ACETYLCHOLINESTERASE ACTIVITIES IN CEREBROSPINAL FLUID OF PATIENTS WITH *PLASMODIUM FALCIPARUM* CEREBRAL MALARIA

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INTRODUCTION

Measurement of serum cholinesterase enzymes had provided a diagnostic clue in hepatitis, myocardial infarction, muscular dystrophy and other somatic diseases (Fremon-Smith *et al.*, 1952; Vorhaus and Klark, 1953; Lehmann *et al.*, 1961). As acetylcholinesterase (ACHE) activity in the cerebrospinal fluid (CSF) indirectly reflects the enzyme state within the central nervous system, determination of these values in cerebral malaria may offer data bearing on the relationship between cerebral symptoms and ACHE activity.

Disturbance of some enzyme activity such as serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH) and cholinesterase occur in malaria (Schnell *et al.*, 1969; Areekul *et al.*, 1980). In acute falciparum malaria, cerebral symptoms of unconsciousness and convulsions occur and the mechanism causing these symptoms are not known. Some alterations of enzymes in the central nervous system may play a role in the pathogenesis. The present study was carried out to investigate the ACHE activity in the CSF of patients with cerebral malaria caused by *P. falciparum*.

MATERIALS AND METHODS

The study was carried out on 30 patients of both sexes who were admitted for *P. falciparum* malaria with cerebral complications to the Provincial Hospital in Chantaburi. Their ages ranged from 14 to 50 years. Venous blood samples were taken from the antecubital vein and simultaneously lumbar punctures were done to obtain the cerebrospinal fluid for determination of ACHE and cholinesterase activities. Determinations of hemoglobin (Hb), hematocrit (Ht), malarial parasite count on thick and thin blood films were also carried out on blood samples. The blood and CSF examinations were repeated in 9 patients after treatment in the hospital for one week.

Serum cholinesterase (CHE) and CSF ACHE were determined by the micro methods of Garry and Routh (1965) and Ellman *et al.*, (1961), repectively, using acetylcholine as a substrate. The cholinesterase and ACHE activity was expressed as micromoles of sulfhydryl groups liberated in one minute from 1 ml of serum or CSF. Hb concentration was determined using the cyanmethemoglobin method and Ht value was obtained after centrifugation the blood for 5 minutes at 10,000 g.

RESULTS

The mean serum CHE and CSF ACHE levels in 30 patients with cerebral malaria were found to be 2.045 ± 0.849 μM/ml/min (range 0.88-4.56) and 0.0195 ± 0.0075 μM/ml/min (range 0.0096 - 0.0352), respectively.

The values of serum CHE and CSF ACHE levels in 9 patients before and after treatment

Table 1

Serum CHE and CSF ACHE (μM/ml/min) values in 9 patients with cerebral malaria before and after treatment.

No.	Before			After		
	Serum	CSF	Ratio	Serum	CSF	Ratio
1	2.40	0.016	0.67	3.09	0.016	0.52
2	1.42	0.010	0.70	2.16	0.012	0.56
3	2.33	0.010	0.43	2.76	0.040	1.45
4	2.40	0.020	0.83	2.72	0.033	1.21
5	2.03	0.010	0.49	3.10	0.024	0.77
6	1.58	0.017	1.08	2.65	0.030	1.13
7	2.76	0.021	0.76	3.39	0.040	1.18
8	1.21	0.009	0.74	1.81	0.022	1.22
9	1.33	0.013	0.98	1.44	0.016	1.11
Mean	1.94	0.014	0.74	2.57	0.026	1.02
S.D.	0.57	0.005	0.21	0.64	0.010	0.30

Paired t-test: Before vs after treatment, serum CHE and CSF ACHE, $p < 0.01$, ratio (expressed as percentage of CSF/serum), $p > 0.05$.

are shown in Table 1. The cholinesterase activity in both serum and CSF increased and were significantly higher ($p < 0.01$) in the convalescent group.

There was no relationship between serum CHE and CSF ACHE levels in patients with cerebral malaria ($r=0.285$, $p>0.05$). However, these relationships were observed in subjects during convalescence ($r=0.866$, $p<0.01$) and the mixed these 2 groups ($r=0.323$, $p<0.05$) as illustrated in Fig 1.

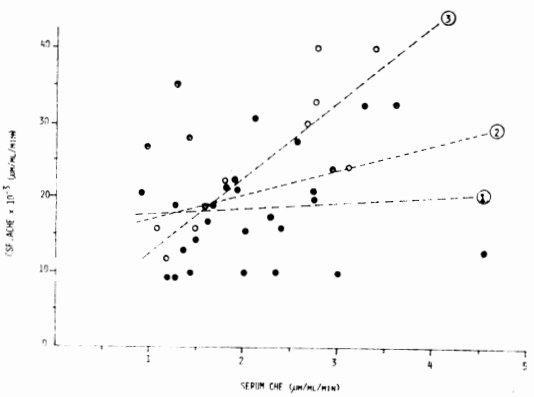

Fig. 1—Relationship between serum CHE and CSF ACHE levels of 30 patients with cerebral malaria (1), 9 convalescence (3) and 39 of both groups (2).

DISCUSSION

The mean value (2.05 μM/ml/min) of serum CHE activity in 30 patients with cerebral malaria was similar to those (2.69 μM/ml/min) reported in 32 patients with *P. falciparum* without cerebral symptoms and was significantly lower ($P<0.01$) than 3.16 μM/ml/min obtained from 80 normal subjects reported by Areekul *et al.*, (1980). Nineteen patients (63.3%) had serum CHE less than 2.16 μM/ml/min which was the lower limit of non-infected subjects. One week post-treatment, serum CHE levels in 9 convalescent patients rose to 2.57 μM/ml/min which was still lower than those of non-infected persons. Low serum CHE in patients with *P. falciparum*

had been reported to return to the normal level relatively slowly during post-treatment in 4-6 weeks (Areekul *et al.*, 1980).

As lumbar punctures were not performed on normal subjects, the normal CSF ACHE levels were taken from the published data. Duvoisin and Duttbarn (1967) found the mean CSF ACHE value of 0.043 ± 0.003 μM/ml/min in normal subjects while Soininen *et al.*, (1981) reported 0.0375 ± 0.0033 μM/ml/min. Thus, the mean CSF ACHE level of 0.0195 μM/ml/min in patients with cerebral malaria was therefore significantly lower ($p<0.01$) than those of normal subjects. However, a much lower value of 0.0113 ± 0.009 μM/ml/min was reported in normal human subjects by Singer *et al.*, (1984). These authors determined CSF ACHE by using radioenzymatic method which probably explained the discrepancy in the results obtained by other workers using the colorimetric methods.

Post-treatment follow up on day 7 showed that CSF ACHE activities in 9 convalescent subjects had significantly increased ($p < 0.01$) to 0.0259 μM/ml/min. These findings indicated that both serum CHE and CSF ACHE levels were depressed in cerebral malaria and increased on recovery. The linear relationship between serum CHE and CSH ACHE levels in convalescent and the combined groups of patients indicated that the elevation of serum CHE was in parallel to that of increased CSF ACHE activities.

Previous studies on the CSF contents in patients with cerebral malaria revealed variable results and different characteristics. Recent studies showed decrease in CSF folic acid but normal vitamin B_{12} levels in patients with *P. falciparum* cerebral malaria (Areekul *et al.*, 1985 a, b).

It has been proposed that alterations in CSF ACHE level could reflect changes in the activity of cholinergic neurons. In an attempt to correlate between pathologic changes with alterations of ACHE in the CSF, ACHE was found to be low in patients with senile dementia of the Alzheimer's type, multiple sclerosis and leukemia lymphoma (Soininen *et al.*, 1981; Plum and Fog, 1960; Johnson and Domino, 1971). However, as the source and mechanism causing the alterations in CSF ACHE levels are still not well established at present, the significance of the present finding of low CSF ACHE levels in patients with cerebral malaria need further studies.

SUMMARY

Serum cholinesterase (CHE) and acetylcholinesterase (ACHE) in cerebrospinal fluid (CSF) were determined simultaneously in 30 patients with *P. falciparum* cerebral malaria. Nineteen patients (63%) had low serum CHE and mean value of this serum enzyme in 30 patients was significantly lower than that of non-infected group. CSF ACHE levels were found to be significantly lower than those of normal subjects reported earlier. Post-treatment in the hospital for one week, both serum CHE and CSF ACHE levels in 9 convalescent subjects increased significantly. These findings indicated that both serum CHE and CSF ACHE levels were depressed in patients with cerebral malaria and increased on recovery.

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