CEREBROSPINAL FLUID ANALYSIS IN EOSINOPHILIC MENINGOENCEPHALITIS

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Abstract. Eosinophilic meningoencephalitis (EME) remains an important neurological disease and is widely distributed in Thailand. We analyzed the cytological specimens of 56 EME cases. Pertinent clinical data were analyzed retrospectively and correlated with the cerebrospinal fluid (CSF)analysis. Headache was the commonest symptom seen in all EME cases. History of raw or partially cooked Pila snail ingestion was elicited from most patients. There was a marked seasonal occurrence between July to January. Patients received specific treatment as supportive therapy, which included spinal taps, analgesics and corticosteroids, was adequate. No fatal cases were seen. The CSF specimens were sorted into two categories: fresh CSF and hematoxylin and eosin (H&E) stained centrifuged CSF sediment. There was a statistically significant difference between the number of eosinophils and lymphocytes of fresh CSF and the H&E stained centrifuged CSF sediment ($p = 0.001$ and 0.001 respectively). The CSF glucose and the number of eosinophils in both methods were significantly correlated ($p = 0.000$, $p = 0.008$ for fresh CSF and the H&E stained centrifuged CSF sediment respectively). Moreover, the number of eosinophils was statistically significant with the protein in the CSF ($p = 0.013$), and intracranial pressure (ICP) ($p = 0.025$). Higher yields of eosinophils, especially in the early course of the disease, can readily be detected in the H&E stained centrifuged CSF sediment, whereas fresh specimens were negative. Further tests may increase the sensitivity and specificity of EME diagnostic results.

INTRODUCTION

Eosinophilic meningitis drew attention when Rosen *et al* (1961) reported an outbreak of the disease in Tahiti in 1961. Eosinophilic meningoencephalitis (EME), eosinophilic meningitis and epidemic eosinophilic meningitis were the first terms used to describe the disease with eosinophilic pleocytosis related to abnormal neurological symptoms before its etiology was known (Jindrak, 1975). The causes of EME fall into 4 major groups: parasitic infections, insect larvae, fungal infections and miscellaneous. Among the parasitic infections are nematodes (*Angiostrongylus cantonensis, Gnathostoma spinigerum, Toxocara canis* and *Baylisascaris procyonis*), cestodes (cysticerci of *Taenia solium*) and trematodes (*Paragonimus westermani, Fasciola hepatica* and *Schistosoma* spp). Insect larvae (*eg Hypoderma bovis*) and fungi, *Coccidioides immitis* and *Cryptococcus neoformans,* have also been mentioned as causes of EME. Miscellaneous causes include lymphoma, hypereosinophilic syndrome, post-rabies vaccination encephalitis, periarteritis nodosa, reaction to ibuprofen, lymphocytic choriomeningitis, Coxsackie B4 viral meningitis, Rocky Mountain spotted fever and foreign substances in the central nervous system (Suntharasamai, 1990).

In Thailand, the parasites that commonly cause neurological diseases in man and that are associated with eosinophilic pleocytosis are *A. cantonensis*, *G. spinigerum* and *T. solium*

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(Vejjajiva, 1978).

An. cantonensis is the most common cause of eosinophilic meningitis in both Thailand (Prommindaroj *et al*, 1962; Benjapongse, 1963) and in the wider world (Legrand and Augibaud, 1998). Since 1962, numerous clinical cases of EME have been reported from Bangkok and northeastern part of Thailand (Prommindaroj *et al*, 1962; Benjapongse, 1963; Punyagupta, 1965). Humans are infected by the ingestion of an infected intermediate host. The relationship of EME to the ingestion of fresh-water snails, especially Pila snails which serve as intermediate hosts of *An. cantonensis*, has been established (Hongladarom and Indarakoses, 1966; Punyagupta *et al*, 1970). The intermediate hosts of *An. cantonensis* in Thailand are *Pila polita, P. ampullacea, P. turbinis, P. gracilis, P. angelica, Sinotiana martensiona, Achatina fulica* and parentenic hosts: land crabs, frogs and fresh-water shrimp (Suntharasamai, 1990). The possible role of water and lettuce in the transmission of *An. cantonensis* has been raised (Richards and Merrtitt, 1987). The disease may present as a transient meningitis or a more severe disease involving the brain, spinal cord and nerve roots, with a characteristic eosinophilia of the peripheral blood and cerebrospinal fluid (CSF) (Kliks and Palumbo, 1992).

G. spinigerum is also known to be a cause of EME. The disease caused by this parasite can be distinguished from that caused by *A. cantonensis* by characteristic acute nerve root pain, signs of spinal cord and cerebral involvement, and the presence of bloody or xanthochromic CSF (Vejjajiva, 1978). Because of its high motility, the parasite causes widespread damage in the spinal cord and brain stem. Man becomes an accidental host by eating infected under cooked fresh-water fish.

The neurocysticercosis of *T. solium* is also associated with EME. Convulsions are a common symptom, occurring more often than headache. The disease can result in epilepsy, and sometimes raised intracranial pressure (ICP) from intraventricular obstruction or from basal arachnoiditis (Vejjajiva, 1978).

In endemic areas, typical clinical signs associated with CSF eosinophilic pleocytosis help to establish the diagnosis. ELISA testing may be useful (Legrand and Angibaud, 1998). Diagnosis is best confirmed by the detection of *A. cantonensis* larvae surrounded by a cluster of eosinophilic cells in the CSF (Dorta-Contreras *et al*, 1987). The detection of *A. cantonensis* larvae in the CSF was reported for 10% of patients in Taiwan (Chen, 1979) and for 0-4% of Thai patients (Punyagupta *et al*, 1975; Jaroonvesama *et al*, 1985). The diagnosis is based upon a history of raw food ingestion, clinical symptoms and the epidemiology of the disease (Suntharasamai, 1990). The disease remains important in tropical areas, considering eating habits and the presence of the intermediate hosts. We attempted to study retrospectively all collectable cases of EME admitted to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand. The main objective was to analyze the CSF findings of EME cases. Fresh CSF and the hematoxylin and eosin (H&E) stained centrifuged CSF sediment were evaluated. The findings were correlated with pertinent clinical data.

MATERIALS AND METHODS

We made a retrospective descriptive study by reviewing the records of the Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Thailand, for the period 1968 to February, 2001. All cytologically diagnosed EME specimens were collected. The H&E stained centrifuged CSF sediments were examined. Cerebrospinal fluid was centrifuged in a conical 15 ml-tube at 1,500 rpm for 10 minutes, using a horizontal-head centrifuge. The sediment was then put into a clean slide, dried at room temperature, fixed with 95% ethanol for 10 minutes, and stained with H&E stain. All slides were kept as permanent records. The H&E stained centrifuged CSF sediment was examined using low power (10x) and high power (40x) light microscopy. Differential counts were based on 500 cells unless very few cells were present. An eosinophilic pleocytosis was defined as 20 or more leukocytes per mm^3 of CSF of which at least 10% were eosinophils (Punyagupta *et al*, 1975). The H&E stained centrifuged CSF sediments were reviewed in detail and the percentages of white blood cells were recorded. Data were gathered from pathological reports and clinical charts. Pertinent clinical features included age, sex, occupation, home province, admission date, discharge date, length of admission, chief complaint, history of ingestion raw or partially-cooked foods, the time between the ingestion of raw or partially cooked foods and the onset of symptoms. Pertinent physical examination, laboratory data, treatment and outcome were recorded. Western blot analyses for *A. cantonensis* and *G. spinigerum* were collected. Results of fresh CSF profiles were collected from the clinical charts. These were compared with the H&E stained centrifuged CSF sediments for matched data.

Data analysis

Data gathered were described by using to descriptive statistics. For comparison of the leukocyte counts from both fresh CSF and the H&E stained centrifuged CSF sediment, data were recorded and analyzed using Wilcoxon Matched-Pairs Signed-Ranks Test. The α level was set at 0.05. Spearman's rho correlation test was applied for possible correlations. All statistical analyses were performed by Statistical Package for the Social Sciences (SPSS) version 7.5 for Windows 98.

RESULTS

Since 1968, fifty-six cases (25.45%) were cytologically diagnosed as EME of the 220 CSF specimens submitted to the Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Thailand. Of these 56 cases, 49 cases were clinically diagnosed as EME (87.5%). Forty-two cases (75%) were clinically suspicious for EME and confirmed by cytology. Fourteen cases (25%) were not clinically suspicious of EME, however they showed eosinophilic pleocytosis. They were suspected of tuberculosis (3), cryptococcosis (2), typhoid (1) and carcinomatous meningitis (2). Other diagnoses included convulsions of unknown cause (1), headache of unknown cause (1), fever (2) and undiagnosed (2). Of the 49 cases of clinically suspected EME, eosinophilic pleocytosis was detected in 42 (85.71%). The other seven cases were diagnosed cytologically as chronic lymphocytic meningitis (4), cryptococcosis (2) and normal (1). The data gathered showed the prevalence of EME to be 25.45%. Taking cytological diagnosis to be the gold standard, the sensitivity and specificity of clinical diagnosis were 75% and 95.73% respectively. The predictive value for true positive and negative diagnosis of EME was 85.71% and 91.81% respectively. The efficiency for clinical diagnosis of EME was 90.45%.

Age, sex and habitat

There were 39 male and 17 female patients. The age range was 8 months to 68 years. The average age of the patients was 31 years. EME was most often seen 20-39 years age range (71.43%).

Regarding patients' habitat, EME is prevalent and widely distributed in the northeastern and central parts of Thailand and in Bangkok. Thirty-two of the 51 patients (62.75%) were from the of northeastern province, whereas 15.69% and 11.76% were from central Thailand and Bangkok respectively. The patients were mostly employees (48%) or house keepers (12%). The rest were farmers, workers, merchants or government servants (2/25 patients or 8% in each occupational group). One patient was unemployed (4%).

Food intake

Most of the patients had a history of ingestion of raw or partially cooked Pila snails (91.66%). Pila snails and other raw or partially cooked food were recorded in 9 of 36 cases (25%). Other food sources included fresh-water shrimps, fresh-water fish, land crabs, pork and other meat. One patient gave a history of ingestion of golden apple snails (*Pomacea canaliculata*) while another patient had ingested only raw or partially cooked pork. Duration of raw or partially cooked food ingestion was, on average, 21 days (range 1 to 90 days).

Seasonal incidence

Thirty-five cases gave an estimate of the date of raw or partially cooked food ingestion and the date of onset of symptoms. Raw or partially-cooked food ingestion was common during the period July to January (91.43%). There was no significant correlation between the incubation period and the percentage of eosinophils in fresh CSF and the H&E stained centrifuged CSF sediment ($p = 0.220$ and 0.192 respectively).

Signs and symptoms

Headache was the commonest symptom (100%). Most of the patients (54 of 56 cases) were admitted with the chief complaint of persistent headache (96.43%). Two patients had intermittent headache (3.57%). Headache was located at the occipital region (32.14%), temporo-parietal region (25%), generalized (10.72%) and combined occipital and temporo-parietal region (32.14%). None reported frontal region headache. Eyeball pain, as an associated symptom of headache was seen in 2 of 28 patients. Degree of headache was either moderate (38.89%) or severe (61.11%); none presented with mild symptoms. Headaches were characterized as dull, sharp or throbbing in 8.33%, 8.33% and 16.67% of patients respectively. The duration of headache varied from 1-90 days, with an average of 12 days.

There was no significant correlation between the duration of headache and the percentage of eosinophils in fresh CSF and the H&E stained centrifuged CSF sediment ($p = 0.886$ and 0.871 respectively). Duration of headache and ICP-open pressure (OP) was not statistically correlated ($p = 0.647$).

Several associated symptoms were noted. Most of the patients had vomiting (96.30%); nausea was found in 19 of 30 patients (63.33%). Nausea and vomiting were not related to increased ICP ($p = 0.959$ and 0.701 respectively) and disappeared after a few days. Other associated symptoms included fever (37.93%), weakness (37.93%), weakness of extremities (6.90%), myalgia (28%), blurred vision (14.81%), convulsions (7.41%), diarrhea (7.41%), constipations (4.17%), anorexia (8.33%) and dizziness (20.83%). Noteworthy edema presented in 4 patients: edema was generalized (1), in both legs (1), facial (1) or migratory (1). Only one case presented with facial edema that was serologically positive for *G. spinigerum* in blood.

There was a significant correlation between the presence of headache and the occurrence of fever ($p = 0.000$). The severity of headache did not appear to be correlated with the number of leukocytes in the CSF ($p = 0.552$) or with the total protein in the CSF ($p = 0.454$) or with the ICP (OP) ($p = 0.384$). The number of eosinophils in fresh CSF and in H&E stained centrifuged CSF sediment were not significantly correlated with the degree of headache ($p=0.782$ and 0.393 respectively).

Stiffness of the neck was found in 65.79% of cases; paresthesia was noted in two patients - at the extremities in one case and affecting the entire body in the other case. Two patients were in coma. One patient presented with drowsiness. All recovered after a few days. Other pertinent findings included fever (21.88%), facial palsy (8.33%), abnormal behavior (8.33%), papilledema (3.57%), blurred discs (3.57%) and backstiffness (2.63%).

The past medical histories of patients were generally unremarkable. There was no previous history of angiostrongyliasis, gnathostomiasis and cysticercosis.

Treatment

Supportive therapy was the main treatment. This included spinal taps, analgesics, and in some cases, corticosteroids (dexamethazone and prednisolone). All of the patients were treated with analgesics. Most of the patients were treated with a combination of analgesics and symptomatic drugs (66.67%). Some of the patients were treated with analgesics only (12.50%). Steroids were given to 3 of 24 patients with severe symptoms secondary to increased ICP (12.50%). They generally were relieved of their symptoms. Three of 24 patients were treated with antihelminthic drugs (12.50%) for intestinal parasites. Almost all patients experienced improvement soon after the lumbar puncture. Repeated lumbar punctures were performed to reduce the ICP in cases with severe headache. One patient required 7 lumbar taps, (an 8-month-old female infant). Generally, patients needed only one lumbar puncture.

Outcome

The EME cases had a generally good outcome. The average admission length was 10 days. All except one patient (23 of 24 cases, 95.83%) had either good symptom control or had fully recovered on discharge. The condition of one patient remained unchanged: he was referred to another hospital and lost to follow up.

Length of admission

All patients needed hospitalization: 70.83% left the hospital within the first 10 days; 20.83% left on the 12th to 17th day; 8.33% stayed in the hospital for some 3 weeks.

Laboratory findings

The average values of the complete blood count and biochemical findings are presented in Table 1. The total white blood cell count was slightly increased. Blood eosinophilia was marked. The liver transaminases and alkaline phosphatase were slightly increased. Other findings were unremarkable. Table 2 shows CSF findings and related biochemical data. Leukocytosis was detected in the CSF (average 963 cells). The CSF leukocyte counts of over 1,000/mm^3 were found in 50% of patients. Eosinophils and lymphocytes were increased. Open pressure was increased (23.83 cm H_2O). Total protein was slightly increased while glucose levels were normal.

There was no significant correlation between blood glucose and CSF glucose (p = 0.094). Blood total protein and CSF total protein was not statistically correlated (p = 0.911).

There were no significant differences between blood glucose and the number of eosinophils in fresh CSF and the H&E stained centrifuged CSF sediment (p = 0.303 and 0.442 respectively). However, there were significant correlations between CSF glucose and the number of eosinophils of fresh CSF and the H&E stained centrifuged CSF sediment (p = 0.000 and 0.008 respectively). No statistical difference between blood total protein and the number of eosinophils of fresh CSF and the H&E stained centrifuged CSF sediment was seen (p = 0.638 and 0.858 respectively). The protein in

Table 1
Average values of blood and biochemical findings.

Blood and biochemical findings	Ratio of collectable cases	Average values	Range	Normal range
Leukocyte count	30/56	9.86x10^9 cells/l	2-18.8x10^9 cells/l	5-10x10^9 cells/l
Neutrophils	29/56	49%	20-87%	60-70%
Lymphocytes	30/56	27%	11-74%	25-33%
Eosinophils	31/56	19%	0-42%	1-4%
Monocytes	29/56	5%	1-9%	2-6%
Platelet count	18/56	285.77x10^9 cells/l	104-404x10^9 cells/l	200-400x10^9 cells/l
Eosinophil count	5/56	4.22x10^9 cells/l	1.37-8.44x10^9 cells/l	0.1-0.3x10^9 cells/l
Alkaline phosphatase	23/56	58.43 U/l	3.2-173 U/l	25-70 U/l
Aspartate aminotransferase	25/56	45.12 U/l	15-202 U/l	5-28 U/l
Alanine aminotransferase	25/56	60.96 U/l	15-299 U/l	25-75 U/l
Blood glucose	25/56	105.36 g/l	69-210 g/l	70-120 g/l
Blood total protein	24/56	72.10 g/l	61-81 g/l	60-80 g/l

Table 2
CSF findings of collectable EME cases (24).

CSF findings	Average values	Range	Normal range
CSF volume	9.91 cc	3-20 cc	-
CSF color	Colorless	Colorless to 'coconut juice'	Colorless
CSF turbidity	Clear	Clear to turbid	Clear
Lumbar puncture, open pressure	22.54 cmH_2O	8-51 cmH_2O	10-20 cmH_2O
Lumbar puncture, closed pressure	11.71 cmH_2O	5-20 cmH_2O	<20 cmH_2O
Pandy's test	+1	Negative to 2+	Negative
CSF glucose	46.75 g/l	27-79 g/l	40-70 g/l
CSF total protein	58.09 g/l	15-116 g/l	20-40 g/l

Table 3
The comparative data of fresh CSF and the H&E stained centrifuged CSF sediment of EME cases.

Leukocytes	Fresh CSF		H&E stained centrifuged CSF sediment		Normal range (%)
	Average values (%)	Range (%)	Average values (%)	Range (%)	
Neutrophils	1	0-14	2	0-67	0-1
Lymphocytes	55	0-100	38	1-88	1-7
Eosinophils	36	0-81	58	10-98	0
Monocytes	0	0-0	2	0-25	0

CSF was significantly correlated with the number of eosinophils of fresh CSF ($p = 0.013$) but did not show significant correlation with the number of eosinophils of the H&E stained centrifuged CSF sediment ($p = 0.072$).

The serological profile in the 10 certain cases confirmed EME due to *A. cantonensis* in both the blood and the CSF specimen in 3 cases. Six cases were positive for *G. spinigerum* in blood: of these, only two were serologically positive in the CSF specimens. Blood and CSF cultures were negative in the pertinent 24 cases.

Cerebrospinal fluid

Open pressure of the first spinal tap was higher than 20 cmH_2O in 17 of 29 cases (58.62%); this included a pressure of more than 60 cmH_2O in one case.

The gross appearance of the CSF samples was not uniformly characteristic: slightly turbid (24%), mild cloudy (20%) and turbid (12%). The rest were clear. Colorlessness was a feature of 83.33% of the samples; a notable coconut juice appearance described as 'something floating' was a feature of 16.67% of the samples.

Red blood cells were seen on microscopic examination in one case and were believed to have been due to traumatic tap. Each specimen of CSF was carefully examined: no parasites were seen. Occasionally, fibrin threads were seen which looked like larvae. Eosinophils usually presented with a bi-lobed nucleus and distinct large granules; some were seen with clumped lobes and large granules. The size of cells was slightly reduced. Degenerated eosinophils were noticed with large, swollen, vacuolated cytoplasm and very loosely packed chromatin. Occasional degranulated eosinophils cells without a cell wall were seen. Neutrophils, lymphocytes and monocytes were easily recognizable.

Table 3 shows the comparative values of leukocytes in fresh CSF and the H&E stained centrifuged CSF sediment. Comparison between the results of fresh CSF and those of the H&E stained centrifuged CSF sediment showed a statistically significant difference in the number of eosinophils ($p = 0.001$) and lymphocytes ($p = 0.001$), but showed no statistically significant difference in the percentage of neutrophils ($p = 0.248$).

There was a statistically significant correlation between the number of eosinophils in fresh CSF and ICP (OP) ($p = 0.025$). However, the correlation between the number of eosinophils in the H&E stained centrifuged CSF sediment and ICP (OP) was not statistically correlated ($p = 0.137$).

DISCUSSION

It is generally accepted that *A. cantonensis* is the cause of a typical form of EME. Characteristically, patients present with a moderate to severe headache and very few focal neurological signs. Eosinophils in the CSF could be detected with or without a history of ingestion Pila snails.

More male patients than female patients were admitted (2.29 : 1). The youngest patient was 8 months old and the oldest was 68 years old. Previously, there had been four reported EME cases due to *A. cantonensis* in infants, one of which was 10 months old and three of which were 11 months old (Graber *et al*, 1997; Cooke-Yarborough *et al*, 1999). Two were reported fatal (Graber *et al*, 1997; Cooke-Yarborough *et al*, 1999). Our case is the youngest EME ever reported. This infection in infants and children remains unavoidable.

The incidence of EME infection was highest in the northeasthern part of Thailand. This may be the result of local eating habits and the increasing popularity of raw fish and snails in rural areas. The history of consumption of raw or improperly cooked food was critical to the diagnosis. A variety of sources were noted, including: Pila snails, golden apple snails, fresh-water shrimp, fresh-water fish, land crab, pork and other meat. Our study revealed a high percentage of Pila ingestion alone, higher than previously recorded (Punyagupta *et al*, 1970). Our study featured one case of EME in a patient with a history of raw pork ingestion and diagnosed as having cerebral cysticercosis. *Pomacea canaliculata* or golden apple snails are of medical importance as the intermediate host for *A. cantonensis* which causes EME in humans in Taiwan and China (Halwart, 1994). However, the local Asians, not liking the taste and consistency of Pomacea meat, prefer Pila instead: this may be related to the low incidence of *Pomacea canaliculata* in this study.

The average time of duration from the ingestion of raw food until the onset of symptoms was 21 days: a little more than previously reported (Yii, 1976; Hongladarom and Indarakoses, 1996). Some of the patients had long incubation periods (more than 7 weeks, 14.29%) which is not often seen. These patients may have suffered headaches of other causes at first; their headache was then aggravated by the disease. On the other hand, the symptoms may have persisted for a period of time but patients were hesitant to seek consultation.

The result indicates clear evidence of seasonal incidence. Raw or partially-cooked food ingestion was common in the months of July to January (91.43%). This is the rainy season and the beginning of the cold season. There is abundant food, which may encourage the snails to leave their usual places and rise to the surface of natural ponds where they were caught.

Headache was mostly occipital and tempero-parietal. It is important to note that no frontal headache was seen in our study: a finding different to that of Yii (1976). The most common associated symptoms were vomiting and nausea, which before 24-48 hours after ingestion of Pila snails. This may be due to an allergic response. The symptom disappeared after 48 hours. This result was slightly different from those of Hongladarom and Indarakoses (1966), who reported an immediate reaction after ingestion of Pila snails. Paresthesia, weakness of extremities and convulsion were found in a small number of cases, similar to the previous study (Punyagupta *et al* 1975).

In our study, all of the patients were treated symptomatically. Lumbar puncture was useful in relieving headache in patients with high CSF pressure. Some patients needed frequent LP. Treatment with corticosteroids has been reported. Corticosteroids have been helpful in severe cases to relieve ICP as well as neurologic symptoms due to inflammatory responses to migrating and eventually, dead worms (Pien and Pien, 1999). The results indicated that a 2-week course of prednisolone was beneficial in relieving headache in the patients with EME (Chotmongkol *et al*, 2000). Antihelminthic drugs have not been recommended for eosinophilic meningitis because of the risk of a reaction to the dying parasites; however, albendazole and levamisole have been used in Taiwan with good results (Hwang and Chen, 1991).

Most of the patients recovered after a few weeks. No fatal case was seen in our study. One comatose patient recovered after supportive treatment. The outcome of eosinophilic meningitis is usually benign althrough death has been reported.

The characteristic of CSF was colorlessness or a 'coconut juice' appearance, similar to the previous study (Punyagupta *et al,* 1975). The appearance of "something floating" in the 'coconut juice' appearance is due to light reflection from the eosinophil granules.

The leukocytes in the H&E stained centrifuged CSF sediment were readily recognized - the eosinophils especially. It is thought that eosinophilic pleocytosis is triggered by the metabolic products of dead worms. The large red granules and bi-lobed nuclei of eosinophils were easily seen. Other inflammatory cells were readily identified. Degenerated cells were rarely seen, due to properly fixed specimens and proper staining procedures. However, for cytological specimens, the fluid should be as fresh as possible to avoid degeneration and artifacts.

Serological tests were possible for only 10 cases: matched positive results for *A. cantonensis*. *G. spinigerum* was detected in the blood and CSF in a ratio of 3:1. Further studies are needed to evaluate the serological profiles before a conclusion can be made.

The clinical symptoms and epidemiology of infection were useful tools for the diagnosis of EME. Of particular importance was the history of Pila ingestion and the eosinophilic pleocytosis. The H&E stained centrifuged CSF sediment was important when confirming the fresh CSF results. We found a highly statistically significant difference between the eosinophils and lymphocytes of fresh CSF and those of the H&E stained centrifuged CSF sediment: We concluded that the H&E stained centrifuged CSF sediment method is more reliable, giving a high yield of cells to be examined by microscopy. Fresh specimen may have a dilutional effect, preventing a proper identification of cells and estimation of the number of eosinophils. Sensitivity and specificity for both methods need to be evaluated. In our study, there was a small number of matched serological profiles, the goal standard. It may be necessary to do further tests to increase the accuracy and the precision of diagnosis.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the staff of The Department of Tropical Pathology , Faculty of Tropical Medicine, Mahidol University, for their kind cooperation in data collection. We are grateful to Professor Wanpen Chaicumpa, for kindly providing the serological results. We also wish to thank Ms Wimon Yomana, Mrs Patcharee Boonachot and Mrs Namvaew Punpongpaew for secretarial assistance. The help of Ms Sompan Srinukham and Mrs Anchan Binmadee in searching for the clinical charts of EME patients and serological profiles is gratefully appreciated. Printing of this manuscript is supported by the Faculty of Tropical Medicine, Mahidol University, Thailand.

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