has been found useful against *B. mandrillaris* in vitro in combination with other drugs [23]. In this study, we found that milfetosin (in combination with other drugs) was used in 6 cases, but only one patient survived (Table 1) [24].

More investigation is needed to establish the appropriate and certain guidelines for this disease and its combination therapy. It should be noted that intracranial pressure should decrease as soon as possible in cases of high intracranial pressure. It has been found that steroids are not indicated because they can trigger both skin and CNS lesions [25].

In conclusion, further investigation is necessary to improve diagnosis and develop more effective therapies. Although it is rare and fatal, early detection and appropriate treatment can provide a chance of survival [26].

Conclusion

The manifestations of BAE may resemble those of any other encephalitis, which could lead to a loss of critical time and misdiagnosis, potentially resulting in the death of patients. Thus, it is recommended that every relevant specialist gain more knowledge and awareness of this lethal infection to facilitate early diagnosis and the best treatment plan. Further investigations into this infection and the development of more effective treatments are recommended to improve the survival chances of these patients.

Limitations

This systematic review has several limitations. First, all the included literature consisted of case reports and case series, which carry a risk of bias due to selective reporting and also lack of a control group. Second, the heterogeneity across articles in terms of patient demographics, diagnostic tools, and treatment regimens limited the ability to conduct a meta-analysis. Additionally, the small sample size in most articles restricted the generalizability of the results.

Despite these limitations, our review highlights the need for early diagnosis and more efficient treatment plans for this encephalitis.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflicts of interest

The authors declared no conflict of interest.

References

1. Roos KL. Encephalitis. Neurol Clin. 1999; 17(4):813-33. [DOI:10.1016/S0733-8619(05)70168-7] [PMID]

2. Martinez AJ, Visvesvara GS. Laboratory diagnosis of pathogenic free-living amoebas: Naegleria, acanthamoeba, and leptomyxid. Clin Lab Med. 1991; 11(4):861-72. [DOI:10.1016/S0272-2712(18)30524-9]

3. Schuster FL. Cultivation of pathogenic and opportunistic free-living amebas. Clin Microbiol Rev. 2002; 15(3):342-54. [DOI:10.1128/CMR.15.3.342-354.2002] [PMID] [PMCID]

4. Dunnebacke TH, Schuster FL, Yagi S, Booton GC. Balamuthia mandrillaris from soil samples. Microbiology. 2004; 150(Pt 9):2837-42. [DOI:10.1099/mic.0.27218-0] [PMID]

5. Schuster FL, Dunnebacke TH, Booton GC, Yagi S, Kohlmeier CK, Glaser C, et al. Environmental isolation of Balamuthia mandrillaris associated with a case of amebic encephalitis. J Clin Microbiol. 2003; 41(7):3175-80. [DOI:10.1128/JCM.41.7.3175-3180.2003] [PMID] [PMCID]

6. Visvesvara GS, Schuster FL, Martinez AJ. Balamuthia mandrillaris, N. G., N. Sp., agent of amebic meningoencephalitis in humans and other animals. J Eukaryot Microbiol. 1993; 40(4):504-14. [DOI:10.1111/j.1550-7408.1993.tb04943.x] [PMID]

7. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol Med Microbiol. 2007; 50(1):1-26. [DOI:10.1111/j.1574-695X.2007.00232.x] [PMID]

8. Zhang Z, Liang J, Wei R, Feng X, Wang L, Wang L, et al. Facial Balamuthia mandrillaris infection with neurological involvement in an immunocompetent child. Lancet Infect Dis. 2022; 22(3):e93-100. [DOI:10.1016/S1473-3099(21)00334-0] [PMID]

9. Visvesvara GS, Martinez AJ, Schuster FL, Leitch GJ, Wallace SV, Sawyer TK, et al. Leptomyxid ameba, a new agent of amebic meningoencephalitis in humans and animals. J Clin Microbiol. 1990; 28(12):2750-6. [DOI:10.1128/jcm.28.12.2750-2756.1990] [PMID] [PMCID]

10. Wang L, Cheng W, Li B, Jian Z, Qi X, Sun D, et al. Balamuthia mandrillaris infection in China: A retrospective report of 28 cases. Emerg Microbes Infect. 2020; 9(1):2348-57. [DOI:10.1080/22221751.2020.1835447] [PMID] [PMCID]

11. Cabello-Vílchez AM, Rodríguez-Zaragoza S, Piñero J, Valladares B, Lorenzo-Morales J. Balamuthia mandrillaris in South America: An emerging potential hidden pathogen in Perú. Exp Parasitol. 2014; 145Suppl:S10-9. [DOI:10.1016/j.exppara.2014.05.007] [PMID]

12. Cope JR, Landa J, Nethercut H, Collier SA, Glaser C, Moser M, et al. The epidemiology and clinical features of balamuthia mandrillaris disease in the United States, 1974-2016. Clin Infect Dis. 2019; 68(11):1815-22. [DOI:10.1093/cid/ciy813] [PMID] [PMCID]

13. Bravo F, Sanchez MR. New and re-emerging cutaneous infectious diseases in Latin America and other geographic areas. Dermatol Clin. 2003; 21(4):655-68, viii. [DOI:10.1016/S0733-8635(03)00090-1] [PMID]

14. Kiderlen AF, Laube U. Balamuthia mandrillaris, an opportunistic agent of granulomatous amebic encephalitis, infects the brain via the olfactory nerve pathway. Parasitol Res. 2004; 94(1):49-52. [DOI:10.1007/s00436-004-1163-z] [PMID]

15. Mungroo MR, Khan NA, Siddiqui R. Balamuthia mandrillaris: Pathogenesis, diagnosis, and treatment. Expert Opinion on Orphan Drugs. 2020; 8(4):111-9. [DOI:10.1080/21678707.2020.1758061]

16. Booton GC, Carmichael JR, Visvesvara GS, Byers TJ, Fuerst PA. Genotyping of balamuthia mandrillaris based on nuclear 18S and mitochondrial 16S rRNA genes. Am J Trop Med Hyg. 2003; 68(1):65-9. [DOI:10.4269/ajtmh.2003.68.65] [PMID]

17. Schuster FL, Yagi S, Wilkins PP, Gavali S, Visvesvara GS, Glaser CA. Balamuthia mandrillaris, agent of amebic encephalitis: detection of serum antibodies and antigenic similarity of isolates by enzyme immunoassay. J Eukaryot Microbiol. 2008; 55(4):313-20. [DOI:10.1111/j.1550-7408.2008.00333.x] [PMID]

18. Guarner J, Bartlett J, Shieh WJ, Paddock CD, Visvesvara GS, Zaki SR. Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. Mod Pathol. 2007; 20(12):1230-7. [DOI:10.1038/modpathol.3800973] [PMID]

19. Yi Z, Zhong J, Wu H, Li X, Chen Y, Chen H,et al. Balamuthia mandrillaris encephalitis in a child: case report and literature review. Diagn Microbiol Infect Dis. 2021; 100(4):115180. [DOI:10.1016/j.diagmicrobio.2020.115180] [PMID]

20. Wu X, Yan G, Han S, Ye Y, Cheng X, Gong H, et al. Diagnosing Balamuthia mandrillaris encephalitis via next-generation sequencing in a 13-year-old girl. Emerg Microbes Infect. 2020; 9(1):1379-87. [DOI:10.1080/22221751.2020.1775130] [PMID] [PMCID]

21. Piper KJ, Foster H, Susanto D, Maree CL, Thornton SD, Cobbs CS. Fatal Balamuthia mandrillaris brain infection associated with improper nasal lavage. Int J Infect Dis. 2018; 77:18-22. [DOI:10.1016/j.ijid.2018.09.013] [PMID]

22. Schuster FL, Yagi S, Gavali S, Michelson D, Raghavan R, Blomquist I, et al. Under the radar: Balamuthia amebic encephalitis. Clin Infect Dis. 2009; 48(7):879-87. [DOI:10.1086/597260] [PMID]

23. Martínez AJ, Guerra AE, García-Tamayo J, Céspedes G, González-Alfonzo JE, Visvesvara GS. Granulomatous amebic encephalitis: A review and report of a spontaneous case from Venezuela. Acta Neuropathol. 1994; 87(4):430-4. [DOI:10.1007/BF00313614] [PMID]

24. Cuoco JA, Klein BJ, LeBel DP, Faulhaber J, Apfel LS, Witcher MR. Successful treatment of a balamuthia mandrillaris cerebral abscess in a pediatric patient with complete surgical resection and antimicrobial therapy. Pediatr Infect Dis J. 2022; 41(2):e54-7. [DOI:10.1097/INF.0000000000003418] [PMID]

25. Doyle JS, Campbell E, Fuller A, Spelman DW, Cameron R, Malham G, et al. Balamuthia mandrillaris brain abscess successfully treated with complete surgical excision and prolonged combination antimicrobial therapy. J Neurosurg. 2011; 114(2):458-62. [DOI:10.3171/2010.10.JNS10677] [PMID]

26. Joo SJ, Thompson AB, Philipsborn R, Emrath E, Camacho-Gonzalez AF, Chahroudi A, et al. An unusual cause of fever and headache in a school-aged male. Clin Pediatr. 2018; 57(11):1359-62. [DOI:10.1177/0009922818772056] [PMID] [PMCID]

27. Tootla HD, Eley BS, Enslin JMN, Frean JA, Hlela C, Kilborn TN, et al. Balamuthia mandrillaris granulomatous amoebic encephalitis: The first African experience. J Pediatric Infect Dis Soc. 2022; 11(12):578-81. [DOI:10.1093/jpids/piac096] [PMID]

28. Tao K, Wang T, Zhang L, Yang XC, Zhai ZF. Fatal balamuthia mandrillaris infection with red plaques on the nasal dorsum as the first presentation. An Bras Dermatol. 2022; 97(4):498-500. [DOI:10.1016/j.abd.2021.12.001] [PMID] [PMCID]

29. Ai J, Zhang H, Yu S, Li J, Chen S, Zhang W, Mao R. A case of fatal amoebic encephalitis caused by Balamuthia mandrillaris, China. Infect Genet Evol. 2022; 97:105190. [DOI:10.1016/j.meegid.2021.105190] [PMID]

30. Yang Y, Hu X, Min L, Dong X, Guan Y. Balamuthia mandrillaris-related primary amoebic encephalitis in china diagnosed by next generation sequencing and a review of the literature. Lab Med. 2020; 51(2):e20-6. [DOI:10.1093/labmed/lmz079] [PMID]

31. Shehab KW, Aboul-Nasr K, Elliott SP. Balamuthia mandrillaris granulomatous amebic encephalitis with renal dissemination in a previously healthy child: Case report and review of the pediatric literature. J Pediatric Infect Dis Soc. 2018; 7(3):e163-8. [DOI:10.1093/jpids/pix089] [PMID]

32. Khurana S, Hallur V, Goyal MK, Sehgal R, Radotra BD. Emergence of balamuthia mandrillaris meningoencephalitis in India. Indian J Med Microbiol. 2015; 33(2):298-300. [DOI:10.4103/0255-0857.154887] [PMID]

33. Krasaelap A, Prechawit S, Chansaenroj J, Punyahotra P, Puthanakit T, Chomtho K, et al. Fatal balamuthia amebic encephalitis in a healthy child: A case report with review of survival cases. Korean J Parasitol. 2013; 51(3):335-41. [DOI:10.3347/kjp.2013.51.3.335] [PMID] [PMCID]

34. Moriarty P, Burke C, McCrossin D, Campbell R, Cherian S, Shahab MS, et al. Balamuthia mandrillaris encephalitis: Survival of a child with severe meningoencephalitis and review of the literature. J Pediatric Infect Dis Soc. 2014; 3(1):e4-9. [DOI:10.1093/jpids/pit033] [PMID]

35. Stidd DA, Root B, Weinand ME, Anton R. Granulomatous amoebic encephalitis caused by Balamuthia mandrillaris in an immunocompetent girl. World Neurosurg. 2012; 78(6):715.e7-12. [DOI:10.1016/j.wneu.2011.10.040] [PMID]

36. Ghosh PS, Ghosh D, Loddenkemper T, Prayson RA, Tekautz T, Sriram CS, et al. Necrotizing granulomatous meningoencephalitis due to Balamuthia in an immunocompetent child. Neurology. 2011; 77(8):801-2. [DOI:10.1212/WNL.0b013e31822b0100] [PMID]

37. Orozco L, Hanigan W, Khan M, Fratkin J, Lee M. Neurosurgical intervention in the diagnosis and treatment of Balamuthia mandrillaris encephalitis. J Neurosurg. 2011; 115(3):636-40. [DOI:10.3171/2011.4.JNS102057] [PMID]

38. Hill CP, Damodaran O, Walsh P, Jevon GP, Blyth CC. Balamuthia amebic meningoencephalitis and mycotic aneurysms in an infant. Pediatr Neurol. 2011; 45(1):45-8. [DOI:10.1016/j.pediatrneurol.2011.05.003] [PMID]

39. Combs FJ Jr, Erly WK, Valentino CM, Rance NE. Best cases from the AFIP: Balamuthia mandrillaris amebic meningoencephalitis. Radiographics. 2011; 31(1):31-5. [DOI:10.1148/rg.311105067] [PMID]

40. Cary LC, Maul E, Potter C, Wong P, Nelson PT, Given C 2nd, et al. Balamuthia mandrillaris meningoencephalitis: Survival of a pediatric patient. Pediatrics. 2010; 125(3):e699-703. [DOI:10.1542/peds.2009-1797] [PMID]

41. Valverde J, Arrese JE, Piérard GE. Granulomatous cutaneous centrofacial and meningocerebral amebiasis. Am J Clin Dermatol. 2006; 7(4):267-9. [DOI:10.2165/00128071-200607040-00009] [PMID]

42. Tavares M, Correia da Costa JM, Carpenter SS, Santos LA, Afonso C, Aguiar A, et al. Diagnosis of first case of Balamuthia amoebic encephalitis in Portugal by immunofluorescence and PCR. J Clin Microbiol. 2006; 44(7):2660-3. [DOI:10.1128/JCM.00479-06] [PMID] [PMCID]

43. Bakardjiev A, Azimi PH, Ashouri N, Ascher DP, Janner D, Schuster FL, et al. Amebic encephalitis caused by Balamuthia mandrillaris: Report of four cases. Pediatr Infect Dis J. 2003; 22(5):447-53. [DOI:10.1097/00006454-200305000-00013] [PMID]

44. Deetz TR, Sawyer MH, Billman G, Schuster FL, Visvesvara GS. Successful treatment of Balamuthia amoebic encephalitis: Presentation of 2 cases. Clin Infect Dis. 2003; 37(10):1304-12. [DOI:10.1086/379020] [PMID]

45. Healy JF. Balamuthia amebic encephalitis: Radiographic and pathologic findings. AJNR. 2002; 23(3):486. [Link]

46. Galarza M, Cuccia V, Sosa FP, Monges JA. Pediatric granulomatous cerebral amebiasis: A delayed diagnosis. Pediatr Neurol. 2002; 26(2):153-6. [DOI:10.1016/S0887-8994(01)00360-5] [PMID]

47. Kodet R, Nohýnková E, Tichý M, Soukup J, Visvesvara GS. Amebic encephalitis caused by Balamuthia mandrillaris in a Czech child: Description of the first case from Europe. Pathol Res Pract. 1998; 194(6):423-9. [DOI:10.1016/S0344-0338(98)80033-2] [PMID]

48. Reed RP, Cooke-Yarborough CM, Jaquiery AL, Grimwood K, Kemp AS, Su JC, et al. Fatal granulomatous amoebic encephalitis caused by Balamuthia mandrillaris. Med J Aust. 1997; 167(2):82-4. [DOI:10.5694/j.1326-5377.1997.tb138785.x] [PMID]

49. Duke BJ, Tyson RW, DeBiasi R, Freeman JE, Winston KR. Balamuthia mandrillaris meningoencephalitis presenting with acute hydrocephalus. Pediatr Neurosurg. 1997; 26(2):107-11. [DOI:10.1159/000121172] [PMID]

50. Riestra-Castaneda JM, Riestra-Castaneda R, Gonzalez-Garrido AA, Pena Moreno P, Martinez AJ, Visvesvara GS, et al. Granulomatous amebic encephalitis due to Balamuthia mandrillaris (Leptomyxiidae): Report of four cases from Mexico. Am J Trop Med Hyg. 1997; 56(6):603-7. [DOI:10.4269/ajtmh.1997.56.603] [PMID]

51. Griesemer DA, Barton LL, Reese CM, Johnson PC, Gabrielsen JA, Talwar D, et al. Amebic meningoencephalitis caused by Balamuthia mandrillaris. Pediatr Neurol. 1994; 10(3):249-54. [DOI:10.1016/0887-8994(94)90034-5] [PMID]

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