

Platelet function in 30 cases of β -Thalassemia Hemoglobin-E

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Abnormal platelet aggregation was found in 21 of 30 patients (70%) and poor platelet adhesiveness was found in 18 of 30 patients with β -Thalassemia Hemoglobin-E. The aggregation defect did not correlate with the liver function tests, splenectomy, degree of iron overload, hematocrit and platelet count. Only 10 patients had prolonged bleeding time and 6 had tourniquet test positive, while only 8 of these suffered from clinically significant hemorrhage.

The results show that a proportion of patients with β -Thalassemia Hb-E have abnormal platelet function. It is possible however, that the in vitro abnormalities might partially be due to artifacts induced by manipulations in removing the abnormal thalassemic red cells, and this may explain the much lower incidence of significant hemorrhage. By and large in this study, 10 out of 21 cases had abnormal platelet aggregation significantly occurring in vivo as demonstrated by prolonged bleeding time.

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จากการศึกษาหน้าที่ของเกร็ดเลือดในคนไข้ β -Thalassemia Hemoglobin-E 30 ราย พบว่า 21 ราย (70%) มีหน้าที่ของเกร็ดเลือดทางด้านการจับกลุ่มน้อยกว่าปกติ และ 18 ราย (60%) เกร็ดเลือดมีหน้าที่ผิดปกติเกี่ยวกับ Adhesiveness ซึ่งความผิดปกตินี้ไม่มีความเกี่ยวข้องกันเลยกับหน้าที่ของตับ หรือจำนวนเกล็ดที่มีคั่งอยู่ หรือจำนวนฮีมาโตคริตหรือแม้แต่ม้ามที่ยังมีอยู่หรือไม่ก็ตาม

ทั้ง 21 ราย ที่มีความผิดปกติของหน้าที่ของเกร็ดเลือดนี้ มีเพียง 10 รายเท่านั้น มี Bleeding time ยาวนานกว่าปกติ และ 8 ราย ให้นอบวของ Tourniquet test และพบว่ามีคนไข้ 8 ราย ที่มีเลือดออกขณะทำการศึกษา

A mild bleeding tendency as characterized by bruising easily, frequent epistaxis and bleeding per gum has been described in a significant minority of our patients with β -thalassemia Hb-E disease. There did not, however, appear to be a close correlation between the severity of the clinical hemorrhagic tendency and the degree of deficiency of plasma coagulation factors which arose in these disorders, due largely to a paren-

chymal liver disease.

Hilgartner et al⁽¹⁾(1963) and Hilgarther and Smith⁽²⁾(1964) found that patients with β -thalassemia major had mild bleeding tendency and Eldor (1987)⁽³⁾ found that 14 of 15 patients with thalassemia major and 4 of 5 with thalassemia minor had in vitro defects of platelet function. The extent of which was directly related to the severity of hemorrhagic symptoms.

Table 1. Hematologic data in 30 beta-thalassemia hemoglobin-E.

Case.	Sex.	Age.	Hct. %	Platelet count $10^9/L$		Hemoglobin %		Total unit. of bl. Tx.	Serum ferritin 10-68ng/ml	Spleen
				in blood	in PRP	E	F			
1	F	25	19	155	230	80.16	6.42	7	160	intact
2	F	21	26	805	915	65.61	10.46	130	1845	out
3	F	32	27	250	310	55.72	21.95	120	1426	"
4	F	42	23	177	385	62.25	11.93	150	1772	"
5	F	33	19	430	275	65.89	15.98	160	1150	out
6	F	31	20	645	680	69.50	21.96	110	2050	"
7	F	20	24	430	720	48.70	24.59	140	1866	"
8	F	22	26	720	510	43.16	10.70	140	2320	"
9	M	18	12	152	384	82.23	5.02	150	4415	intact
10	F	18	24	325	380	70.73	16.52	120	2285	"
11	M	17	16	229	504	37.69	33.85	140	3775	"
12	M	20	24	155	290	38.91	23.03	100	3750	"
13	F	23	28	653	685	59.56	16.44	30	363	out
14	F	24	25	456	760	64.02	23.06	100	1886	"
15	M	16	26	1130	1250	48.26	23.63	100	492	"
16	F	34	18	890	386	44.15	21.69	16	351	"
17	F	42	23	1040	968	37.7	40.07	60	514	"
18	M	18	20	580	660	82.33	12.31	120	1950	"
19	F	38	23	408	740	59.16	6.40	80	1275	"
20	F	22	27	546	335	64.80	10.46	100	1105	"
21	M	38	26	330	410	84.29	7.06	80	1327	intact
22	F	16	23	152	240	73.14	10.83	100	1516	"
23	M	24	26	826	923	58.90	20.01	40	1130	out
24	F	16	28	256	470	51.41	24.32	110	1960	intact
25	F	19	24	164	320	70.46	6.24	24	940	"
26	F	21	20	201	314	64.50	11.65	60	1064	"
27	F	24	26	645	789	66.72	5.66	120	1845	out
28	F	31	25	210	342	74.16	7.45	20	745	intact
29	F	19	20	160	316	72.4	11.41	40	986	"
30	M	20	26	152	317	56.90	9.25	16	310	"
Mean value			22.6	442.06	523.93	58.31	25.29	91.16	1614.3	-

The purpose of the present study was to explore the frequency and cause of platelet qualitative defects in Thai β -thalassemia Hb-E and to determine whether these in vitro defects have any relation to other variables such as clinical bleeding and prolongation of the bleeding time.

Patients and methods

Thirty cases (22 females, 8 males, 15 splenectomized, age 16-42 years) of β -thalassemia Hb-E were included in this study (Table 1). The total amount of blood transfusion received ranged from 7 to 150 units. (mean 91.16 units) and the value of serum ferritin determined fell within the range of 160 to 4415 ngm/ml. (mean 1614.3 ngm/ml). Among these, 8 patients had active bleeding at the time of study, 12 had previous history of bleeding and 10 had no episode of bleeding at all. Their clinical bleeding included epistaxis, bleeding per gum, tongue and acne. All cases denied recent ingestion of any drugs known to influence platelet function for at least 2 weeks prior to the laboratory assessment.

Platelet function tests

Blood was collected in a polystyrene tube containing 1:9 volume of 3.2% sodium citrate, and centrifuged at 1000 rpm for 10 minutes at room temperature to obtain platelet-rich plasma (PRP). However in some patients, this procedure was unable to remove the contaminated red cells, so an aliquot of supernatant PRP was collected and the remaining portion was recentrifuged as described above. An aliquot of the PRP, now almost free of red cells was obtained, and the remaining, blood was recentrifuged at 3000 rpm for 10 minutes at 4° C to yield platelet-poor plasma. Platelet count was carried out on both samples. Platelet aggregation was measured within 2 hours of the blood sampling time by the method of Brecher and Born. (4, 5) The PRP (0.45 ml) was incubated at 37°C in a dual-channel independent aggregometer elvi 840, with 0.05 ml of aggregation agent. The stirring speed was 1000 rpm. The aggregation agents used at their final concentration were adenosine diphosphate (ADP) 2 μ M, adrenaline 2 μ M, ristocetin 1.5 mg/ml, thrombin 0.2 unit/ml and collagen (bovine, Achilles tendon) suspension in buffered saline (pH 7.35) of a concentration approximately 1 mg/ml. With all these reagents, platelet aggregation

was precisely quantitated by measuring the descendant percentage in the optical density of the PRP at 3 minutes after the addition of the aggregating agents.

Results

The clinical presentations and hematological assessment data are shown in the table 1. There were 22 females and 8 males, in the age range between 16-42 years (mean = 26.04). Their hematocrit values fell within 12-26% (mean = 22.6%), all of them having received blood transfusion at the maximum volume of 7-150 units (mean 91.16 units). The quantitative hemoglobin F range were between 5.02-24.32% (mean = 25.29%) and hemoglobin E findings from 44.15 to 84.29 % (mean = 58.31%). One half of cases had splenectomy. Of these patients, 8 had active bleeding at the time of study while 12 had only previous history of recurrent attack; the clinical features included 8 epistaxis, 6 bleeding per gum, 4 bleeding per nose and gum and 2 bleeding from injury.

The coagulation studies showed a mild prolongation of prothrombin time and activated partial thromboplastin time as demonstrated in only 3 cases. All patients presented with jaundice, had total bilirubin of 1.6-9.3 mg% (mean = 3.09); 13 cases had high SGOT level of 46-176 sigma U/ml (mean = 91.76); 12 cases had elevated SGPT 41-127 sigma U/ml (mean = 60.25) and 9 cases had rising serum alkaline phosphatase between 38.5-80 IU (mean = 53.5). There was neither correlation between liver function abnormalities and clinical bleeding nor with abnormal platelet aggregation tests.

As shown in table 2, ten patients showed prolonged bleeding time (case 1-10) and 6 (case 1-6) had also positive tourniquet test. Those with prolonged bleeding time showed reduced platelet aggregation when applied to 2 or more of the aggregation agents. The defect was maximum when ADP, adrenaline and collagen were used, however, less consistent results were obtained with thrombin and ristocetin. Every case of platelet aggregation defect was characterized by the absence of, or reduction in the secondary curve aggregation and 2 additional patients (case 9 and 12) had reduced aggregation reaction with ristocetin.

Finally the aggregation defect was not correlated with the degree of hepatic function tests, splenectomy, units of blood transfusion, serum ferritin, hematocrit or platelet count.

Table 2. Platelet function tests in 30 patients.

Case	Bleeding time (min.)	Tourniquet test	Platelet adhesiveness (40 - 60%)	POOR (Decreased) Platelet aggregation test*				
				Adr.	ADP.	Thr.	Col.	Ris
1	23	+	12	+	+	+	+	-
2	20	+	8	+	+	+	+	-
3	16	+	12	+	+	+	+	-
4	24	+	8	+	+	+	+	-
5	16	+	16	+	+	+	+	-
6	20	+	10	+	+	+	+	-
7	18	+	8	+	+	+	+	-
8	15	-	12	+	+	+	+	-
9	22	-	10	+	+	+	+	+
10	16	-	15	+	+	+	+	-
11	5	-	10	+	+	+	+	-
12	4	-	9	+	+	+	+	+
13	3	-	72	-	-	-	-	-
14	6	-	50	+	+	-	+	-
15	5	-	48	-	-	-	-	-
16	3	-	60	-	-	-	-	-
17	4	-	68	-	-	-	-	-
18	5	-	9	+	+	-	+	+
19	6	-	15	+	+	-	-	-
20	7	-	11	+	+	-	-	-
21	3	-	12	+	-	-	-	-
22	4	-	14	+	-	-	-	-
23	3	-	56	-	-	-	-	-
24	6	-	64	+	+	-	+	-
25	5	-	60	-	-	-	-	-
26	4	-	15	+	-	-	-	-
27	7	-	50	+	+	-	+	-
28	3	-	48	-	-	-	-	-
29	5	-	46	-	-	-	-	-
30	5	-	52	-	-	-	-	-

*Adr. = adrenaline, ADP = Adenosine diphosphate, Thr. = Thrombin, Col. = Collagen, Ris. = Ristocetin

Discussion

The etiology of platelet dysfunction appears to be quite variable. The association of scurvy and functional platelet defect is well established.⁽⁶⁾ Although iron overload and reduced levels of leukocyte ascorbate were frequently observed in the majority of the β -thalassemia HbE patients, there were no correlation of these findings with the functional platelet defect.^(7, 8)

Vitamin E deficiency has also been well documented in patients with β -thalassemia major,⁽⁹⁾ although little is known about the role of vitamin E in throm-

bopoiesis or platelet function. However, high platelet counts have been reported in vitamin B deficiency.^(10, 11) Interestingly Khurshid et al⁽¹²⁾ reported impaired platelet function with ristocetin in an infant with vitamin E deficiency in whom the platelet function returned to normal after vitamin E treatment. In 1979 Hussain et al⁽¹³⁾ studied 18 cases of β -thalassemia major, all of which were deficient in vitamin E; nevertheless their severity did not correlate with the platelet aggregation results, although they may have contributed to the thrombocytosis observed in some of their patients.

Because of the reduced size and volume of the red cells mass in β -thalassemia hemoglobin E it is rather difficult to prepare PRP sufficiently free of red cells in order to satisfy the platelet aggregation test. In many instances, prolonged centrifugation resulted in lower platelet count in platelet-rich-plasma. It is possible that prolonged centrifugation selectively removes the young metabolically active platelets which, by morphology are larger and denser than older ones. The latter might be expected to show somewhat reduced ability to aggregate, particularly to ADP, adrenaline and collagen.

It is possible that the high incidence of abnormal platelet aggregation in thalassemic patients in previous reports, (44% for Hussain and 93% for Eldor's group) may have been due partly to artifacts induced by in vitro manipulations. This suggestion is to some extent supported by the low incidence of clinically significant hemorrhagic symptoms in such patients. Nevertheless, excessive bleeding can occur in association with platelet disorders (Lusher and Barnhart 1977).

References

- Hilgartner MW, Erlandson ME, Smith CH. The coagulation mechanism in patients with thalassemaia major. *J Pediatr* 1963 Jul; 63(1) : 36-45
- Hilgartner MW, Smith CH. Coagulation studies as a measure of liver function in Cooley's anemia. *Ann NY Acad Sci* 1964; 119 : 631-640
- Elder A. Abnormal platelet functions in beta-thal assemia. *Scand J Hematol* 1978; 20 : 447-452
- Bracher GA, Cronkite EP. Morphology and enumeration of human platelets. *J Appl Physiol* 1950; 3 : 365-369
- Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962 Jun; 194 (4832) : 927-929
- Wilson PA, McNicol GP, Douglas AS. Platelet abnormality in human scurvy. *Lancet* 1967 May; 1 (7497: 975-978)
- Lynch SR, Seftel HC, Torrance JD, Charlton RW, Bothwell TH. Accelerated oxidativ catabolism of ascorbic acid in siderotic Bantu. *Am J Clin Nutr* 1967 Jun; 20 (6) : 641-647
- Wapnick AA, Bothwell TH, Seftel H. The relationship between serum iron levels and ascorbic acid stores in siderotic Bantu. *Br J Hemato* 1970 Aug; 19(2) : 271-276
- Hyman CB, Landing B, Alfin-Slater R, et al. D1-alphatocojpheraliron and lipofuscin in thalassemia. *Ann NY Acad Sci* 1974; 232 : 211-220
- Ritchie JH, Fish MB, McMasters V, Grossman M. Edema and hemolytic anemia in premature infants: a vitamin E deficinecy syndrome. *N Engl J Med* 1968 Nov 28; 279 (22) : 1185-1190
- Keeton BR. Vitamin E and thrombocytosis in Collye's disease. *Arch Dis Child* 1976 May; 51 (5) : 393-395
- Khurshid M, Lee TJ, Peake IR, Bloom AL. Vitamin E deficiency and platelet functional defect in a jaundiced infant. *Br Med J* 1975 Oct 4; 4(5987) : 19-21
- Hussain MA, Hutton RA, Pavlidou O, Hoffbrand AV. Platelet function in beta-thalassemia major. *J Clin Pathol* 1979 May; 32(5) : 429-433