

SPONTANEOUS GRANULOMATOUS AMEBIC ENCEPHALITIS : REPORT OF FOUR CASES FROM THAILAND

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Abstract. Granulomatous amebic encephalitis (GAE), or meningoencephalitis caused by *Acanthamoeba* sp and leptomyxid amebae are uncommon CNS infections that usually occur in an immunocompromised host. From 1990 to 1992, 4 patients with GAE were treated at Siriraj Hospital, Bangkok. One case was diagnosed antemortem, from a brain biopsy. The other three cases were diagnosed as GAE postmortem. Pathological findings included acute and subacute granulomatous inflammation with extensive cerebral necrosis, angiitis, fibrinoid necrosis and fibrin thrombi. One patient had a chronic skin ulcer in which free-living amebic trophozoites were found. No visceral involvement was observed. All patients developed "spontaneous" GAE, but we suspect an undiagnosed abnormality in cell mediated immunity or a deficient humoral immune response.

INTRODUCTION

Entamoeba histolytica infection is a common infection in Thailand (Viranuvatti, 1992). By contrast, primary amebic meningoencephalitis (PAM) due to *Naegleria fowleri* has rarely been reported (Jariya *et al*, 1983; Charoenlarp *et al*, 1988; Pongvarin and Jariya, 1991). In addition to *Naegleria fowleri*, *Acanthamoeba* sp and leptomyxid amebae are free-living amebae that may cause CNS involvement chiefly in immunosuppressed patients, resulting in granulomatous amebic encephalitis (GAE) (Ma *et al*, 1990; Visvesvara *et al*, 1990; Anzil *et al*, 1991). As of January 1990, 56 cases of GAE had been reported from different countries (Visvesvara and Stehr-Green, 1990). The first case of GAE in Thailand was diagnosed in 1987 (Jariya *et al*, 1992). This is a report of the next 4 cases of GAE; the patients were treated at the same hospital over a 3-year period.

Clinical and demographic data

Age, sex, clinical and other pertinent information are given in Table 1.

Pathologic findings

The neuropathological findings of the 4 cases were similar. The brain weight ranged from 1,160

to 1,600 g with moderate to marked swelling. One case showed uncal and cerebellar tonsillar herniations. The external surface of all 4 cases showed multiple, discrete pale-gray to dark-brown soft necrotic areas, 2 to 4 cm in diameter. Most of the lesions involved the cerebral cortex and the subcortical white matter. Lesions were found in the cerebral hemispheres, the brainstem, and the cerebellum (Figs 1, 2).

Microscopic examination disclosed the following histopathological findings:

1. Amebic trophozoites and cysts within the lesions
2. Modest, acute and chronic inflammation with multinucleated giant cells
3. Necrosis and hemorrhage of CNS parenchyma
4. Modest angiitis and occasional fibrin-platelet thrombi

Free-living amebic trophozoites and cysts

Amebic trophozoites were numerous in each case. They measured 30 to 45 μ m, and were spherically shaped with centrally placed single prominent nucleoli surrounded by a clear nuclear zone. Trophozoites were scattered in the necrotic areas within the leptomeninges; they accumulated around blood vessels and occasionally invaded the vascular wall (Fig 3). Fewer amebic cysts were found; they were smaller, 15-20 μ m, spherically

Table 1

Granulomatous amebic encephalitis in Thailand : epidemiologic/demographic/clinical/pathologic data.

Case# Autopsy#	Age/sex occupation	Date of death	Clinical features	Pathological findings	Etiologic ameba
1 A34-66	26 F worker	02/27/91	Systemic lupus erythematosus Tx: Prednisone Fever and oral candidiasis, Focal seizures, confusion Died 8 days after admission	Brain wt : 1,250 g Multiple areas of hemorrhagic encephalomalacia Pulmonary aspergillosis Lupus nephritis	<i>A. castellanii</i>
2 A34-234	20 F worker	08/02/91	Chronic ulcer on right ankle × 2 months with amebic troph, Fever, headaches, and drowsiness Crania 1 nerve palsies and stiff neck. Died 5 days after admission	Brain wt : 1,450 g Multiple areas of hemorrhagic encephalomalacia Bronchopneumonia	Leptomyxid
3 A33-114	20 M farmer	05/08/90	History of aplastic anemia and paroxysmal nocturnal hemoglobinuria. Tx: Prednisolone Headaches; vomiting Died 2 days after admission	Brain wt : 1,600 g Multiple areas of hemorrhagic encephalomalacia Uncal and cerebellar tonsil herniation.	<i>Acanthamoeba</i> sp (<i>A. healyi</i>)
4 A35-2	48 F worker	01/05/92	Chronic alcoholism; history of headaches, drowsiness and left hemiparesis × 5 days. Left facial palsy. Tx: Dexamethazone Brain bx: GAE Died 4 days after biopsy	Brain wt : 1,160 g Multiple areas of hemorrhagic softening	Leptomyxid

F = Female

M = male

Tx = Treatment

Troph = Trophozoites

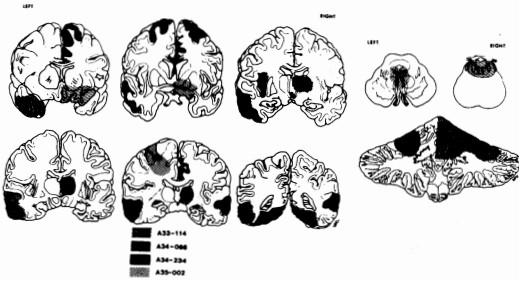


Fig 1—Coronal sections of cerebral hemispheres and horizontal sections through midbrain, pons and cerebellum showing the location and distribution of lesions in the 4 cases of GAE.

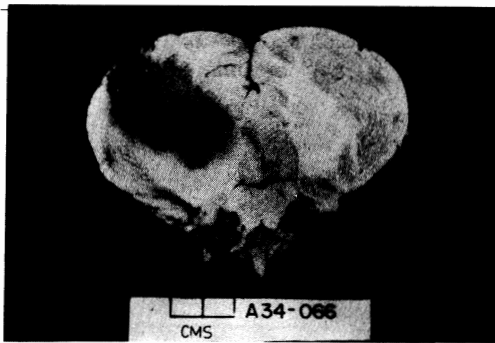


Fig 2—The cerebellar lesion caused by *A. castellanii* from case 1

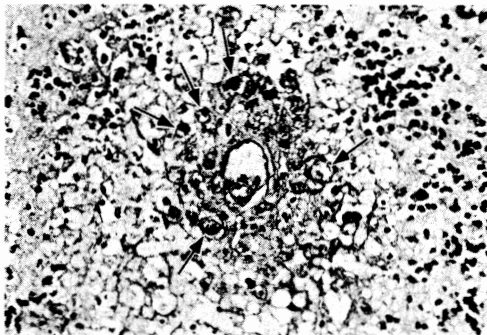


Fig 3—Amebic trophozoites (arrow) around the vessel (A33-114, H and E x 200)

to polygonally shaped. The cyst wall was thick and refractile. They usually accompanied the trophozoites (Fig 4).

Acute and chronic inflammation

The inflammatory reaction varied from acute

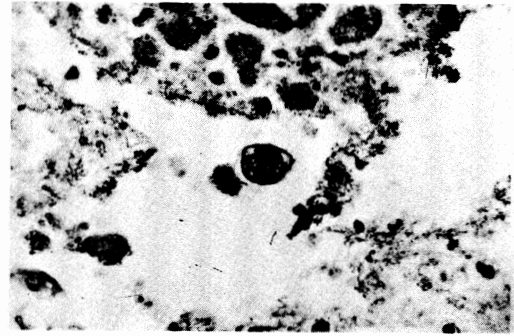


Fig 4—Leptomyxid amebic cyst within edematous CNS tissue (A35-002, H and E x 400)

to chronic. Diffuse and rather modest polymorphonuclear cell infiltration with scanty lymphocytes and mononuclear cells were noted in 2 cases (cases 1, 3) without granulomatous reaction or multinucleated cells. In the other 2 cases (case 2, 4) numerous mononuclear cells, plasma cells, foamy macrophages and multinucleated giant cells were observed. We also found perivascular cuffing, atypical lymphocytes, plasmacytoid cells, and immunoblasts with angiocentric patterns. The leptomeninges covering cortical lesions show focal inflammatory reaction.

Necrosis and hemorrhage of CNS parenchyma

CNS parenchymal necrosis, infiltrated by acute and chronic inflammatory cells, was seen in all cases. Within necrotic tissue, blood vessels occluded by fibrin-platelet thrombi were usually present. Reactive astrocytosis at the periphery of the lesions was observed in 2 cases (case 2, 4). Microglial nodules were not present.

Modest angiitis

Necrosis of the blood vessel wall with polymorphonuclear cell infiltration and nuclear debris were noted in all cases (Fig 5). Small leptomeningeal vessels and cortical capillaries were affected even in the blood vessels that were devoid of trophozoites. Blood vessels invaded by trophozoites and cysts were occasionally seen. Aneurysmal dilatation of blood vessels was also seen occasionally. Fibrin-platelet thrombi were noted in blood vessels with or without angiitis.

The chronic skin ulceration found in case 2 revealed chronic inflammation deep into the sub-

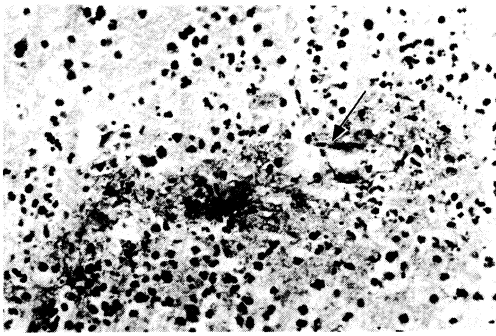


Fig 5—Necrotizing vasculitis (arrow) with numerous polymorphonuclear cells (A35-002, H and E \times 200)

cutaneous tissue, with caseous necrosis, surrounded by chronic inflammation and multinucleated giant cells. Angiitis was noted with many amebic trophozoites nearby. The pulmonary nodules found in case 1 revealed multiple *Aspergillus* abscesses and evidence of bronchopneumonia. We were unable to find trophozoites, cysts, or even a granulomatous reaction in the visceral organs in any of the cases.

Identification of the etiologic amebae

We used the indirect immunofluorescence (IIF) test on the tissue sections on all 4 cases to identify the etiologic agent. Rabbit antiserum was made against several species of *Acanthamoeba* (*A. castellanii*, *A. polyphaga*, *A. quina*, *A. lugdunensis*, *A. rhyssodes*, *A. palestinensis*, *A. divionensis*, *A. culbertsoni*, *A. lenticulata*, *A. healyi*) and the leptomixid ameba. We found the following results:

Case 1 was positive for *Acanthamoeba castellanii* (Table 1).

Case 2 was positive only for the leptomixid ameba.

In case 3, amebae in the tissue sections reacted with all of the anti-*Acanthamoeba* sera but not with the anti-leptomixid ameba serum. The amebae, however, reacted most strongly with the anti-*A. healyi* and produced bright apple green fluorescence.

Case 4 was positive only for leptomixid ameba. No fluorescence was seen when the sections were reacted with any of the other sera, indicating that the ameba involved belong to the leptomixid ameba group.

DISCUSSION

GAE usually occurs in compromised patients, or in patients with associated underlying diseases; however, it can occur in normal hosts as well. Unlike PAM, where the portal of entry is the nasal passage, the skin, open wound, lower respiratory tract and nasal mucosa are thought to be portal of entry for GAE. (Visvesvara and Stehr-Green, 1990). Only case 2 from this report demonstrated a possible portal of entry and the route of infection. (skin lesion)

The incubation period and the duration of GAE are difficult to determine. The patient in case 2 had a skin lesion for 2 months. Clinically, all patients had insidious onset of fever, headache, meningism, or CNS localizing signs. When changes in consciousness occurred, either drowsiness or confusion, the patients all died within 10 days.

Antemortem diagnosis of GAE is quite difficult. A CSF examination should be done for all patients for whom lumbar puncture is not contraindicated. Our findings showed CSF pleocytosis (44-468 cells/mm³), predominantly lymphocytes (54%-100%). The protein was increased (117-251 mg/ml) and sugar was quite low (14-62 mg/ml). CT scan or MRI may expedite diagnosis. Histopathological examinations of the CNS shows multiple discrete necrotizing hemorrhagic areas, usually involving the cortex and subcortical white matter (Wiley *et al*, 1987). Using CT scanning, multiple hyperdense mass lesions may be seen. Brain edema and hydrocephalus are quite severe. Results of a brain biopsy can be diagnostic. Because amebic trophozoites are present in the lesions, even a small amount of necrotic tissue may be very helpful in the diagnosis. The amebic trophozoites stained with H and E, resemble large mononuclear cells or gitter cells, and the perivascular infiltration with lymphocytes may mimic CNS lymphoma. When granulomatous inflammation is evident, tuberculosis or fungal infection should be considered in the differential diagnosis (Martinez, 1980; Taratuto *et al*, 1991; Gonzalez-Alfonso *et al*, 1991).

The pathogenesis of *Acanthamoeba* and leptomixid ameba disease is not well understood; certainly the immune response, including cellular and humoral factors, play an important role (Martinez, 1985). Inflammatory reaction varies from acute to chronic; only scattered multinucleated giant cells were seen in these cases without definite

granuloma. These patients could have had undiagnosed immunodeficiency, from steroid treatment, or due to an impairment of cell mediated immune response, or an abnormal humoral immune response that had not been demonstrated by specific laboratory tests.

Angiitis was a constant finding in cases in which the patients did not receive specific treatment for free-living amebic infection (Martinez, 1980). In these cases, angiitis was seen in blood vessels without surrounding amebic trophozoites. Necrosis of the blood vessel wall, with polymorphonuclear cells and nuclear debris, were similar to those seen in allergic angiitis elsewhere (Ferrante, 1991). Aneurysmal dilatation, ruptured and obstructed blood vessels, may aggravate tissue necrosis and hemorrhage, producing hemorrhagic infarcts. In case 2, the portal of entry into the CNS most likely was the chronic ulceration in the patient's ankle; it contained multiple amebic trophozoites.

GAE almost always results in death, because of difficulty in early diagnosis, poor penetration of drugs into the CNS tissue, and the ability of *Acanthamoeba* and leptomyxid ameba to form cysts. Unlike PAM, which can be successfully treated with Amphotericin B (Seidel *et al*, 1982) no successful treatment for GAE exists. Ketoconazole, however appears to be effective both *in vitro* and *in vivo* (Martinez and Janitschke, 1985).

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