

# Amoebic meningoencephalitis in Samarinda East Kalimantan

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**Abstract.** Meningoencephalitis is a disease of central nervous system caused by s microorganism such as bacteria, Rickettsiae, Spirochaeta, virus, fungus, protozoa and helminth. Free living amoeba protozoa has been reported as an etiology agent of meningoencephalitis. Amoebic meningoencephalitis is a very rare condition and often fatal because of late diagnosis. We reported a 24 years old woman, hospitalized at Abdul Wahab Syahrani's Hospital, Samarinda East Kalimantan with decreased of consciousness and fever. The brain MRI showed focal lesion suggested as a granuloma. ed Microorganisms like cyst and trophozoite with pseudopodia amoebic form were identified from the cerebrospinal fluid. Based on these findings the patient was diagnosed with amoebic meningoencephalitis infection and got intravenous metronidazole. The patient died on the twenty third days of hospitalization.

## 1. Introduction

Meningoencephalitis could be caused by microorganism such as bacteria, Rickettsiae, Spirochaeta, virus, fungus, protozoa and helminth. Some of free living amoeba protozoa like Acanthamoeba, Naegleria and Balamuthia genus have been reported as an etiology of central nervous system infection in human. Amoebic meningoencephalitis is very rare but often fatal because of late diagnosis. In this patient, the formation resembles protozoa found in cerebrospinal fluid (CSF), suggesting an amoebic protozoa. They found as the formation of cysts, and trophozoite with the presence of pseudopodia. We reported a fatal case with amoebic meningoencephalitis.

## 2. Case Report

A 24-year-old woman came to the Emergency Unit of AW. Sjahrani Hospital Samarinda East Kalimantan on February 23, 2016 with a major complaint of decreased consciousness since 4 hours before admission. The patient had fever since one week before admission. Approximately one month before admission, the patient had severe headache and was hospitalized for two weeks. On physical examination there was no meningeal excitatory sign nor focal neurologic deficits. Magnetic



Resonance Imaging (MRI) angiography was performed on January 29, 2016. The results did not indicate the existence of focal lesions of the parenchyma and blood vessels. The patient discharged from the hospital with a diagnosis of migraine headaches and treated with Depakote 250 mg two times daily, and the headache did not improve. On physical examination, the Glasgow Coma Scale (GCS) score was E1V1M5, diameter of pupil 2.5 mm bilaterally, normal light reflex bilaterally, blood pressure 120/80 mmHg, pulse frequency 96 times per minute, respiratory rate 20 times per minute, temperature of 38,4°C, Babinski and clonus positive on the left side. Laboratory examination on 23 February 2016 was o Hb 12.8 gr/dL, leucocytes 10.780 /  $\mu$ L, platelet 284.000 /  $\mu$ L, blood glucose 121 mg / dL, albumin 4,2 g / dL, ureum 24,0 mg/dL, creatinine 0.5 mg/dL, sodium 133 mEq/L, potassium 4.0 meq L, chloride 100 mEq/L, magnesium 2.3 mEq/L, procalcitonin <0.05 ng/mL, non reactive HBsAg, non reactive HIV antibodies, negative IgM dengue, negative IgG, negative NS1 dengue. Radiological examination of chest X-ray showed normal. Head CT scan on February 23, 2016 showed a lateral ventricular dilatation that impressed an obstructive hydrocephalus. Patients are diagnosed with Hydrocephalus as a result of bacterial meningoencephalitis. The patient was treated with intravenous Ceftriaxone two grams twice daily intravenous and Dexamethasone 10 grams four times daily. On February 25, 2016, the installation of Extra Ventricular Drainage (EVD) was performed and found an intracranial pressure increased. The analysis of cerebrospinal fluid (CSF) obtained clear color, cloudy, cell count 7 cells/mL with MN cells 85% and PMN 15%, protein 170 mg/L, glucose 10 mg dL, None test (+), Pandy test (+). There was no growth of germs in the CSF culture. TORCH immunological examination on February 26, 2016 showed Rubella IgG positive (186 IU/mL) and CMV IgG positive (205.2 IU/mL) and CMV IgG avidity was 72.2%. The patient was diagnosed temporarily with Fungal and TB Meningoensefalitis and the presence of reinfection of the Cytomegalovirus virus. The patient was given additional injection therapy of Flukonazole 450 mg once daily and Gancyclovir 250 mg twice daily and combination of antituberculosis drugs (Rifampicin 450 mg, INH 300 mg, Etambutol 750 mg, Pyrazinamide 1000 mg. Provision of Amphotericin B Injection 0.5 g/kg body weight day but the drug was not available. On February 29, 2016 sputum culture examination was performed and *Acinetobacter baumannii* was found, in addition nasal swab culture obtained *Staphylococcus haemolyticus*. On March 1, 2016, analysis of CSF showed clear and slightly cloudy fluid, 150 leucocytes/mL (of with MN 85% and PMN 15%), Pandy test (+), protein 4,232 mg/L and glucose 71 mg/dL.



**Figure 1.** MRI results on the 8th day of an impressive treatment of a granuloma

No growth of germs was found from CSF culture. In sputum culture examination, *Pseudomonas aeruginosa* was obtained and in left and right axillary swap cultures, *Klebsiella penumonia* was obtained. On March 3, 2016, patient's GCS score was E1M5Vx (tracheostomy was performed). Microbiological examination of CSF obtained negative acid fast bacilli and examination of real time PCR showed positive DNA *Mycobacterium tuberculosis complex*. The patient was given additional therapy of 1 gram Streptomycin once daily. In addition to the contrasted MRI results in the presence of focal lesions in T1 and T2 in the distal regions of the ganglia dextra basal which suggest a granuloma (figure 1).

Laboratory examination on March 4, 2016 was showed Hb 11.3 gr/dL, leucocytes 19,440/ $\mu$ L, 261,000  $\mu$ L platelets, sodium 128 mEq/L, potassium 4.6 mEq/L, chloride 97 mEq/L, and Procalcitonin 0.79 ng mL., SGOT 112 mIU/mL, SGPT 256 mIU/mL. In the direct preparation of CSF with ink parker, a formation of microorganisms was found, did not absorb the color, three times erythrocytes size, with cyst (figure 2), with the existence of pseudopodia and found amoeboid formation. It was something like amoeba (figure 3 and figure 4) On March 7, 2016, patient was given additional treatment of Metronidazole 500 mg injection three times daily. Patient was diagnosed with amoebic Meningoencephalitis.



**Figure 2.** Amoebic cyst

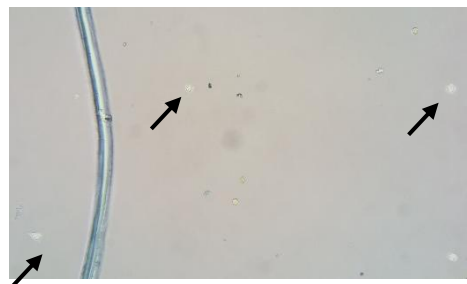


**Figure 3.** (a) Trophozoite with pseudopodia. (b,c) amoebic cysts

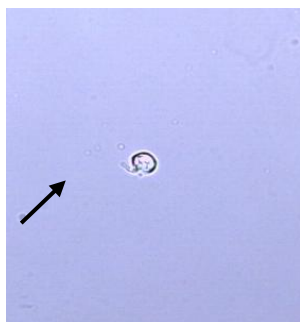


**Figure 4.** Amoeba with pseudopodia

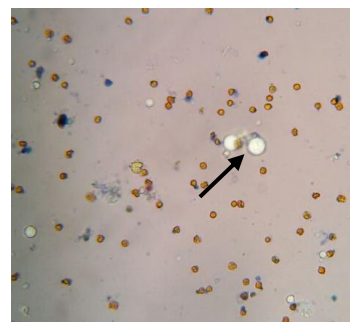


**Figure 5.** Cysts with nucleus

### 3. Discussion

Meningoencephalitis can be established clinically through anamnesis and neurological physical examination. The presence of fever, headache, behavioral changes, meningeal excitatory signs and focal neurologic deficits suggest an inflammatory process in the meninges region accompanied by focal lesions in the brain parenchyma. Acute meningoencephalitis can be caused by various microorganisms such as gram-positive bacteria, gram-negative bacteria, anaerobic bacteria and *Mycobacterium tuberculosis*, Rickettsiae, Spirochaeta, viruses, fungi, protozoa and helminth. In the initial phase of the course of the patient's illness, the presence of meningeal excitatory signs or focal

neurologic deficits was not found, only headache and fever was exist. Symptoms of meningeal excitatory and even global focal neurologic deficits was found in the advanced phase of the disease course. The patient had decreased of consciousness approximately 1.5 months from the first time complaining of headaches. Etiology of meningoencephalitis was established through examination of CSF and microbiological analysis and contrast head imaging. Since the patient was admitted to the hospital, several times the CSF was examined but until day 18 showed no bacterial growth but showed a significant increase in protein levels since admission. The results indicated the possibility of infection caused by tuberculosis, fungi or parasite that is with the increase of protein content, decrease of glucose level and cell count is dominated by MN (85%) than PMN (35%). Meningoencephalitis can also be caused by frequent fungal infections due to *Cryptococcus neoformans*. In this patient, we performed a microbiological examination for *Cryptococcus* with direct preparation using ink parker but no *Cryptococcus* was found. According to the reference, *Cryptococcus* identification uses Indian ink. Immunologic examination results showed positive CMV IgG antibody and continued with an avidity test, indicating the possibility of reinfection or past infection. CMV infection may manifest as interstitial pneumonia, hepatitis, Guillain-Barre Syndrome, retinitis, myocarditis, colitis, thrombocytopenia and haemolytic anemia, skin eruption, and meningoencephalitis.

Meningoencephalitis due to CMV generally occurs in immunocompromised patients. In AIDS patients, CMV infection is the most common viral opportunistic infection (21-44%). Meningoencephalitis due to CMV is characterized by sensory impairment and motor weakness such as radiculopathy, severe headache, photophobia, lethargy. CSF shows a slight increase in lymphocytes. The exact diagnosis is based on the discovery of CMV DNA by PCR examination. However, in this patient there is no CMV DNA testing due to equipment limitations. Antiviral drugs that are effective in the treatment of CMV infections are ganciclovir, valganciclovir, foscarnet, cidofovir, maribavir [1] Ganciclovir has been given in this patient. Based on results of brain fluid analysis indicating the possibility of tuberculosis or fungal infection, the patient was given antituberculous therapy but did not give any improvement. *Mycobacterium tuberculosis complex* positive DNA test results can be interpreted as an infection of other *Mycobacterium* species, namely *M. bovis*, *M. bovis Bacillus Calmette-Guerin*, *M. africanum*, *M. canetti*, *M. microti* [2]. The 11th day of treatment, from the microscopic examination of the direct supply of cerebrospinal fluid found a formation that resembles protozoa. There are several types of protozoan infections that can infect central nervous system, such as amoebiasis, cerebral malaria, Toxoplasmosis, Tripanosomiasis, Microsporidiosis, Leishmaniasis<sup>2</sup>. In this patient, the formation resembles protozoa found in CSF, suggesting an amoebic formation as it is found in the formation of cysts, amoeboid formations and the presence of pseudopodia. Some types of free living amoeba from the genus *Acanthamoeba*, *Balamuthia* and *Neigleria* have been known to infect the central nervous system of humans [2,3]. It could be manifest as opportunistic infections mainly in immunocompromised hosts or as non opportunistics [4] Some *Acanthamoeba* species cause chronic infections ie. granulomatous amoebic encephalitis (GAE) particularly in immunocompromised individuals. *Balamuthia* can cause GAE in immunocompromised and immunocompetent individuals. *Neigleria fowleri* has been reported to infect children and immunocompetent young adults and cause acute and fulminant illness called Primary Amoebic Meningoencephalitis (PAM) [3].

Free living amoeba can live in soil and water such as lake water, swimming pool water or water for domestic. A study in Malaysia detected *Acanthamoeba naegleria* species from some swimming pool of four sites in Petaling Jaya and Kuala Lumpur [5]. PAM cases caused by *Neigleria fowleri* that contaminate domestic water used for bathing have been reported. Infection can occur when amoeba enters through the nose, attaches to the olfactory mucosa, and migrates through the cribriform plate along the olfactory nerve. When entering the olfactory bulb in the brain, this amoeba will divide rapidly and death occurs within 7-10 days. *N.fowleri* may result in fulminant hemorrhagic brain necrosis [6]. Symptoms of PAM infection are characterized by severe and sudden headache, fever, nausea and vomiting and rhinitis. The symptoms will next followed by tense neck, diplopia, diminished olfactory sensations, confusion and convulsions, which develop rapidly into coma and

result in death. Diagnosis with Head CT scan or MRI, indicating lesion but not specific. This condition is often indistinguishable from meningoencephalitis due to bacteria, fungi or viral[4]. Microscopic examination with direct preparations of CSF is the choice because the presence of moving amoebae can be observed with a light microscope. Amoeba can be misinterpreted with macrophages, but the *N. Fowlerii* nucleus contains a large nucleus located centrally that distinguishes it from host cells <sup>[6]</sup>. *Acanthamoeba* is the most commonly isolated amoeba from the environment. Amoeba is often present in domestic water reservoirs, hospital water, air and soil. It has two morphological forms, trophozoites and cysts, in which the trophozoite form is an infective form. Some *Acanthamoeba* species cause GAE which is a progressive chronic infection and rarely in the central nervous system. Symptoms of GAE include headache, slight fever, seizures, hemiparesis, personality disorders, nausea, strained neck, decreased consciousness and coma. The clinical symptoms of GAE are not specific so often previously diagnosed as bacterial leptomeningitis, tuberculosis meningitis, viral encephalitis, toxoplasmosis, fungal infections, neurosistiserkosis or brain tumors. On brain imaging studies, hydrocephalus found with meningens thickening, multifocal lesions and edema [2,6] The definitive diagnosis of GAE is confirmed by the discovery of amoebae in tissue or amoebic isolation. Microscopic examination with a light microscope and electron can detect *Acanthamoeba* from brain fluids or brain tissue. The shape of trophozoite can be distinguished from macrophage cells in the presence of a nucleus structure with a large central nucleolus that forms a halo image. Biopsy examination of brain tissue by HE staining will show a picture of brain granuloma, necrosis with multinucleated cells, inflammatory infiltrate and amoeba [2,6]. *Acanthamoeba castellanii*, *A. polyphaga*, and *Balamuthia mandrillaris* are the species that cause brain infections. These amoeba can be found on the ground and have been reported to be found in water. *Balamuthia*-induced encephalitis presents symptoms that are not specific, chronic in the form of headache, nausea, vomiting, fever, myalgia, seizures, weight loss, hemiparesis, speech disorders and are usually associated with previous lesions of skin granuloma. *Balamuthia* is often identified as *Acanthamoeba* because both cause GAE. *B. mandrillaris* is not visible in the fluid reservoir of the brain but can only be isolated from the biopsy of brain tissue [2,6].

This patient showed a course of sub-chronic disease. A contrast head imaging examination results in an impressive granuloma. With the discovery of a cyst formation, trophozoite and the presence of protozoan pseudopods in CSF, it may be suspected of an *Acanthamoeba* protozoal infection in the central nervous system. This patient had been given antifungal, antiviral, antituberculosis, and metronidazole but the patient's condition has not shown any improvement since hospitalization. Survival rate is determined by the speed of diagnosis and immediate management. Administration of Amphotericin B, Fluconazole IV followed by oral Rifampicin was reported to show improvement in protozoan meningoencephalitis cases <sup>[6]</sup>. In this patient, Amphotericin could not be administered because of inavailability of the drug. This condition could be contributed to the patient's worsening, since it had been proven that only amphotericin B has in vitro and in vivo amoebicidal effect <sup>[7]</sup>. On the 19th day of treatment, this patient had nosocomial pneumonia in which sputum culture was obtained *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is one of the major causes of nosocomial infections and is currently feared for resistance to various antibiotics, including the carbapenem (MDR/XDR *P. aeruginosa*) group [6,8]. This patient suffered from a condition of acquired or secondary immunodeficiency resulting in infection of various microorganisms such as bacteria, fungi, viruses, parasites. Acquired immunodeficiency occurs through two major pathogens: biological complications of a disease (infection, malnutrition, cancer) or complications of therapy against other diseases (immunosuppressive therapy). Immunodeficiency occurs due to impairment of cellular mediated immunity (CMI), ie. reduced number of lymphocytes or decreased lymphocyte activation (CD4 and CD8 effector cells) that play a role in the adaptive immune response to intracellular microorganisms [9,10].

#### 4. Conclusion

A case of a 24-year-old woman with meningoencephalitis is probably caused by amoeba. Clinical symptoms are not specific early in the course of the disease, making the diagnosis difficult to enforce. In the advanced phase, after being given anti-amoeba therapy, the patient did not show clinical improvement. Anti-amoebic therapies such as Amphotericin B and fluconazole were required. Amoebic meningoencephalitis is a rare case and the diagnosis is difficult to establish. For that reason, consider the development of immunological examination methods to help detecting the presence of amoeba in brain fluids since amoebic morphological examination is highly subjective and needs special skills.

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