PLATELET DYSFUNCTION IN MALARIA

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INTRODUCTION

Thrombocytopenia has long been known to occur during malarial infection. Studies since 1966 have demonstrated that thrombocytopenia may occur in both *P. vivax* and *P. falciparum* infection and varies according to the severity of the disease. A mild to moderate degree of thrombocytopenia usually occurs in non-complicated malaria whereas a marked fall in platelet count is observed in severe complicated falciparum infection (Hill, 1964; Shulman, 1970; Beale, 1972; Skudowitz, 1973). Two mechanisms are involved in the occurrence of thrombocytopenia, namely: immunodestruction by IgM antibodies (Shulman, 1970; Kelton *et al.*, 1983) and disseminated intravascular coagulation (Dennis *et al.*, 1967; Punyagupta *et al.*, 1974). The latter mechanism was seen only in severe complicated falciparum infection.

Recently, several studies have indicated that platelet hyperactivity occurs during acute malarial infection. The line of this evidence includes increased release of beta-thromboglobulin, and platelet factor 4 (Essien and Ebhota, 1983); enhanced thromboxane B_2 production (Essien *et al.*, 1984) and *in vitro* hypersensitivity of platelet response to ADP stimulation (Inyang *et al.*, 1987). Increased intravascular lysis of platelets was also observed during *P. falciparum* infection (Essien *et al.*, 1983). So far, studies concerning the interrelationship between platelet dysfunction and clinical significance of malarial infection has never been reported. In this communication, we consider the relationship between platelet function and other clinical manifestations including bleeding during *P. falciparum* and *P. vivax* infection.

MATERIALS AND METHODS

Patients with malaria (falciparum 38; vivax 8; mixed 2), 45 males and 3 females aged between 20–30 years admitted to the Department of Medicine, Pramongkutklao Hospital, Bangkok were studied. Of these 48 patients, 21 had systemic complications and 27 had no systemic complications. Sixteen patients were studied both during and after parasitemia, and 32 either during or after parasitemia.

Systemic complications included CNS, renal, lung, liver, bleeding and DIC. They were graded as mild/moderate to severe degree namely: CNS complications (cerebral malaria): impaired sensorium, stuporous, semiconscious to coma and/or convulsion; renal failure: increased BUN, and creatinine without or with acidosis to oliguria with severe acidosis or creatinine above 10 mg/dl;

pulmonary insufficiency: decreased PO_2 below 60 mm Hg, with symptoms varied from mild dyspnea to orthopnea; liver dysfunction: hepatomegaly and increased SGPT above 500 units/l; bleeding: at skin and mucous membrane to profused bleeding from internal organs. Diagnostic criteria for DIC: multiorgan anoxia with thrombocytopenia, prolongation of partial thromboplastin time (PTT), or low fibrinogen and increased fibrin monomer (FM) or increased fibrinogen degradation product (FDP).

Antimalarial drugs included oral or parenteral quinine sulfate or quinine hydrochloride 30 grains or adjusted dosage according to renal status given daily for 7 days in patients with *P. falciparum* infection. Chloroquine total dose of 2.5 grams within 3 days was given in *P. vivax* infection followed by a total dose of primaquine 225 mg for the prevention of relapse.

Adequate supportive treatment included intravenous fluids, hemodialysis in severe acute renal failure and respiratory assist in patients with pulmonary insufficiency. Heparin was given in 2 patients who had DIC with systemic complications and hyperparasitemia.

No drugs with antiplatelet effect including heparin were given before or during the studies (Carvalho and Rao, 1987). Ten patients received quinine hydrochloride intravenously prior to the study of platelet aggregation. In the other sixteen, in both during and after parasitemia groups, the antimalarial drugs were given immediately after the initial platelet function studies were performed. Since the decreased platelet aggregation by chloroquine has been demonstrated *in vitro* (Carter *et al.*, 1971); we therefore studied the effect of chloroquine on platelet aggregation in normal subjects who received the same dosage of chloroquine and quinine as given to malaria patients.

Daily complete blood count including malarial parasites counted per 100 red blood cells were performed by standard method. Laboratory biochemistries, including BUN, creatinine, electrolyte, SGOT and SGPT, alkaline phosphatase and film chest, were recorded on the first day of admission and were repeated as necessary. Platelet function studies including platelet aggregation stimulated by ADP 5 μM and epinephrine 25 μM (Day and Holmsen, 1972), (automated platelet aggregation recorder Model PA-3210 Kyoto, Daiichi); platelet factor 3 (PF_3) (Abildgaard and Harrison, 1974); clot retraction and bleeding time (Mielke, 1969), were performed on admission and repeated within 14 days afterwards. Coagulation studies namely partial thromboplastin time (PTT) (Miale, 1982); prothrombin time (PT) (Miale and Winningham, 1960); thrombin time (TT) (Brodsky *et al.*, 1986); fibrinogen (Ellis and Stransky, 1961); fibrinogen degradation product (FDP) (Hawiger *et al.*, 1970); euglobulin clot lysis (ECL) (Sherry *et al.*, 1959) and fibrin monomer (FM) (Watanabe and Tullis, 1978) were performed simultaneously with platelet function tests.

The control subjects were divided into 3 groups: Group I consisted of 40 healthy volunteers; Group II 5 febrile patients with upper respiratory tract infection; Group III, 15 healthy volunteers; 10 received quinine sulfate 30 grains daily for 3 days, and 5 received chloroquine hydrochloride total dose of 2.5 gm for 3 days. Group III was studied twice prior to and after the administration of antimalarial drugs.

RESULTS

Systemic complications including CNS, renal, lung, liver, bleeding and DIC in 21

malaria patients are shown in Table 1. Only nine patients presented with one organ complication (7-brain, 1-kidney, 1-lung) whereas twelve had multiorgan complications (7-brain plus kidney; 1-brain plus lung; 1-brain plus liver; 1-brain + kidney + lung and 2-brain + kidney + lung + liver). Bleeding complications occurred late following multiorgan dysfunction in 8 cases (40%) (7 with brain and kidney and one with lung). Two DIC patients with 15% and 40% of parasitemia, had four major complications namely brain, kidney, lung and bleeding. Despite all these complications, these 2 patients survived. In the two fatal cases (9.6%), one had pulmonary edema and the other had both brain and pulmonary complications. Of 27 cases with no systemic complications, none died.

Table 1
Clinical and laboratory manifestations in 21 patients with malarial systemic complications.

Manifestations	No. cases	Percent
Total	21	100
Cerebral malaria	19*	90
coma, convulsion	4	19
Renal insufficiency	11	52
actue renal failure	5	24
Pulmonary edema	5**	24
Liver enlargement, SGPT > 500 units/l	3	15
Bleeding	8	40
DIC	2	9.6
Hyperparasitemia (> 30%)	2	9.6
Hematocrit (%) mean ± SD		
initial	35.4 ± 7.7	
reduction during studies	7.7 ± 4.7	

* One fatal case (6.0%)
** Two died (40%)

Coagulation studies are presented in Table 2. During parasitemia, abnormalities of a few tests namely mild prolongation of PTT, a slight increase in fibrin monomer, fibrinogen and fibrinogen degradation products (FDP) were observed in 36, 42, 43, and 71% of the cases with complications. After parasitemia, only mild increase in FDP and fibrinogen level were observed in a few cases with complications.

During parasitemia, abnormal clot retraction, prolonged bleeding time and decreased release of platelet factor 3 were observed in a few cases from both groups with or without systemic complications (Table 2). Decreased platelet aggregation stimulated by ADP, and epinephrine were observed in all cases studied during parasitemia. The characteristic of the abnormalities were as severely depressed primary aggregation with the absence of secondary aggregation (Fig. 1). In comparing the groups with and without systemic complications, the suppression of aggregation was more severe in the former group (Table 3, Fig. 2), and the most severe suppression was observed in the patients who had bleeding accompanied by systemic complications (Table 4). In the patients who either had DIC or died from complications, the degree of suppression were similar to that observed in the other non DIC patients who survived (Fig. 1). The abnormalities as such were also observed in the vivax and falciparum patients who had no complications (Table 5).

The recovery in number and aggregation of platelets was observed in both groups who presented with or without systemic complications studied after parasitemia (Table 3). Complete recovery were observed at 7 and

Table 2

Hematological studies in 43 malaria patients.

Studies	During parasitemia		After parasitemia	
	$\bar{c}$ complications n = 14	$\bar{s}$ complications n = 13	$\bar{c}$ complications n = 15	$\bar{s}$ complications n = 22
PTT sec (normal 37–55)	51(38–58)*	47(40–55)	45(39–51)	42(37–47)
(% prolonged)	36	0	0	0
Fibrinogen gm/l (normal 2.39–5.58)	5.3(2.17–7.7)	5.6(3.04–8.4)	5.1(3.9–6.3)	4.9(4–5.62)
(% > 5.59)	43	38	33	45
FDP µg/ml (normal 0–5)	22.5(5–80)	7.5(5–10)	4(2.5–5)	4(2.5–5)
(% > 5)	71	57	0	0
FM (% positive)	42	8	0	0
BT (% > 5 minutes)	50	8	0	0
CR (% reduced)	28	16	0	0
PF_3 (% reduced)	7	0	0	0

* mean (range)
Tests for prothrombin time, thrombin time and euglobulin clot lysis were normal in all cases.
PTT = Partial thromboplastin time, FDP = fibrinogen degradation product,
FM = fibrin monomer, BT = bleeding time, CR = clot retraction
PF_3 = platelet factor 3,

14 days after parasitemia in the groups without and with systemic complications respectively (Fig. 2).

The results of the studies in the control group showed normal platelet aggregation along with other hemostatic tests. None of the febrile controls or the healthy subjects who took antimalarial drugs demonstrated any abnormalities in platelet aggregation stimulated by ADP, epinephrine during the study (Table 3).

DISCUSSION

In this study, we have demonstrated that during malarial infection there are platelet function abnormalities as indicated by the impaired platelet aggregation in response to ADP, and epinephrine. These changes, however, were transient, and then gradually return to normal in the period of 7–14 days after clearance of parasitemia. The degree of suppression was more severe in the patients who had systemic complications and bleeding. All of these above findings indicated that the impaired platelet aggregation occurring in malarial patients are indeed the result of malarial infection.

The mechanism causing the impaired platelet aggregation occurring during malarial infection is unclear at present. However, immune injury as a basic mechanism is the most probable one. Previous studies have demonstrated that platelet antibodies arise during malarial infection. These may activate the platelets, which are then removed by the liver and spleen (Beale, 1972; Skudowitz, 1973; Kelton *et al.*, 1983). FDP have been

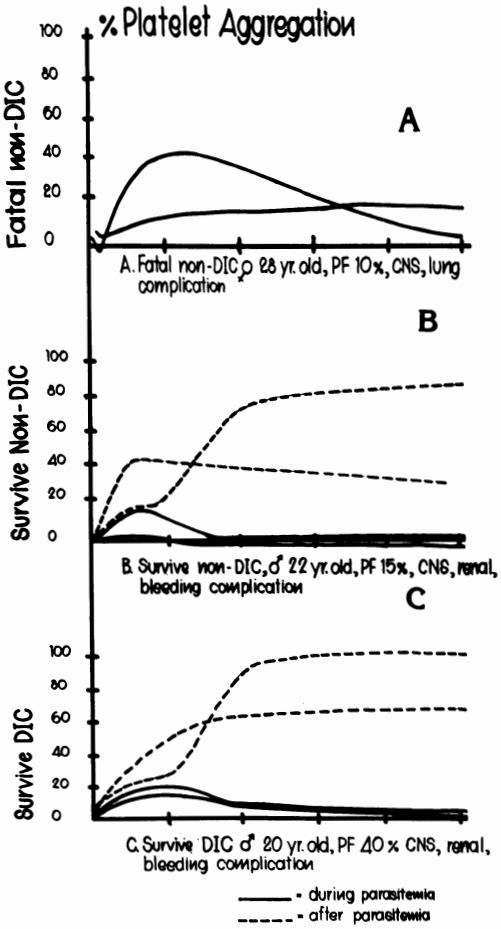

Fig. 1–Platelet aggregation in 3 falciparum malaria cases: Case A, non-DIC (fatal); Case C, non-DIC (survived); Case C, DIC (survived).

shown to inhibit platelet function (Stachurska *et al.*, 1970) but this is an unlikely explanation in the present study because only a very modest elevation of FDPs was noted (Solum *et al.*, 1973). Previous studies also demonstrated that the acquired storage pool deficiency of platelets could be induced by circulating platelet antibodies (Pareti *et al.*, 1976, 1980). A similar situation may prevail in malarial patients, where the damaged platelets may release ADP from their granules, and then circulate as "exhausted" platelets (Weiss *et al.*, 1980). This postulation is strongly supported by a previous study which demonstrated the increased release of beta thromboglobulin and platelet factor 4 in plasma during malarial infection (Essien and Ebhota, 1983).

The recovery from impaired platelet aggregation and thrombocytopenia which was simultaneously observed in the period of 7 to 14 days after the disappearance of parasitemia may reflect the new forming platelets from the bone marrow at this recovery stage. The correlation between the degree of thrombocytopenia and the impairment of platelet aggregation in the group with complications also possibly reflects the same pathogenetic mechanism of platelet destruction and platelet dysfunction in malaria caused by immune injury at the same time.

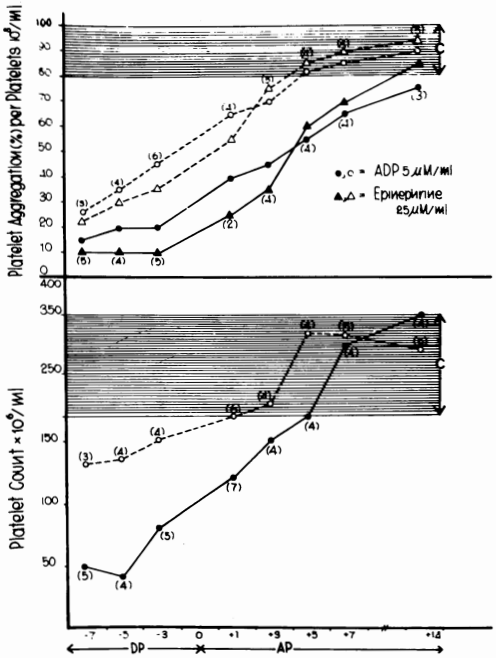

Fig. 2– Platelet aggregation and platelet count in malaria patients during and after parasitemia (DP and AP), C = control, ●——● with complications, o- - - - -o without complications. No. of cases studied shown in parenthesis.

Table 3

Platelet aggregation studies in 48 malarial patients during and after parasitemia.

Cases studied	No. cases	Platelets ($x10^6$/ml)	Maximum platelet aggregation (%) in platelets 10^8/ml	
			ADP 5μm/ml	Epinephrine 25μM/m
Control	40	280 ± 100*	90 ± 7	90 ± 7
Control ε antimalarial drugs	15	215 ± 110	80 ± 7	88 ± 7
		p = 0.05	p = 0.05	p = 0.05
Febrile Control	5	272 ± 120	81 ± 6	87 ± 8
		p = 0.05	p = 0.05	p = 0.05
During parasitemia (DP)				
With complications	14	82 ± 30	22 ± 10	10 ± 10
No complications	13	137 ± 75	46 ± 25	45 ± 31
		p = 0.005	p = 0.005	p = 0.005
After parasitemia (AP)				
With complications	15	170 ± 80	44 ± 17	29 ± 21
No complications	22	250 ± 100	75 ± 27	79 ± 26
		p = 0.005	p = 0.005	p = 0.005

* Mean ± S.D.

Table 4

Results of platelet aggregation in 21 malaria patients with complications related with bleeding.

Group Studied	No. cases	Platelets ($x10^6$/ml) Mean ± S.D.	Maximum Platelet Aggregation (%) ADP 5 μM/ml	Epinephrine 25 μM/ml
During parasitemia				
with bleeding	6	91 ± 80	13 ± 3	7 ± 3
no bleeding	8	101 ± 80	25 ± 12	17 ± 5
			p = 0.005	p = 0.005
After parasitemia				
with bleeding	6	100 ± 30	50 ± 16	60 ± 31
no bleeding	9	119 ± 80	57 ± 31	66 ± 30

Table 5

Platelet aggregation in *P. vivax* and *P. falciparum* patients without complications studied during parasitemia.

Patients studied	No. cases	Platelets ($x10^6$/ml)	Maximum Platelet Aggregation (%) ADP 5 μM/ml	Epinephine 25 μM/ml
P. vivax	4	140 ± 85	41 ± 20	38 ± 17
P. falciparum	7	131 ± 65	49 ± 22	47 ± 28
		p = 0.05	p = 0.05	p = 0.05

The results of this study also show that clot retraction and release of platelet factor 3 are insensitive tests for the early detection of abnormal platelet function. Bleeding time correlated with clinical bleeding resulting from severe thrombocytopenia. Platelet aggregation is a sensitive test for the early detection of platelet function abnormality since the defect is constant throughout malarial infection. Futhermore, the correlation between the degree of suppressed aggregation and the clinical severity suggests a significant role of platelet function alteration in the pathogenesis of bleeding and systemic complications in malaria.

The incidence of DIC (9.6%) observed in this study is quite low when compared to previous reports in which DIC was observed in 17% of 42 falciparum patients with renal insufficiency (Stone *et al.*, 1972); and 55% of 22 autopsy cases with severe systemic complications (Boonpucknavig *et al.*, 1984). The discrepancy found in this present and the previous studies may be due to two factors: hyperparasitemia and immunologic status of the hosts. In the previous study hyperparasitemia and the non-immune status were the main features of the infected patients (Boonpucknavig *et al.*, 1984); whereas in this study the majority of the cases came from an endemic area of malaria, and only 9.6% had hyperparasitemia. Nevertheless, the prolongation of partial thromboplastin time found in 36% of the patients with complications in this study suggests that the low grade consumptive coagulopathy might occur during the infection. The increased fibrinogen a common finding in malaria patients with or without complications (Jaroonvesama, 1972; Punyagupta *et al.*, 1974, Srichaikul *et al.*, 1975), as observed in over 40% of cases in this present study reflects an increase of protein synthesis during the acute phase of malarial infection. The high plasma fibrinogen, along with the platelet hyperactivity during malarial infection (Essien and Ebhota, 1983; Essien *et al.*, 1984; Inyang *et al.*, 1987) can lead to the process of hypercoagulability, increased intravascular coagulation and fibrin formation. This may become clinically significant particularly in the non-immune cases with high parasitemia.

In conclusion, this study indicates that severe impairment of platelet aggregation is a common finding in malarial infection with systemic complications and may lead to bleeding. To clarify, the role of platelet dysfunction on pathogenesis of bleeding and systemic complications in malaria should be studied further.

SUMMARY

Platelet function tests including platelet aggregation, PF_3, bleeding time and clot retraction were studied in 48 malarial patients. The suppression of platelet aggregation was demonstrated in both *P. vivax* and *P. falciparum* infection. However, this abnormality was more prominent in malarial patients who had systemic complications and bleeding. The recovery of the impaired platelet aggregation was observed at period of 7 and 14 days after parasitemia in malarial patients without and with systemic complications. The correlation between the supression of platelet aggregation and thrombocytopenia was observed. From this study, bleeding in malaria are operated by two mechanisms: thrombocytopenia and severely depressed platelet aggregation.

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