PLATELET FUNCTION DURING THE ACUTE PHASE OF DENGUE HEMORRHAGIC FEVER

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INTRODUCTION

In dengue hemorrhagic fever (DHF), bleeding is an important complication which is associated with a high mortality, (Srichaikul *et al.*, 1975). The pathogenesis of bleeding in DHF involves several factors. During the early febrile phase of the disease, vasculopathy and platelet dysfunction play major roles whereas in the pre-shock or shock phase thrombocytopenia becomes more important (Mitrakul *et al.*, 1977; Isarangkura *et al.*, 1987). Along with thrombocytopenia, a mild to moderate degree of consumptive coagulopathy associated with mild hyperfibrinolysis can occur (Mitrakul *et al.*, 1977; Srichaikul *et al.*, 1977; Funahara *et al.*, 1987). Despite their apparently mild presentation, the latter two abnormalities can progress so that fatal bleeding results, particularly in patients who present with severe and intractable shock (Srichaikul *et al.*, 1975). Despite the fact that several extensive studies have examined the role of bleeding induced by thrombocytopenia and clotting defects in DHF, little is known of the nature of platelet dysfunction in this disease.

We have therefore, studied in-vivo and in-vitro aspects of platelet function during the acute phase of DHF.

MATERIALS AND METHODS

Thirty five patients (16 females, 19 males) aged 5–12 years with serologically-confirmed dengue infections were studied. Twenty-seven cases were admitted to the Department of Pediatrics, Pramongkutklao Hospital and eight to the Children's Hospital. They were subdivided into four groups on the bases of their clinical features (WHO, 1986). Seventeen patients had grade 2 DHF, and 18 had grade 3 and 4. All patients received the same treatment (WHO, 1986).

All laboratory tests were performed at the Department of Medicine, Pramongkutklao Hospital. Blood was taken from each patient at the time of admission and then daily for hematocrit, platelet count, partial thromboplastin time (PTT) (Miale, 1982), quick one-stage prothrombin time (PT) (Miale and Winningham, 1960), thrombin time (TT) (Brosky *et al.*, 1968), fibrinogen (Ellis *et al.*, 1961), fibrinogen degradation products (FDP) (Hawiger *et al.*, 1970), euglobulin clot lysis time (ECL) (Sherry *et al.*, 1959), and fribrin monomer (FM) (Watanabe *et al.*, 1978). Platelet aggregation studies were performed using an automated aggregometer (Model PA-3210, Kyoto Daichi). Platelet-rich plasma, prepared by centrifugation of

citrated blood, was corrected to a standard count of 10^8 platelets/ml with platelet-poor plasma. A single dose of ADP (5μ mol/l final concentration) was used as proaggregating agent. Aggregation studies were performed on two occasions: in the acute phase of infection and then in convalescence (14–21 days after defervescence or shock).

In 12 of 35 patients, serial blood samples were taken for plasma betathromboglobulin (BTG) and platelet factor (PF_4) measured by the sandwich enzyme immuno assay (Asserachrome BTG and PF_4 – Diagnostic Stago) using a modification of the method of Kerry and Curtis (1985).

In five patients, platelet aggregation, in convalescence was also studied after addition of acute phase autologous plasma (stored at −70 ° c) to fresh platelet-rich plasma.

Two groups of age-matched children acted as controls: group I consisted of ten normal subjects and group II of 4 febrile patients with upper respiratory tract infection. All the controls underwent the same tests as the DHF patients.

RESULTS

The clinical details of the patients with DHF are summarised in Table 1. The age and sex distribution, and admission hematocrit in grade 1–2 and grade 3–4 patients were similar. However, clinical evidence of bleeding, thrombocytopenia, prolongation of PTT and TT with hypofibrinogenemia were more severe in grade 3–4 patients.

Thrombocytopenia was found as early as one to two days prior to the day of shock or subsidence of fever and more severe on the day of shock. This change was observed for only few days during the acute phase and then gradually returned to normal within the first week of the recovery phase (Fig. 1).

A decrease in platelet aggregation stimulated by 5 μM ADP was observed in every patient studied at the time of the acute phase of the disease relative to that in convales-

Table 1

Clinical details and hemostatic findings in 35 DHF patients.

Findings	DHF patients	
	Grade 2	Grade 3 + 4
Number of cases	17	18
Age range (yrs)	5 – 12	6 – 14
Hct % (42 ± 5)	45 ± 7	45.3 ± 8
Platelets/mm^3 (280 ± 110)	39 ± 28	20 ± 26
PTT seconds (46 ± 9)	56 ± 22	60 ± 16
TT seconds (9 ± 3)	12 ± 2	15 ± 7
Fibrinogen mg% (390 ± 150)	370 ± 170	300 ± 150
Severe bleeding-cases	1	3

cence. The characteristic abnormality was severely depressed showing primary aggregation and the absence of secondary aggregation (Fig. 2). The degree of suppression of platelet aggregation observed in the patients with shock and bleeding were similar to those in non-shock without bleeding (Fig. 3). The convalescent study showed normal platelet aggregation in the majority of the cases 14 to 21 days after shock or subsidence of fever (Fig. 3).

Plasma BTG and PF_4 concentrations during acute illness in 'febrile' controls were not significantly different from the "normal" controls. In the acute phase of DHF, there were markedly increased plasma PF_4 and BTG concentration to values 4 to 5 times those of the controls. These increased plasma BTG and PF_4 concentrations occured at the same time as the decrease in platelet aggregation induced by ADP (Fig. 4). The concentrations of these two substances declined towards normal within 3–4 days of shock or subsidence of fever.

The most marked coagulation abnormalities observed were prolongation of PTT, TT and low fibrinogen on the day of shock or subsidence of fever. In the shock group, 7, 5 and 4 out of 11 cases had prolonged PTT, prolonged TT and hypofibrinogenemia respectively whereas in the non-shock group 4, 2 and 3 out of 11 studies cases had the same abnormalities. These changes were transient and returned to normal a day after shock (Fig. 5). Despite these coagulation abnormalities, PT and FDP remained normal. The fibrin monomer and euglobulin clot lysis were also normal throughout the study.

Table 2 shows the stimulatory effect of the autologous acute phase DHF plasma on the aggregation of the convalescent platelets in all 5 cases studied.

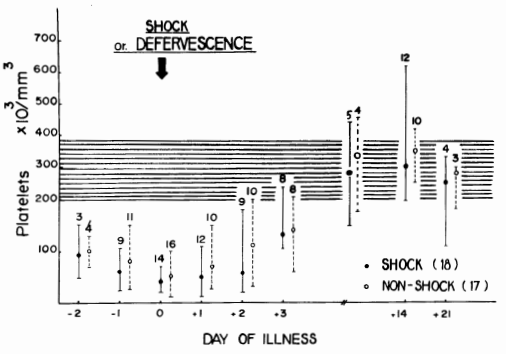

Fig. 1–Serial platelet count in 35 DHF patients number above each column = No. of cases studied, ▤ = control value.

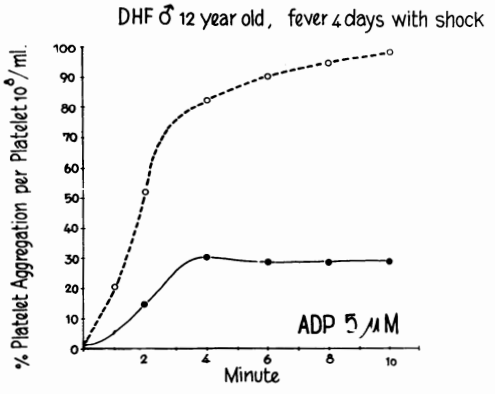

Fig. 2–Platelet aggregation in DHF patient studied at 1 day before shock and 14 days post-shock ● ——— ● = before shock; ○ - - - - - ○ = post-shock.

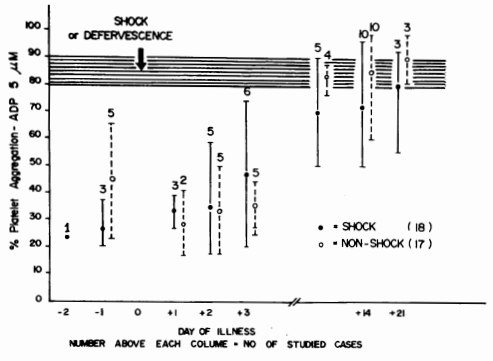

Fig. 3–Serial platelet aggregation in 35 DHF patients number above each column = No of cases studied, ▤ = control value.

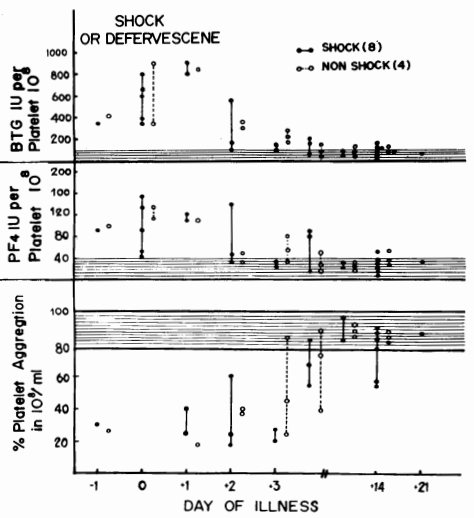

Fig. 4–Platelet aggregation, BTG PF_4 in 12 DHF Patients ▭ = control value.

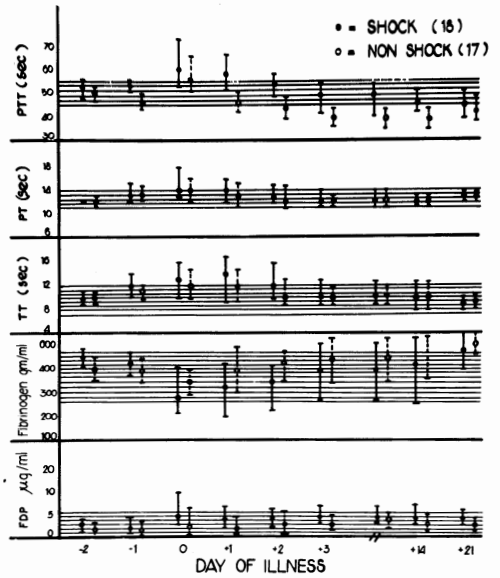

Fig. 5–Serial coagulation studies in 35 DHF patients number of cases studied on each day are similar to those of platelet count studies. ▭ = count studies.

Table 2
Effect of autologous acute phase plasma on convalescent platelet aggregation in 5 DHF patients

Grade	%Platelet aggregation obtain from		
	Acute phase platelets in acute plasma (same day)	Convalescent platelets mixed with	
		Convalescent plasma (same day)	Acute plasma (different day)
2	–	60.3 (+ 7)	80 (0) 79 (+ 3)
1	26 (– 2) 55 (+ 1)	64 (+ 8)	70 (– 2) 70 (+ 1)
3	66 (+ 4)	51 (+ 11)	74 (+ 4)
3	–	58 (+ 11)	77 (+ 0)
3	–	54 (+ 10)	69 (0)
Control	85 ± (day 0)	86 ± 7 (day 14)	85 ± 6 (day 0)

( )Number in parentheses indicate day of illness related to shock or subsidence of fever; 0 = shock or subsidence of fever; – = before, + = after.

DISCUSSION

In this study, simultaneously decreased platelet aggregation and increased plasma betathromboglobulin (BTG) and platelet factor 4 (PF_4), together with thrombocytopenia and abnormal indices of coagulation were demonstrated during the acute phase of DHF. These abnormalities occurred transiently and then gradually returned to normal at the end of the first or second week of convalescence. These findings indicate that all of these changes are a direct result of dengue hemorrhagic fever *per se*.

The impaired ADP-induced platelet aggregation found during the acute phase of DHF in the present study confirms the results of previous author (Suvatti *et al.*, 1975). The mechanism of platelet hypoaggregation is unclear at present. It has been found that platelet aggregation can be inhibited *in vitro* by the presence of immune complexes consisting of dengue viral antigen and antibody (Suvatti *et al.*, 1975). It has also been shown that platelet function can be inhibited by FDP; 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 have demonstrated dengue antigen and antibody complexes on the surface of platelets during acute infection (Boonpucknavig *et al.*, 1979). These immune complexes may cause damage to platelets which are then removed by the liver and spleen (Mitrakul *et al.*, 1977). Thrombocytopenia can also be induced by a circulating platelet antibody and platelet 'exhaustion' (Pareti *et al.*, 1980). A similar situation may prevail in DHF. Dengue-spciefic immune complexes or platelet antibodies may cause intially *in-vivo* aggregation but then render platelet less sensitive to pro-aggregating agents such as ADP. Platelets which have degranulated may have accerelated clearance with resultant thrombocytopenia. Consistent with this hypothesis was the effect of acute phase plasma on platelet aggregation. Previous studies have shown that an increase in platelet aggregation can be induced *in vitro* by mixing platelets with dengue antibody, which may then lead to platelet lysis and thrombocytopenia *in vitro* (Funahara *et al.*, 1987).

Coagulopathy in DHF has been studied extensively during the past 20 years. The results indicate that during acute phase of DHF, there are increased intravascular coagulation and fibrinolysis. There is rapid elimination of radioactive fibrinogen (Srichaikul *et al.*, 1977), prolongation of the partial thromboplastin and thrombin time; decreased fibrinogen and increased FDP accompanied by variable reduction in many hemostatic factors including antithrombin III, α_2 antiplasminogen (Mitrakul *et al.*, 1977; Isarangkura *et al.*, 1987; Funahara *et al.*, 1987). In the present study, prolonged partial thromboplastin and thrombin time and reduced fibrinogen were found mainly in DHF grade 3–4 patients at time of shock, in accord with these previous studies and suggesting a mild to moderate degree of consumptive coagulopathy during the acute phase of DHF.

SUMMARY

Platelet aggregation, plasma betathromboglobulin (BTG) and platelet factor 4 (PF_4) were studied in 35 children with dengue hemorrhagic fever. The suppression of platelet aggregation was demonstrated during acute phase of DHF in both shock and non-shock patients. Simultaneous with abnormal plate-

let aggregation, there was increased release of BTG and PF_4 from platelets into plasma during the acute phase which lasted only 3–4 days after shock or subsidence of fever. Acute phase plasma during DHF infection was also shown to have a stimulatory effect on the aggregation of autologous platelets. In this study we showed that there was an increase in platelet secretory activity of BTG and PF_4 along with an impairment of the platelet aggregation during acute phase of DHF.

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