Levels of Serum Tumor Necrosis Factor Alpha in Relation to Clinical Involvement and Treatment Among Thai Adults with *Plasmodium falciparum* Malaria†

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Abstract

Concentrations of tumor necrosis factor alpha (TNF-α) in serum were measured in 17 Thai men infected with *Plasmodium falciparum* malarial infections to determine whether they were affected by severity of infections or exchange transfusions. Twelve patients were considered having complicated malarial infections, eight of whom had cerebral malaria. Five patients had uncomplicated malarial infections. The results showed that malarial infection markedly raised TNF-α level above normal values (mean ± SEM 406 ± 38 *vs* 15 ± 5, p = 0.004). In complicated malaria, cerebral involvement appeared to significantly increase concentration of TNF-α when compared to values in uncomplicated malaria (mean ± SEM 496 ± 64 *vs* 339 ± 12, p = 0.01). Degree of parasitemia, intravenous quinine (day 0 value *vs* day 7 value) and exchange transfusion did not significantly affect TNF-α levels.

Conclusion : Serum level of TNF-α is increased in *Plasmodium falciparum* malarial infections and may be a useful index to predict severity of malarial infection, cerebral malaria in particular.

Malaria is an important cause of death in Thailand. Causes of death in complicated malaria include cerebral, renal and pulmonary involvement. Previous studies have shown that in complicated malaria severity of metabolic and inflammatory changes were associated with levels of interleukin-1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in particular[1-3]. Exchange transfusion is an adjunct therapy in severe malaria; however, it does not affect the level of serum TNF-α[5].

To further ascertain the role of TNF - α in the clinical manifestation of malaria and the effect of exchange transfusion on its level, serum TNF - α was determined and compared in Plasmodium falciparum infected patients one week apart and before and after exchange transfusions.

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MATERIAL AND METHOD

This study involved 17 men, aged 21 to 53 years. They were admitted to Ramathibodi Hospital or to Pramongkutklao Hospital between 1992 and 1994. All patients had *P. falciparum* infection and were treated with intraveneous quinine. The patients were classified as uncomplicated (mild) or complicated malaria in accordance with the absence or presence of cerebral, renal and/or pulmonary involvements. Cerebral malarial infection was diagnosed when there was a convulsion and/or change in conciousness such as delirium, semicoma and coma.

Laboratory evaluation

The sera were obtained, stored at - 70°C for up to 6 months and determined for TNF-α with a commercial ELISA kit (Predicta^{TM} Gemzyme diagnostic, Minnapolis, U.S.A). The detection limit of the assay was 10 pg/ml. Control serum values of TNF - α were obtained from 10 normal adults.

Paired *t*-test was used to compare mean values of TNF - α at days 0 and at 7 of admission and those before and after exchange transfusions. Comparisons of the other mean values, namely, patient *vs* control, complicated malarial *vs* uncomplicated malarial, cerebral malarial *vs* uncomplicated malarial infections, and parasitemia >10 per cent *vs* parasitemia <10 per cent were carried out employing the nonparametric Mann Whitney U test.

RESULTS

Of the 17 patients, 12 had complicated malarial infections and 5 had uncomplicated malarial infections. Two thirds of the complicated malarial infections had cerebral involvement. Table 1 presents the clinical manifestation of the 17 patients with Plasmodium falciparum malarial infection. Six patients underwent exchange transfusions. All 17 patients were successfully treated. Table 2 summarizes the mean ± SEM of the concentrations of serum TNF-α. TNF-α values were drastically increased above control values during Plasmodium falciparum infection (406 ± 38 *vs* 15 ± 5, p = 0.004). Complicated malarial infections had higher levels of TNF-α than uncomplicated malarial infections although it is not statistically significant. However,

Table 1. Clinical presentation of 17 patients infected with *Plasmodium falciparum* malaria.

Clinical feature	n	Systemic complications (Number of patients)			
		Brain	Renal	Lung	DIC*
1. Uncomplicated malaria	5	0	0	0	0
2. Complicated malaria without cerebral involvement	4	0	4	2	0
3. Cerebral malaria	8	8	6	4	1

DIC* = Disseminated intravascular coagulopathy

Table 2. Serum TNF - α levels in patients with malaria.

Variable	N	TNF - α level (pg/ml) mean ± SEM	p value
1. Control subjects	10	15 ± 5	0.004*
Patients (day 0)	17	406 ± 38	
2. Non-complicated malaria	5	339 ± 12	0.11
Complicated malaria	12	434 ± 51	
3. Uncomplicated malaria	5	339 + 12	0.01*
Cerebral malaria	8	496 + 64	
4. Parasitemia <10%	11	406 + 35	0.24
Parasitemia >10%	6	406 + 91	

* Statistically significant

Table 3. Serum TNF-α levels in malarial infected patients before and after treatment.

Treatment	N	TNF - α level (pg/ml) mean ± SEM	p value
1. Before quinine treatment (Day 0)	17	406 ± 38	0.17
After quinine treatment (Day 7)	17	371 ± 30	
2. Pre- exchange	6	498 ± 87	0.25
Post- exchange	6	462 ± 63	

in cerebral malarial infections, a subgroup of complicated malarial, the difference reached statistical significance (496±64 *vs* 339±12, p = 0.01). Treatment with intraveneous quinine and exchange transfusion did not affect TNF-α values (Table 3).

DISCUSSION

Tumor necrosis factor-alpha is an inflammatory cytokine released by monocytes / macrophage and T lymphocyte in response to inflammation and infection. Elevated levels of serum TNF-α have been reported in septicemia and parasitic infection including malaria(1). *Plasmodium falciparum* was found to stimulate TNF-α production by human mononuclear cells(5). Previous studies have reported an association between high serum TNF-α concentration and cerebral malarial infections and mortality in falciparum malarial infections(2,6).

In the present study, serum TNF-α concentrations were markedly elevated in patients with malaria. The mean concentration of TNF-α was significantly higher in the patients with cerebral malaria than in patients who had no systemic complications. There was no correlation between the TNF-α level and the degree of parasitemia. The findings that neither quinine treatment nor exchange transfusion significantly decreased TNF-α values suggest that the extravascular distribution space of TNF-α is large. The findings that TNF-α level was elevated in cerebral malarial infections and was not affected by plasma exchange were in accordance with the results of previous studies (1,2,4,6). However, such a relation is purely speculative and remains to be elucidated. In summary the results of the present study substantiate the findings that serum TNF-α level is increased in malarial infections and is not affected by exchange transfusion. In addition, intravenous quinine administration and degree of parasitemia do not significantly influence the serum TNF - α concentrations.

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ความสัมพันธ์ของระดับ tumor necrosis factor ในซีรั่มและอาการแสดงทางคลินิกในผู้ป่วยไทยที่ติดเชื้อ มาลาเรียชนิด *Plasmodium falciparum*

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การศึกษาระดับ tumor necrosis factor alpha (TNF-α) ในซีรั่มผู้ป่วยไทยที่ติดเชื้อมาลาเรียชนิด *Plasmodium falciparum* จำนวน 17 ราย ซึ่งมีภาวะแทรกซ้อนทางสมอง 8 ราย มีภาวะแทรกซ้อนอื่น ๆ 4 ราย ไม่มีภาวะแทรกซ้อน 5 ราย ในระหว่าง พ.ศ. 2535 ถึง พ.ศ. 2537 ด้วยวิธี ELISA พบว่า การติดเชื้อมาลาเรียทำให้ระดับ TNF-α ในซีรั่มผู้ป่วยเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ (ค่าเฉลี่ย ± ค่าเบี่ยงเบนมาตรฐาน 406 ± 38 *vs* 15 ± 5, p = 0.004) โดยเฉพาะผู้ป่วยที่มีอาการแทรกซ้อนทางสมองจะมีระดับ TNF-α สูงกว่าผู้ป่วยที่ไม่มีอาการแทรกซ้อนต่าง ๆ อย่างชัดเจน (ค่าเฉลี่ย ± ค่าเบี่ยงเบนมาตรฐาน 496 ± 64 *vs* 339 ± 12, p = 0.01) แต่จำนวนเชื้อมาลาเรียและความเข้มข้นของยา quinine ในเลือดระหว่างวันแรกและวันที่ 7 ตลอดจนการเปลี่ยนเลือด (exchange transfusion) ไม่ทำให้ระดับของ TNF-α ในซีรั่มผู้ป่วยเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติ

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