SIGNIFICANCE OF BLOOD COAGULATION AND PLATELET PROFILES IN RELATION TO PULMONARY THROMBOSIS IN β-THALASSEMIA/Hb E

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Abstract. In β-Thalassemia hemoglobin E (β-thal Hb E), hypoxemia with abnormal lung function was described and postmortem examination in these patients showed organized pulmonary trombi with thicked arterial wall, particularly in post-splenectomized cases. Coagulation and platelet profiles were studied in 58 β-thal Hb E patients. In 35 cases with intact spleen, the fibrinolytic activity was significantly decreased with high antithrombin III activity, while coagulation tests revealed mild abnormality. The platelet aggregation to ADP, adrenaline, collagen and ristocretin were defective and platelet 5-hydroxytryptamine content was lower than normal. Twenty-three patients who had been splenectomized for 5-18 years, decreased fibrinolytic activity and high antithrombin III activity were also observed. The coagulation profiles and platelet aggregation in response to ADP, adrenaline and collagen showed better results. Fourteen cases exhibited thrombocytosis and their thrombin generation was in the hypercoagulable range. Platelet aggregation in response to ristocetin remained defective and platelet 5-hydroxytryptamine content was lower than in cases with intact spleens. Defective aggregation to ristocetin would indicate abnormal von Willebrand's factor (vWF). Decreased fibrinolysis should very likely have a role in the occurrence of thrombosis and the better hemostatic profiles in post-splenectomized cases would contribute to the more frequent thrombotic incidence in these cases.

INTRODUCTION

Hypertransfusion programs have been established for children with thalassemia major to improve the oxygen carrying capacity of their blood. In spite of their stable clinical features and ability to attend school regularly, these cases were found to have hypoxemia and showed abnormal lung function tests (Cooper *et al,* 1980; Keen *et al,* 1980). It was interesting to note that on postmortem examination in 8 cases who died of congestive heart failure, the lung histology in 2 cases showed organizing old thrombi and thickened arterial walls (cooper *et al,* 1980). Further studies were subsequently performed in the same cases (Keen et al, 1980) and after under going splenectomy, hypoxemia was still observed and the total lung capacity was further reduced compared with the presplenectomized findings (Grant *et al,* 1986).

In Thailand, double heterozygosity of β-thalassemia and hemoglobin E alleles, β-thalassemia hemoglobin E (β-thal Hb E), is prevalent (Wasi, 1981). The clinical feature of progressive hypoxemia in association with cardiac failure is frequently found, especially in post-splenectomized cases, and their clinical course is always a deteriorating state. In reviewing lung histology from 43 autopsied β-thal Hb E patients (Sonakul *et al,* 1980), organized and recanalized thrombi in pulmonary arteries were demonstrated in 19 cases; 17 of them had been splenecotomized for 5-32 years. Thrombosis was found only in pulmonary arteries. Therefore the possibility of embolism was raised, however, there was no evidence of thromboembolism elsewhere in these cases. Organizing thrombi and some fresh thrombi in pulmonary arteries were also demonstrated on postmortem examination of a post-splenectomized β-thal Hb E patient who died from hypoxemia and refractory heart failure (Sukpanichnant *et al,* 1988). No thrombus in the deep vein of legs and pelvic cavity was found. Furthermore, arterial hypoxemia was described in 22 of 30 post-splenectomized β-thal Hb E patients and there were indications of abnormal ventilatory functions which were restrictive, obstructive or with combined defects (Youngchaiyud *et al,* 1988). Thrombosis in pulmonary arteries could cause pulmonary engorgement leading to small airway obstruction and hypoxemia in β-thal Hb E. The

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formation of pulmonary thrombi as yet has no explanation. The purpose of this study, therefore, was to investigate aspects of coagulation and platelet profiles in β-thal Hb E to determine whether There would be any relationship to the occurrence of pulmonary thrombosis in these patients.

MATERIALS AND METHODS

Patients

Fifty-eight β-thalassemia hemoglobin E patients who attended hematology clinic for regular checkup were studied. There were 13 males and 10 females, ages ranging from 13-41 years, post-splenectomized for 5 to 18 years. The remaining 35 cases had intact spleens, there were 20 males and 15 females, 13 to 48 years of age. None took aspirin for at least 4 weeks prior to the study.

Methods

Sodium citrate 3.8% was used anticoagulant and equipment for collecting blood was all siliconized. Blood was withdrawn from the cubital vein by a two-syringe technique between 09:00 to 11:00 AM. Blood from the first syringe was tested for hemoglobin and antithrombin III determinations. Antithrombin III was measured by functional assay, based on the progressive neutralization of thrombin by tested serum (von Kaulla *et al,* 1972). Lee-White clotting time (VCT) was measured in blood from third syringe.

Coagulation studies

Ten ml of citrated blood were centrifuged at 1500*g* for 5 minutes to obtain plasma and the following tests were performed on fresh plasma: thrombin generation test (TGT) (von Kaulla and von Kaulla, 1964a), recalcification (RT) and prothrombin consumption test (PCT), activated partial thromboplastin time (PTT) (Proctor and Rappaport, 1961), one-stage prothrombin time (PT) which was expressed as a percentage of prothrombin complex (Tocantins, 1964), thrombin time (TT) (von Kaulla and von Kaulla, 1964b) and fibrinogen level (Ellis and stransky, 1961). The fibrinolytic activity in plasma was measured by euglobulin lysis time test (ELT) (von Kaulla and Schultz, 1958).

Platelet studies

Platelets were directly counted under phase contrast microscope (Brecher *et al,* 1953). Platelet aggregation was determined by the method described by Baumgartner and Born (1968) using a Paton aggregometer (Peyton Associated Ltd, Toronto). Blood was centrifuged at 150-170*g* for 10 minutes to obtain platelet rich plasma containing < 3% red blood cells. The aggregating agents and their final concentrations were as follows: 2-5 μM adenosine diphosphate (ADP) (Sigma, London Ltd), 0.2-50 μM adrenaline (British Drug House). I mg/ml collagen (Bovine achilles tendon, Sigma Chemical Co, St Louis, MO, USA), and 1.25 mg/dl ristocetin (Ristocetin sulfate, H Lundbeck and Co A/S Copenhagen). Platelet aggregation was recorded for at least 3 minutes and expressed as rate of platelet aggregation or aggregation velocity in mm per minute, measured as the tangent to the steepest slope in the light transmittance records during the first phase of platelet aggregation (Baumgartner and Born, 1968). Good aggregation (G) represented complete aggregation (with second phase aggregation); poor aggregation (P) referred to those presenting only first phase or with deaggregation during 3 minutes observation; delayed aggregation (D) represented the aggregation which occurred after one minute. 5-Hydroxytryptamine (5-HT) content in the platelets was measured by the method of Crosti and Lucchelli (1962). The difference of findings beteen groups was assessed by student's *t*-test.

RESULTS

Coagulation profiles

Thrombin generation test (TGT): As illustrated in Fig. 1, TGT of 21 β-thal Hb E with intact spleen (a) was delayed as compared to normal (dotted area). While in post-splenectomized cases, TGT (b) was within normal limits, particularly in the 12 splenectomized cases with thrombocytosis, (c) an accelerated TGT into hypercoagulable state was demonstrated. The other profiles are summarized in Table 1.

Antithrombin III (AT III)

A long AT III time represented a high AT III activity while a short time showed low activity. In

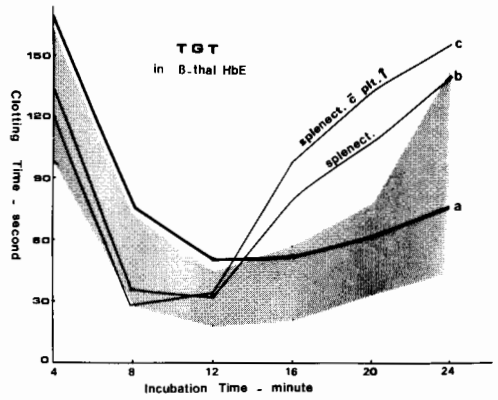

Fig 1–The mean thrombin generation test (TGT). Dotted-area represents the range of TGT in normal (mean ± SD). a - TGT in 21 β-thalassemia hemoglobin E (β-thal Hb E) with intact spleen, β-17 β-thal Hb E post-splenectomy and c-12 β-thal Hb E post-splenectomy with thrombocytosis.

Table 1, β-thal Hb E patients with both intact spleens and post-splenectomy exhibited higher AT III activity than normal, as demonstrated by prolonged AT III time [$p < 0.001$ ($t = 15.992$)], but the AT III activities of post-splenectomized cases and those with intact spleens was not significantly different [$p > 0.3$ ($t = 0.839$)].

Fibrinogen

β-thal Hb B patients with intact spleens had higher fibrinogen levels than normal than post-splenectomized cases, [$p < 0.001$ ($t = 7.585$)].

Prothrombin complex

There was no difference between cases with and without spleen [$p > 0.5$ ($t = 1.982$)]. However, the prothrombin complex of both groups was significantly lower than normal [$p < 0.001$ ($t = 5.365$)].

Table 1

Coagulation profiles, fibrinolytic activity, platelet count, platelet 5-hydroxytryptamine (5-HT) content in β-thal Hb E.

Tests	β-thal Hb E, intact spleen		β-thal Hb E, post-splenectomy		Normal	
	Total no.	Mean ± SD	Total no.	Mean ± SD	Total no.	Mean ± SD
Platelet × 10^9/l	28	195.8 ± 110.1	20	573.0 ± 293.9	85	199.8 ± 97.5
5-HT - ng/10^8 Plt	11	152.9 ± 63.6	19	121.7 ± 43.4	20	182.6 ± 57
At III time - sec	14	175.6 ± 96.4	13	204.9 ± 79.7	99	110.0 ± 49.3
ELT - min	33	211.9 ± 93.1	23	243.3 ± 90.7	88	167.0 ± 66.8
Fibrinogen - mg/dl	31	342.1 ± 167.6	22	274.6 ± 147.6	85	251.7 ± 56.7
Proth comp - %	35	77.2 ± 9.2	23	76.3 ± 7.7	88	92.5 ± 8.23
VCT - min	32	15.7 ± 4.5	23	15.2 ± 3.9	80	14.1 ± 2.9
PTT - sec	30	73.8 ± 12.2	22	64.9 ± 12.5	87	61.3 ± 20
PTT - sec	18	63.3 ± 32.1	14	116.02 ± 25.4	81	82.7 ± 19.9
Hemoglobin - gm/dl	28	6.6 ± 1.7	21	6.5 ± 1.03	-	12 ± 16

Table 2

Platelet aggregation velocity (mm per minute) in β-thal Hb E and normal.

Subject	β-thal Hb E, intact spleen											β-thal Hb E, post-splenectomy											Normal
Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Mean ± SD
AD 2 μM	0	0	0	0	0	42P	0	0	0	0	0	-	-	-	-	80G	80G	150G	330G	-	-	-	
5 μM	18P	30P	44P	58P	75P	-	D	70G	100G	-	-	72P	102G	126G	152G	-	-	-	-	-	-	-	221.4G ± 48.5
Adren 0.2 - 0.5 μM												22G	32G	14G	0	38G	56G	0	0	D	30G	36G	0
1 - 10 μM	0	D	0	0	22P	0	D	8P	12G	D	52G	-	-	-	14G	-	-	10G	40G	27P	-	-	38G ± 12.9
20 - 50 μM	40P	-	60P	5P	-	D	-	-	-	-													
Collagen 1.0 mg/ml	66G	0	0	0	0	0	-	6P	-	0	-	2G	0	60G	16G	40G	22G	2G	44G	0	56G	56G	24.6G ± 23.8
Rist 1.25 mg/ml	-	D	20G	-	30G	7P	D	6P	-	60G	40G	2G	5G	4G	D	10P	18G	D	8G	72P	60G	4G	38G ± 16.1
5-HT ng/10^8 Plt	204	55	149	185	228	60	252	99	114	202	134	137	123	174	88	188	68	85	200	59	87	93	182.6 ± 57
Plt × 10^9/l	-	70	200	-	70	225	195	-	200	-	300	775	-	575	555	755	775	280	725	535	450	515	199.8 ± 97.5

G = Good-with 2nd phase aggregation
P = Poor-with only 1st phase or with deaggregation
D = Delayed - aggregation after one minute

Fibrinolytic activity

Measured by euglobulin lysis time (ELT) test, a significantly prolonged ELT was found in both groups of β-thal Hb E patients, with intact spleens and post-splenectomy, [$p < 0.02$ ($t = 2.53$) and $p < 0.001$ ($t = 3.77$), respectively]. Decreased fibrinolytic activity in β-thal Hb E patients was demonstrated, yet there was no difference of fibrinolytic activity between the two groups, [$p > 0.3$ ($t = 0.875$)].

Other coagulation profiles

Venous clotting time (VCT) of both groups was not different and was within normal limits. Partial thromboplastin time (PTT), and prothrombin consumption test (PCT) showed higher values in post-splenectomized cases than those with intact spleen.

Platelet profiles

Platelet count: A count above 400×10^9/l would be considered as having thrombocytosis. In Table 1, the counts in β-thal Hb E patients with intact spleen were 50 - 530 ₮ 10^9/l with a mean of $195 \pm 110.1 \times 10^9$ /l, and only one case had thrombocytosis. In post-splenectomized cases, the counts were $280 - 1{,}610 \times 10^9$/l with a mean of $573 \pm 293.3 \times 10^9$/l and thrombocytosis (platelet counts $425 - 1{,}610 \times 10^9$/l) was found in 14 of 20 cases.

Platelet 5-HT content

In Table 1, β-thal Hb E patients with intact spleen had reduced 5-HT in platelets compared with normal cases, [($p < 0.001$ ($t = 4{,}823$)] and those post-splenectomized cases exhibited lower 5-HT values than those with intact spleens, [($p < 0.001$ ($t = 5.082$)].

Platelet aggregation

As summarized in Table 2, β-thal Hb E patients with intact spleens (No. 1-11) exhibited profound defective platelet aggregation. The aggregation response to ADP, with obviously low aggregation velocity and with no second phase aggregation or "P" aggregation was demonstrated and "D" aggregation (no aggregation during first minute) was found in one case (No. 7). To adrenaline, the aggregation response was normal only in one case (No. 11), while the remaining cases exhibited low aggregation velocity and "P" aggregation. Increased concentrations of adrenaline up to 20 - 50 μM still exhibited "p" aggregation and "D" agregation. Aggregation response to ristocetin, 4 cases (No. 3, 5, 10, 11) was normal, but there was "D" aggregation in 2 cases (No. 2, 7) and low aggregation velocity with "P" aggregation in another 2 cases (No. 6, 8). Aggregation to collagen in 6 out to 8 β-thal Hb E patients was zero, one had a low aggregation velocity with "P" aggregation, and normal findings were demonstrated in one case (No. 1).

In post-splenectomized β-thal Hb E patients (Table 2, No. 12-22), platelet aggregation to ADP, adrenaline and collagen showed better results compared to cases with intact spleens and 9 out of these 10 cases had thrombocytosis. In the aggregation response to ADP, 3 cases (No. 15, 18, 19) had normal aggregation, 4 cases showed slightly low aggregation velocity with "G" aggregation and "P" aggregation was found in one case (No. 12). Aggregation response to adrenaline was demonstrated as follows: normal aggregation was found at concentrations below 1 μM in 7 out of 11 cases, "G" aggregation was seen in 3 cases and "P" aggregation was present in only one case. Aggregation response to collagen was normal in 8 out of 11 cases. However, the aggregation to ristocetin was defective in 8 cases and normal in 3 cases. It was interesting that a case with nonthrombocytosis (No. 18) showed normal aggregation only to ADP.

Hemoglobin levels were not different between those with intact spleens and post-splenectomized patients, [$p < 0.2$ ($t = 1.156$)]. A large numer of nucleated red blood cells, as many as 500 per 100 leukocytes, wee found in the blood smears of post-splenectomized cases. In addition, we also measured lupus anticoagulant in 10 other β-thal Hb E patients by performing diluted tissue thromboplastin inhibition test, and all gave negative findings.

DISCUSSION

Our study showed that β-thal Hb E patients with intact spleens exhibited defective hemostasis both

in coagulation profiles and platelet aggregation, while in post-splenectomized cases, better findings for several hemostatic parameters were observed, particularly in cases with thrombocytosis. In Fig. 1 a delayed thrombin generation test (TGT) is demonstrated in β-thal Hb E with intact spleen while the post-splenectomized cases exhibited normal TGT and hypercoagulable range TGT in those with thrombocytosis. Moreover, as shown in Table 2, the better aggregation response of platelets to ADP, adrenaline and collagen was found in post-splenectomized cases. It was very interesting that the aggregation response to adrenaline in these cases could be achieved at much lower concentrations than normal. However abnormal parameters were demonstrated in post-splenectomized cases as well as in cases with intact spleens. These abnormalities were decreased fibrinolytic activity as measured by euglobulin lysis time, high antithrombin III activity, defective platelet aggregation response to ristocetin and low 5-HT content of platelets.

In homozygous β-thalassemia (β β-thal) patients, there are several reports on the role of the hemostatic profiles in relation to easy infection of bruises and epistaxis. In these reports, there was no particular emphasis on the findings in splenectomized cases compared with those with intact spleens. Hilgartner *et al* (1963) reported mild to moderate deficiency of prothrombin complex, Factors IX and XI in a number of 21 thalassemia major cases, 17 of which were post-splenectomy; cases with epistaxis had normal tests. Platelet function studies in 15 β-thalassemia major (2 cases being post-splenectomy were described by Eldor (1978) who found diminished platelet aggregation responses to ADP, epinephrine, collagen and ristocetin with normal platelet factor 3 availability tests and suggested that the abnormal findings could be responsible for the bleeding. Another study by Hussian *et al* (1979) in 18 β-thalassemia major patients of which 11 cases were post-splenectomy found prolonged prothrombin time and PTT, together with abnormal platelet aggregation response to ADP, adrenaline, collagen and ristocetin in certain cases, but they did not correlate epistaxis and bruising in their cases.

Based on the above information together with the results of this present study, it is evident that hemostatic defects, particularly defective platelet aggregation, could be another consistent abnormality in β-thalassemia diseases. However, none of the previous reports mentioned fibrinolytic activity. Brusising and epistaxis were also observed in β-thal Hb E cases, yet no other bleeding tendency was noted. On the contrary, thrombosis in pulmonary arteries was described as being of significant incidence and more frequently occurred in post-splenectomized cases, presumed to be due to thromboembolism. However, dissection of the let vein was not performed in those cases (Sonakul *et al,* 1980).

An important question should be raised, how could these cases develop thrombosis in the presence of defective coagulation and platelet profiles and increased antithrombin III activity? In particular, the increased antithrombin III should be a protective mechanism against thrombosis (Girolani *et al,* 1984; von Kaulla *et al,* 1972). Goodnough *et al* (1983) described thromboembolism in cases with pre-existing hemostatic defects. In certain diseases such as myeloproliferative disorders, the patient could develop bleeding and thrombosis (Schafer, 1984). Pulmonary thrombosis was also demonstrated in β-thal (Cooper *et al,* 1980) and in β-thal Hb E patients being more frequent in post-splenectomized cases (Sonakul *et al,* 1980).

Could fibrinolytic activity be a causative factor in β-thal Hb E? The decreased fibrinolytic activity as illustrated by prolonged euglobulin lysis times (ELT) was found in both with intact spleens and post-splenectomized patients. ELT has been described as measuring mainly plasminogen activator (von Kaulla and Schultz, 1958). The decreased plasminogen activator from endothelial cells has been described as being associated with venous thrombosis (Korninger *et al,* 1981; Petaja *et al,* 1989). Therefore the findings of decreased fibrinolytic activity in β-thal Hb E cases could be significant and a role in thrombosis is possible. The better hemostatic profiles described above in post-splenectomized cases should trigger the more frequent occurrence of thrombosis in these cases causing a higher in cidence of thrombosis than in those with intact spleens. Benson *et al* (1990) demonstrated low plasma von Willebrand factor (vWF) antigen conventration and decreased ristocetin of cofactor activity in 6 cases with hemoglobin E-D-β°-thalassemia. Our finding of a defective platelet aggregation response to ristocetin in β-thal Hb E both with intact spleens and post splenectomy could be due to the defect of vWF in our cases. Plasminogen activator and vWF are known to be synthesized by endothelial cells (von Kaulla

and Wasantapruek, 1969; Wehrmacher, 1988). Therefore, an abnormality of endothelial cells in β-thal Hb E could possibly be indicated.

In normal persons, venous occlusion causes the release of plasminogen activator and vWF from endothelial cells (Petäjä *et al,* 1989) while patients with von Willebrand disease exhibit an impairment of fibrinolytic response to venous occlusion (Korninger *et al,* 1981). If this endothelial defect exists in β-thal Hb E, any event which produced endothelial damage, *ie* infection, would predispose to the occurrence of thrombosis. Pulmopary thrombosis in β-thal Hb E perhaps could occur through these mechanisms.

Our study is a preliminary presentation, further investigation on the releasing ability of plasminogen activator and vWF from vascular walls in β-thal E should be pursued. Splenectomy has been described to improve the pre-existing hemostatic defects especially with regard to platelet function (Visudhiphan *et al,* 1985), as in patients with hairy cell leukemia (Rosove *et al,* 1980) and in cirrhosis with portal hypertension (Kamisasa *et al,* 1980). The better hemostatic profiles found in post-splenectomized β-thal Hb E emphasize the effect of the spleen on hemostasis.

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