

Haemostatic disorders in nonsplenectomized and splenectomized thalassaemic children

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SUMMARY A group of 40 thalassaemic patients (20 splenectomized and 20 nonsplenectomized) from the Haematology Unit of Tanta University Hospital (age range: 3–14 years) were studied to identify the mechanisms by which haemorrhagic and thrombotic complications occur in thalassaemic patients. The patients' levels of protein C, antithrombin III and *in vitro* platelet aggregation in response to collagen were compared with those of 20 controls. The study suggests that thrombocytosis, increased platelet aggregation and decreased natural coagulation inhibitors (protein C and antithrombin III) in splenectomized thalassaemic children may be significant in thrombotic complications in such patients. Defective platelet aggregation and prothrombin activity in nonsplenectomized children may also give rise to haemorrhagic tendencies.

Introduction

Thalassaemias are a group of heritable anaemias of varying degrees of severity, occurring early in life. They are caused by a decreased rate of synthesis of one or more globin chains, resulting in chronic haemolysis [1]. The aim of our study was to evaluate the effects of splenectomy on platelet aggregation and on natural coagulation inhibitors in thalassaemia, and to identify the possible mechanisms by which thrombotic and haemorrhagic complications occur in such patients.

Haemostatic disorders have been observed in patients with β -thalassaemia. A mild haemorrhagic tendency, characterized by easy bruising and frequent epistaxis, has been reported by Eldor [2]. Clinical symptoms related to the occurrence of thrombo-

sis, especially in the lungs after splenectomy, have also been observed, although infrequently [3].

Different mechanisms have been suggested to explain haemostatic disorders in thalassaemic patients. Factors related to platelet function and natural coagulation inhibition may be involved in cases of severe splenomegaly or hypersplenism, or following splenectomy [4,5]. The mechanisms by which haemostatic disorders arise are, however, still unclear, and the roles played by protein C, antithrombin III (AT III) and platelet aggregation have yet to be fully studied.

Patients and methods

A group of 40 β -thalassaemia major patients, aged 3–14 years, were selected for

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Received: 10/09/98; accepted: 27/05/99

the study. The patients consisted of both inpatients and outpatients from the Haematology Unit of Tanta University Hospital's Paediatrics Department. All cases were treated optimally regarding transfusion and chelation. The group consisted of 20 nonsplenectomized patients (14 males, 6 females) and 20 patients who had undergone splenectomy at least a year prior to the commencement of the study. Each group had average haemoglobin levels. The therapy regimen for all patients was the same. A group of 20 healthy children matched by age and sex served as the control group.

As most of the β -thalassaemic patients required periodic blood transfusions, blood samples for the study were collected as late as possible after the preceding transfusion. Patients received no antiplatelet medication for 2 weeks prior to the study. Patients with associated renal trouble, chronic active hepatitis or diabetes were excluded from the study. For each of the selected patients and controls, a complete history was taken, a thorough clinical examination was carried out and the following analyses were performed in order to diagnose thalassaemia:

- complete blood picture
- serum ferritin [δ]
- haemoglobin electrophoresis.

A more specific investigation then followed, involving:

- liver function;
 - bilirubin
 - enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transferase (γ -GT), serum alkaline phosphatase (ALP), and α -fetoprotein
- virus markers against hepatitis B virus, hepatitis C virus and human immunodeficiency virus (HIV) [negative by polymerase chain reaction (PCR)]

- echocardiogram and kidney functions (urea, creatinine);
- platelet count, bleeding time, clotting time and prothrombin activity;
- AT III and protein C levels;
- *in vitro* study of platelet aggregation carried out on platelet concentrations using collagen reagent.

Results

The patients showed classic clinical features of children with thalassaemia major. Recurrent epistaxis was observed in two nonsplenectomized children. Cerebral thrombosis was found in one splenectomized child.

Laboratory results are summarized in Tables 1–4. It should be noted that the mean value \pm standard deviation of the pre-transfusion haemoglobin level was 7.5 ± 1.6 g/dL, and for serum ferritin, 731.19 ± 708.95 mg/L. Liver function tests carried out on these groups of patients explored the extent of impairment compared to the control group (Table 1).

Discussion

We attempted to evaluate precisely the mechanisms of haemostatic disorder by examining a number of parameters, prothrombin activity, protein C, AT III, as well as platelet count and aggregation, and by exploring the role of the spleen in such patients.

Our results showed certain hepatic dysfunction, mainly due to iron overload. This finding agrees with that reported by Khalifa et al. [7] and Shebl et al. [8]. Others have reported how thalassaemic patients are often affected to varying degrees by chronic hepatic problems due to iron overload,

Table 1 Liver function tests in nonthalassaemic and thalassaemic (splenectomized and nonsplenectomized) children

Group	Total serum bilirubin (mg/dL)	ALT (U/L)	AST (U/L)	Alkaline phosphatase (U/L)	γ GT (U/L)
Controls					
Range	0.50-1.00	6.00-13.00	3.00-12.00	4.90-15.00	6.00-22.00
Mean	0.78	8.93	6.70	9.16	12.96
\pm s	0.17	2.90	2.90	3.62	5.23
Splenectomized					
Range	0.50-3.90	20.00-53.00	16.0-50.00	14.0-51.00	17.0-45.00
Mean	2.30	34.05	31.40	31.42	29.92
\pm s	0.95	9.01	9.94	11.29	8.96
Nonsplenectomized					
Range	0.40-4.50	20.00-60.00	18.00-43.00	14.00-49.00	17.00-54.00
Mean	2.27	37.15	30.37	29.81	28.87
\pm s	1.70	12.35	7.38	11.15	9.09
F	11.86	59.39	72.45	34.90	28.53
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Scheffe post-hoc test	(II and III) > I	(II and III) > I	(II and III) > I	(II and III) > I	(II and III) > I

ALT = alanine aminotransferase
GT = glutamyl transferase

AST = aspartate aminotransferase
s = standard deviation

chronic hepatitis, hepatic stasis, protein and folate deficiency, and repeated viral infection [9,10]. In our study, patients with viral infection or marked hepatic damage with disturbed clotting function were excluded.

As with the findings of Ayad, there was no significant difference in bleeding and coagulation time between patients and controls [11]. Our results also showed that patients' prothrombin activity (Table 2) was significantly lower than that of controls ($P < 0.001$), with no difference between splenectomized and nonsplenectomized patients ($P > 0.05$). This agrees with Shirhata [4] and Visudhiphan et al. [12]. Decreases in prothrombin activity in thalassaemic patients may be due to parenchymatous liver damage as a result

of iron deposition, leading to a decrease in prothrombin synthesis [8].

Red blood cells (RBCs) from thalassaemia major patients have been shown to enhance thrombin generation in a prothrombinase assay. It has also been shown that the procoagulant effects of thalassaemia major RBCs are abrogated by annexin V, which binds tightly to anionic membrane phospholipids [13]. The increased annexin V binding observed in thalassaemia major patients is not due to the transfused blood but reflects the intrinsic anomalies of thalassaemic RBCs which are related to haemoglobinopathy [13].

The platelet count showed a slight (statistically insignificant) tendency to decrease. This could be attributed to hypersplenic activity and to increased sequestration of platelets in enlarged spleens [14]. In

Table 2 Haemostatic variables carried out on platelet concentrations using collagen reagent for nonthalassaemic and thalassaemic (splenectomized and nonsplenectomized) children

Group	Bleeding time (minutes)	Prothrombin activity (%)	Clotting time (minutes)	Platelet count ($\times 10^3/\mu\text{L}$)
<i>Controls</i>				
Range	1.50-5.50	90.00-100.00	3.00-6.00	175.00-300.00
Mean	3.45	97.45	4.25	231.5
$\pm s$	1.29	3.30	0.98	39.4
<i>Splenectomized</i>				
Range	2.50-5.50	50.00-100.00	3.60-6.00	100.00-500.00
Mean	3.70	78.15	4.73	340.30
$\pm s$	1.11	12.34	0.85	142.25
<i>Nonsplenectomized</i>				
Range	2.00-5.50	45.00-100.00	3.00-5.50	100.00-496.00
Mean	3.78	78.15	4.50	208.65
$\pm s$	1.11	13.41	0.87	83.19
<i>F</i>	0.43	21.72	1.42	10.34
<i>P</i> -value	> 0.05	< 0.001	> 0.05	< 0.001
Scheffe post-hoc test	None	(II and III) < I	None	(I and III) < II

s = standard deviation

the splenectomized thalassaemic patients, there was a significant incidence of thrombocytosis in comparison with the control group (Table 2). Increased platelet counts in these patients were due to hypercellular marrow and splenectomy. These results agree with Eldor et al. [15] and Visudhipan et al. [12].

Platelet aggregation is the best single screening test for platelet function [16]. We studied *in vitro* platelet aggregation using an aggregometer, which measures the transmission of light through platelet-rich plasma. In this work, we selected the collagen challenge as an example of a strong agonist [17]. The platelet aggregation results showed insignificant elevation in the splenectomized group compared with the control group, while there was a significant decrease of platelet aggregation in the non-

splenectomized group compared with the control group (Table 3). These results accord with those of Eldor et al. [15] and Visudhipan et al. [12].

There are two physiological explanations for defective platelet aggregation in nonsplenectomized thalassaemic patients. First, it can be explained by the release of adenosine diphosphate (ADP) from the haemolysed RBCs, leading to defective platelet aggregation; and secondly, by the presence of two platelet populations in the circulation of the patients. The more active platelets present as circulating aggregates and are not detected by *in vitro* study, and the less active platelets, detected by *in vitro* study, are poorly aggregable [11].

Studies on platelet aggregation *in vivo* have shown results contrary to studies on *in vitro* aggregation. Opartkiattikul et al. [5]

Table 3 Platelet aggregation carried out on platelet concentrations using collagen reagent in nonthalassaemic and thalassaemic (splenectomized and nonsplenectomized) children

Group	Platelet aggregation (%)
<i>Controls</i>	
Range	56.80-100.00
Mean	65.34
± s	10.13
<i>Splenectomized</i>	
Range	20.00-110.00
Mean	72.37
± s	14.40
<i>Nonsplenectomized</i>	
Range	28.60-57.30
Mean	45.77
± s	7.46
F	31.18
P-value	< 0.001
Scheffe post-hoc test	(I and II) > III

s = standard deviation

and Ayad [11] reported an increase in circulating platelet aggregates and spontaneous platelet aggregation in all thalassaemic patients, whether splenectomized or not. Increased platelet aggregation in splenectomized patients can be attributed to young and more active platelets, as well as to hypercellular bone marrow [18]. Eldor et al. observed a significant increase in the platelet metabolites (thromboxane A₂ and 11 dehydrothromboxane B₂) in thalassaemia major, which can lead to increased platelet aggregation [15].

Increased *in vivo* platelet aggregation in splenectomized patients results from the change in lipid status (dyslipidaemia) found in thalassaemic patients due to liver changes [19]. Abnormal erythrocytes and leukocytes found in thalassaemic patients,

Table 4 Antithrombin III and protein C concentrations in nonthalassaemic and thalassaemic (splenectomized and nonsplenectomized) children

Group	Antithrombin III (%)	Protein C (%)
<i>Controls</i>		
Range	85.00-100.00	77.00-200.00
Mean	94.45	88.10
± s	4.59	16.97
<i>Splenectomized</i>		
Range	52.00-76.00	20.00-67.00
Mean	62.35	53.25
± s	9.10	14.13
<i>Nonsplenectomized</i>		
Range	55.00-96.00	33.00-62.00
Mean	93.10	46.05
± s	17.26	10.63
P-value	< 0.001	< 0.001
Scheffe post-hoc test	(I and III) > II	(II and III) < I

s = standard deviation

especially after splenectomy, form such factors as thrombin-activating platelets [5].

In addition, platelets of thalassaemic patients rapidly form pseudopodes after their activation and are less able to resume their original shape compared with platelets of normal subjects [18]. The procoagulant surface of thalassaemic RBCs may accelerate thrombin generation *in vivo*, which in turn triggers platelet activation [20].

The difference between *in vivo* and *in vitro* platelet aggregation in thalassaemic patients is explained by the effect of centrifugation on platelets, which eliminates the more active platelets [5], and by the absence of the platelet-activating role of RBCs, which secrete ADP and thrombin due to rapid haemolysis in thalassaemic children [21].

We found a significant decrease in levels of protein C and AT III activity for all patients compared with controls (Table 4), with no significant difference between the splenectomized and nonsplenectomized groups ($P > 0.05$). Decreased levels of protein C and AT III in thalassaemic patients can be explained by vitamin K deficiency, liver dysfunction due to haemosiderosis, and the increased turnover rate of protein C [22]. However, Shirahata et al. found that liver damage was not the only cause of the reduction in these anticoagulant proteins [4]. One alternative explanation for the significant reduction in protein C may be that this type of protein binds to phosphatidylserine, or other negatively charged phospholipids, abnormally present in the external membrane of the thalassaemia erythrocytes [23].

These results agree with Shirahata et al. [4] and Eldor et al. [15]. Their studies found a significant decrease in protein C, protein S and AT III levels in thalassaemic patients, whether splenectomized or not. This decrease was reported to be the cause of thromboembolic manifestations in thalassaemia by Leonardi et al. [24] and Eldor et al. [15].

Visudhiphan et al [12] found that AT III activity was high in both splenectomized

and nonsplenectomized thalassaemic patients, while Leonardi et al. [24] found protein C to be slightly increased in splenectomized and decreased in nonsplenectomized patients. The inconsistency between the different studies can be explained by the presence of different degrees of liver damage resulting in lower levels of protein C and AT III [24], as indicated by higher serum transaminases shown in our study.

We conclude that decreased platelet aggregation and prothrombin activity in nonsplenectomized thalassaemic children may contribute to haemorrhagic tendency. Splenectomy in thalassaemic children will be accompanied by thrombocytosis, increased platelet aggregation, and a decrease in natural coagulation inhibitors. These factors, in addition to others such as hypercoagulability, may lead to thrombotic complication.

We recommend administration of small doses of salicylates (aspirin) to decrease platelet aggregation in splenectomized thalassaemic children (especially in instances of hypercoagulability), in addition to full iron chelation to decrease hepatic haemosiderosis. We also recommend administration of vitamin K in cases of severe hepatic dysfunction.

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