

CASE REPORT

IS THERE ANY ARTEMISININ RESISTANCE IN FALCIPARUM MALARIA ?

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Abstract. We reported two cases of complicated falciparum malaria who had poor response to artesunate with delayed parasite clearance times. They were splenectomized patients who were treated with high doses of artemisinin derivatives. Our cases showed the importance of the spleen in the clearance of malaria parasites and had different clinical outcome, one fatal and one recovery. The host factors, the parasitemia count, the quality of antimalarial chemotherapy and blood level of the antimalarial drugs must be considered in relation to the causes of the delayed clearance of parasitemia.

INTRODUCTION

Artesunate has been used extensively to treat multi-drug resistant *Plasmodium falciparum* malaria both in clinical trials involving many thousands of patients and in general use in over a million patients (Looareesuwan, 1994; Looareesuwan *et al*, 1998). It has proved to be rapidly effective and safe. Recently, there was a report of a poor response to artesunate in two patients in Thailand (Luxemberger *et al*, 1998). Both cases were children, 2 and 5 years old, who received a 7 days artesunate-mefloquine combination. Their clinical course had no complications but the parasitemia were positive on day 7 after treatment. Artemisinin and its derivatives usually clear the parasitemia within 48 hours even in Thailand (Wilairatana *et al*, 1998). Studies of *in vitro* susceptibilities of *P. falciparum* to artesunate and mefloquine in patients coming from the Thai-Myanmar and Thai-Cambodia borders where highly mefloquine resistant *P. falciparum* is prevalent compared to isolates from the southern part where *P. falciparum* is still sensitive to mefloquine, showed that the isolates from the mefloquine resistant area had a higher geometric mean IC₅₀ for artesunate than isolates from the mefloquine sensitive area. Nevertheless, all of the patients were still highly responsive to artesunate (Wongsrichanalai *et al*, 1999).

This information is important for the careful regulation of the large scale use of artemisinin derivatives. However the definitive diagnosis of resistance to artemisinin derivatives is very difficult due to their pharmacokinetic properties and there are many confounding factors. Thus we reported another two cases of complicated falciparum malaria which had poor response to artesunate in our hospital.

Case 1

A 61 year old Thai man from Kanchanaburi Province, an endemic area of malaria of Thailand was admitted to the Bangkok Hospital for Tropical Diseases, with high grade fever for 7 days, rigors, headache and deterioration of consciousness. Details of his medical history are reported in Phongponratn *et al* (2000). He expired on the 13th day of admission from multiple organ failure the last parasitemia was 1,195,335/ μ l.

Autopsy and electron microscopy were done and showed cerebral malaria, congestive heart failure dilated gall bladder with gall stone and post splenectomy. The microscopic findings were positive for the *P. falciparum*-parasitized red blood cells (PRBC) in different organs (Pongponratn *et al*, 2000)

Case 2

A 61 years old Thai man who lived in Bangkok, was admitted to our hospital with high grade fever for 3 days with jaundice and drowsiness. He had no history of malaria infection or traveling to any forest area in the previous 3 months. A month previously, he had the motorcycle accident with

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fracture his left tibia. He also had splenic and bowel injury so splenectomy, left nephrectomy, colostomy, and open reduction with internal fixation of the left tibia were done in Rajavithi Hospital.

During the 2 weeks in the hospital, he received 2 units of whole blood. Three days before, he was referred to our hospital, he looked drowsy and fatigued. The temperature was 38°C, his pulse was 128/minute and the blood pressure was 80/40 mmHg. Lungs were normal. Abdomen showed surgical scars of nephrectomy and splenectomy at left upper quadrant area with colostomy bag and mild tenderness at the epigastrium. A blood smear for malaria showed 30 ring forms of *P. falciparum* per 1,000 red blood cells with 2 shizonts and 26 gamete forms; the parasite count was 84,900/ μ l. His hemoglobin was 8.2 g/dl, the white blood cell count was 13,900/mm³ and his platelet count was 157,000/ μ l. The blood urea nitrogen and creatinine were rising (120.2 and 3.2 mg/dl, respectively). The liver function tests were normal. The septic work up for bacterial infection and HIV status was normal. He was treated with intravenous artesunate 120 mg, then 60 mg every 12 hours. His clinical condition improved, the fever subsided and the urine output was controlled by the diuretic given regularly. On the third day of admission, the blood urea nitrogen and creatinine returned to normal level but the parasitemia was still high (132,620/ μ l). The 6 hourly parasite count was done until the day 15 or 368 hours after treatment started, the parasitemia was cleared. During the 2 weeks course, there was no clinical signs of any complication. He received a total dose of 600 mg of artesunate intravenously and mefloquine 750 mg salt initially, then 500 mg salt was given on the following day orally. Because of the clinical well-being, there was no indication to add any further antimalarial drugs during the positive parasitemia. The clinical condition was observed closely until discharge from the hospital. There was no further recrudescence during the 90 days of follow up.

The spleen plays an essential role in the immune response against many infections including malaria. Our previous report indicated that the parasite clearance time was delayed only in nonimmune splenectomized patients with uncomplicated falciparum malaria infection. All of them were responded very well with a standard course of quinine and tetracycline (Loareesuwan *et al*, 1993). From previous studies, the humoral and cellular immune responses to blood-stage antigens

during the acute infection in splenectomized patients compared with ten patients whose spleens were intact showed similar responses. The absence of the spleen may affect the sequestration process of infected red blood cells, so that these parasite stages remained in the circulation for a longer time. However, the course of malaria in splenectomized patients still needs to be clarified. This issue is still controversial. Some reports showed splenectomy did not affect the clinical status or the parasite clearance because the immune system was able to maintain the immunity against malaria infection (Petersen *et al*, 1992). A few years ago, there was a report of delayed parasite clearance in a splenectomized patient with falciparum malaria who was treated with high doses of artemisinin derivatives (Le *et al*, 1997). They reported two cases with delayed parasite clearance times (17 and 11 days) and believed that the efficacy of the artemisinin drugs relied on the intact splenic function which can mimic high grade drug resistance (RIII response). They suggested alternative antimalarial drugs such as quinine might be the better choice, especially if there were complications. Nevertheless, all of the previous report cases were uncomplicated patients (Table 1).

In this report, one patient died despite artesunate being given in a higher dose (3.4 g). An inadequate therapeutic blood level of artesunate could not explain the poor clinical outcome. The quality of drug is another issue but more than 1,000 patients with falciparum malaria in our hospital responded completely to the artesunate from the same batch. In an *ex vivo* study of blood schizontocidal activities of artemisinin derivatives against *Plasmodium falciparum*, parasites still persisted despite the serum level of drug falling to the limits of quantification (2-3 ng/ml) (Ubalee *et al*, 1999). The malaria culture in this fatal case, done before death, showed no growth. Electron microscopy was also done and showed only dead parasites. All of the result of investigations support the thesis that there is no RIII resistance response in this case. The appearance of an RIII resistance response-like activity in this fatal case may be explained by the absence of a spleen (World Health Organization, 1973). In general, drug resistance has been defined as the ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication. Drug resistance was initially classified by using *in vivo* test regarding to chloroquine as the choice of treatment at that time. The RIII resistance response means parasitized red

Table 1
The pattern of delayed parasite clearance in the falciparum malaria infected patients.

Study	Number of cases	Age (years)	Clinical complicated (yes/no)	Splenectomy (yes/no)	Parasitemia at admission	Parasite clearance time (hr)	Treatment	Outcome
Maharaj <i>et al</i> (1982)	1	61	Yes (renal failure)	Yes	26% of RBC	120	Chloroquine	Recovered
Israeli <i>et al</i> (1987)	1	34	No	Yes	5% of RBC	288	Quinine + tetracycline (standard dose)	Recovered
Petersen <i>et al</i> (1992)	1	55	No	Yes	60/ μ l	No data	No data	Recovered
Looareesuwan <i>et al</i> (1993)	4	19-24	No	Yes	0.1-2.5% of RBC	124	Quinine + tetracycline (standard dose)	Recovered
Le <i>et al</i> (1997)	1	43	No	Yes	256,000/ μ l	408	Artesunate (6.57 g) then quinine	Recovered
Luxemburger <i>et al</i> (1998)	2	2, 5	No	No	42,339 and 65,337/ μ l	1,032 and 96	Artesunate + mefloquine add quinine and atovaquone, proguanil (in one case)	Recovered
Treeprasertsuk <i>et al</i> (2000)	2	61	Yes (cerebral, renal failure)	Yes	1,316,600/ μ l	>164	Artesunate (3.4 g) + mefloquine	Died
		61	Yes (renal failure)	Yes	84,900/ μ l	368	Artesunate + mefloquine (standard dose)	Recovered

blood cells (PRBC) were still high after 48 hours of the standard treatment or the PRBC at 48 hours after treatment were higher than 25% of the initial parasite count. The assessment of the sensitivity of plasmodium to blood schizontocides is limited by many factors such as the concentration of antimalarial drugs in plasma, the correlation of *in vivo* and *in vitro* tests in semi-immune patients or longstanding contact with malaria. However, many observations should be required before the conclusion of drug resistance, especially the physical condition of the patients, the regimen employed and the duration of post-treatment must be at least 28 days depend on the antimalarial drugs (World Health Organization, 1984).

In the clearance of malaria infected erythrocytes, the spleen has an essential role, especially in the malaria patient with splenomegaly (Looareesuwan *et al*, 1987). In patients with *P. falciparum* malaria, IgG, complement components and also splenic Fc receptor mediated phagocytosis are the key factors to eliminate the infected erythrocytes (Merry *et al*, 1986; Abdella, 1986; Ho *et al*, 1990). The complicated *P. falciparum* infections which have considerable malaria antigenemia may fail to increase the Fc receptor-mediated erythrocyte clearance (Ho *et al*, 1990), so that, it is more likely to develop uncontrolled and complicated course of disease especially in the splenectomized host.

Our cases showed the importance of the spleen in the clearance of malaria parasites and had different clinical outcome, one fatal, one recovery. The host factors, the parasitemia count, the quality of antimalarial chemotherapy and blood level of the antimalarial drugs must be considered in relation to the causes of the delayed clearance of parasitemia before the diagnosis of the artesunate resistance was concluded. However, the artesunate resistance by WHO criteria is still essential and should observe carefully especially in the area of multidrug resistant *falciparum* malaria.

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