FUNCTIONAL PLATHLET DEFECTS: A CLINICOPATHOLOGICAL STUDY OF 10 CASES

Pages with reference to book, From 223 To 228

Nusrat Zaffar, Muhammad Saleem, S. Azhar Ahmed (Armed Forces Institute of Pathology, Rawalpindi.)

Abstract

Five cases of Bernard Soulier Syndrome (BSS) and 5 cases of thrombasthenia were diagnosed at the Armed Forces Institute of Pathology, Rawalpindi during the year 1986. All cases presented with life-long bleeding diathesis. Investigations in all the 10 cases revealed prolonged bleeding time and normal or below normal platelet counts with a normal coagulation profile. Normal clot retraction (CR) with defective prothrombin consumption index (PCI) was seen in 5 cases of Bernard Soulier syndrome whereas the former was below normal/lowest limit of normal with normal PCI in thrombasthenia cases. The diagnosis was finally confirmed by platelet aggregation studies using ADP, adrenaline, collagen and ristocetin in varying dilutions. In thrombasthenia, platelet count and morphology was normal and there was no aggregation in response to any concentration of ADP, Collagen or adrenaline whereas aggregation was normal in response to ristocetin. In BSS, platelet count was below normal with giant platelets in peripheral blood film. There was no aggregation with any concentration of ristocetin with mild response to other reagents like ADP, adrenaline and collagen in these cases. The bleeding episodes in some of these patients were successfully managed by transfusion of platelet concentrates obtained from normal donors(JPMA 37: 223, 1987).

INTRODUCTION

Hereditary functional platelet disorders are a rare group of platelet defects which are characterized by a life-long bleeding diathesis with normal or low platelet counts[1]. The abnormality in platelet functions is produced due to one of the following:

1. Membrane defects

The structure of the platelet surface mem. brane is defective in this group, the usual finding being the absence of one or more of the membrane glycoproteins. The examples in this group are:-

* Thrombasthenia
* Bernard Soulier syndrome
* Pseudo von-willebrand's disease
* Psue
* Primary piocoagulant defect

2. Deficiency of Storage organelles

a) Dense body deficiency-Wiskott-Aldrich syndrome
Hermansky-Pudlak syndrome

b) Alpha granule deficiency

* Grey platelet syndrome

3. Defects of thromboxane synthesis

4. Defects of response to thromboxane A_2

5. Miscellaneous

* Montreal platelet syndrome
* Epstein's syndrome
* May Heggliut anomaly

The two commonest defects are Thrombasthenia and Bernard soulier syndrome. Thrombasthenia, an autosomal recessive disorder, is characterized by absence of surface membrane glycoproteins II and lila causing complete failure of such platelets to aggregate with ADP, adrenaline and collagen.
In Bernard soulier syndrome, again a disorder of autosomal recessive inheritance, there is lack of lb and Is glycoproteins. These proteins contain large amounts of sialic acid and have been identified as the absent membrane markers in such cases causing no aggregation with ristocetin but normal aggregation with other reagents.
These defects clinically produce a tendency to bleed either spontaneously or after minor trauma. Even when the platelet count is low in BSS patients, the severity of bleeding is out of proportion to the reduction in platelet count. The bleeding episodes in these patients can be corrected only by administration of normal platelets in the form of platelet concentrates. There is no curative treatment except for allogenic bone marrow transplant which may produce cure in some recipients.

MATERIAL AND METHODS

All the patients were referred to AFIP Rawalpindi for coagulation studies from Mifitary Hospital Rawalpindi, Combined Military Hospital Rawalpindi and from civilian hospitals of Rawalpindi and surrounding areas. Complete history was taken and thorough physical examination was carried out in all cases.

METHODS

Bleeding time was done by Ivy's method.
Platelet count was done visually in neubauer chamber. Platelet morphology was seen using a light microscope. Coagulation profile including elotting time, Hess test. prothrombin time, partial thromboplastin time with Kaoline, thrombin time, coagulation factor Assay. Prothrombin consumption index and clot retraction were performed using standard mefhods.[2]
Bone marrow aspirations were done with standard needles and the smears were stained with Leishman stain.

REAGENTS

ADP, adrenaline, collagen and ristocetin were obtained from SIGMA. The working dilutions of ADP used were 10.0, 5.0, 2.5. 1.0/ pmol/l, collagen 4.0 and 2.0 adrenaline 20 and 2 pmo!/l and ristocetin 1.5, 1.2, 1.0mg/mi.

PLATELETS PREPARATION

Nine parts of venous blood were taken in plastic tubes from the patients and normal control into one part 3.8% trisodium citrate for the preparation of platelet rich plasma (PRP) and platelet poor plasma (PPP). All cases were not taking any medicines for at least 02 weeks prior to these tests. PRP was prepared by centrifuging whole citrated blood at 140g for 10 min and PPP by centrifuging at 1300 g for a further 10 min.

PLATELET AGGREGATION

Aggregation was performed at 37°C in a chrono-log dual aggregometer manufactured by Coulter electronics and coupled to an Omnis. cribe recorder.

RESULTS

All cases of BSS were from Rawalpindi. Out of 5 cases of thrombasthenia two belonged to Rawalpindi and other three were from Distt. Chakwal, Gujrat and Faisalabad each. S cases (Case 1-V), 3 males and 2 females, whose ages ranged from 04-8Yz years (Median 05 years) showed markedly prolonged bleeding time (>15 minutes) with a normal platelet count (Range 188 - 425 x l0~/l -Median 375 x l09/l) and normal morphology (Table-I).

TABLE – I
Coagulation Profile in Thrombasthenia.

	CASE I	CASE II	CASE III	CASE IV	CASE V
Age (Yrs)	06	05	04	04	8½
Sex	M	F	F	M	M
Platelet Count ($N = 150–400x10^9/1$)	375	425	235	375	188
Platelet Morphology	Normal	Normal	Normal	Normal	Normal
Hess Test	–	+	+	–	–
Bleeding Time (N= 2–11 min)	>15	>15	>15	>15	>15
Clotting Time (N = 4 –11 min)	5'–0	4'–20	4'–30	5'–15	4'25
Prothrombin Time N= (12–14 Sec)	14	14	14	14	15
P T T K N= (32–36 Sec)	36	34	34	36	38
Thrombin Time (Normal 9–10 Sec)	09	10	10	9	9
Factor VIII Assay % (N = upto 150%)	90	105	88	95	110
PCI (%) N = (0–30)	25	20	30	20	28
Clot Retraction % (N = 48 –64%)	30	35	48	40	48

These cases, diagnosed as thrombasthenia, had normal PCI (Range 20-30%). CR in 3 cases was abnormal (Range 39-40%) and at the lower limit of normal in 2 cases (48%). Clotting time (CT), Prothrombin time (PT), Partial thromboplastin time with Kaoline (PTTK), Thrombin time (TT) and Factor VIII assay were normal. Hess test was positive in 2 cases (Table I). Platelet aggregation studies In all the 05 cases showed aggregation with varying dilutions of ADP. Adrenaline and Collagen and normal aggregation‘with ristocetin (Table III, Figure 2).

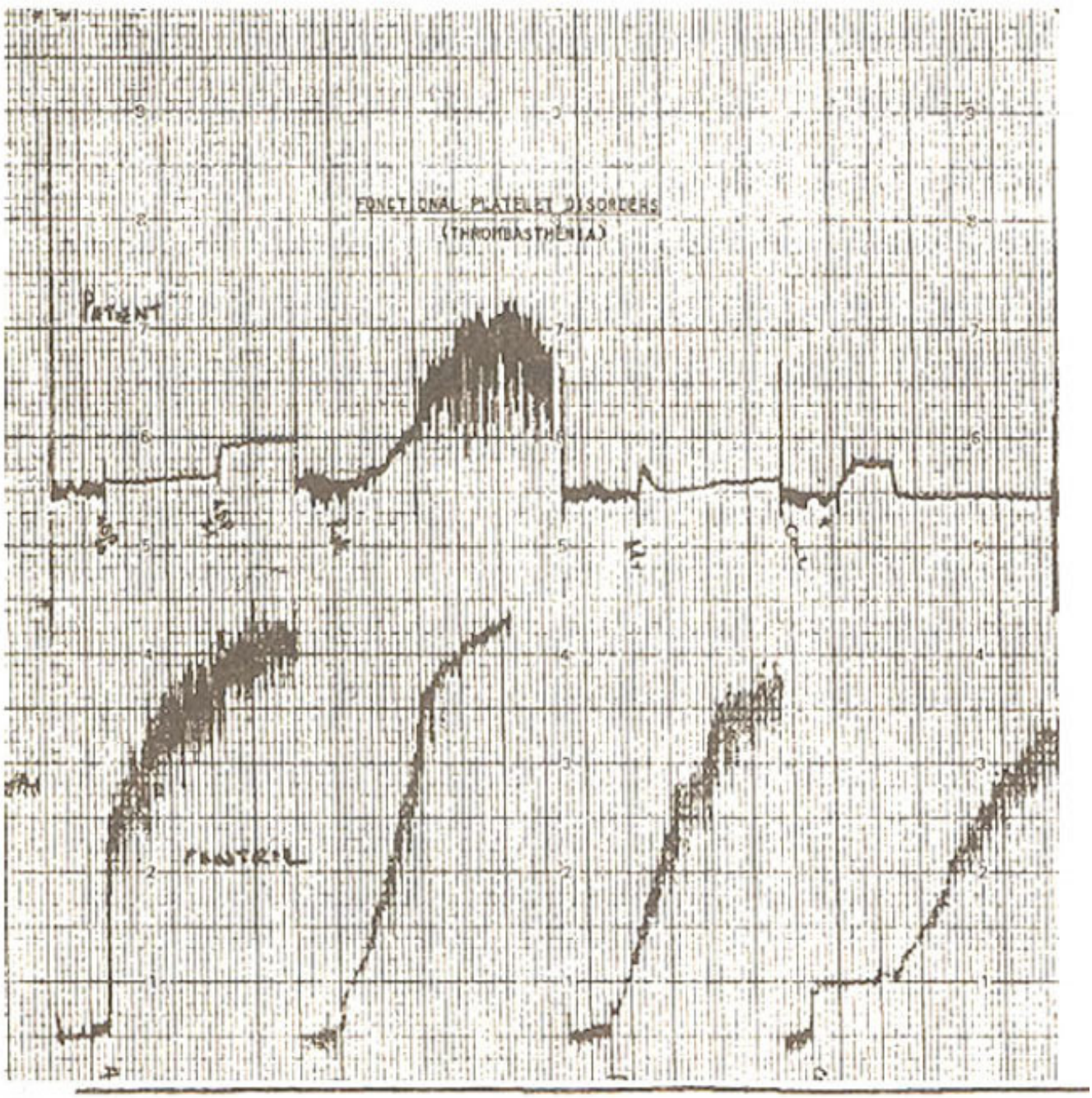

Figure 2. Functional Platelet disorders (Thrombasthenia).

Consan guinity was present in ali these cases. The parents of 03 cases were first cousins and in other 2 cases were among near relatives.

Five cases (case VI to X), one male and 4 females, whose ages ranged from 03-12 years (Median 4 years) bad low platelet counts (Range) 35-75 x 10 9/i, Median 60 x 10 9/l) with markedly prolonged bleeding time (>15 minutes) and giant platelets in the peripheral blood film (Table II).

TABLE –II
Coagulation Profile in Bernard Soulier Cases.

	CASE VI	CASE VII	CASE VIII	CASE IX	CASE X
Age (yrs)	12	08	03	04	04
Sex	F	F	M	F	F
Platelet Count (N=150–400x10^9/1)	60	35	75	36	60
Platelet Morphology	Giant Platelets	Giant Platelets	Giant Platelets	Giant Platelets	Giant Platelets
Hess Test	–	+	–	+	–
Bleeding Time (N=2–11 min)	>15	>15	K15	>15	>15
Clotting Time N=(4–11 min)	5.0	4'–15	4'–20	3'–56	4'–50
Prothrombin Time (N=12–14 Sec)	15	14	15	16	14
P T T K (N=32–36 Sec)	36	38	36	36	36
Thrombin Time (N=9 –10 Sec)	09	09	10	09	10
P C I (%) (N = 0–30%)	58	56	50	56	56
Clot Retraction (N = 48 – 46%)	50	50	60	50	50

CT, PT, PTTK, TT and Factor VIII levels were normal. Hess Test was positive in 2 cases only. PCI in all these cases was abnormal (50-58%) with normal CR (50-60%) (Table -II). Platelet aggregation studies showed no aggregation with ristocetin and mild aggregation with ADP, Adrenaline and collagen (Table III, Figure 3).

TABLE –III
Aggregation Studies in Functional Platelet Defects.

Case No.	AGGREGATION REAGENTS										
	ADP (μmol/l)			Adrenaline (μmol/l)		Collagen (μg/ml)		Ristocetin Diagnosis (mg/ml)			
	1.0	2.5	5.0	2.0	20	2	4	1.0	1.2	1.5	
I	–	–	–	–	–	–	–	++	++	++	Thrombasthenia
II	–	–	–	–	–	–	–	++	++	++	"
III	–	–	–	–	–	–	–	++	++	++	"
IV	–	–	–	–	–	–	–	++	++	++	"
V	–	–	–	–	–	–	–	++	++	++	"
VI	+	+	+	+	+	+	+	–	–	–	Bernard Soulier Syndrome
VII	+	+	+	+	+	+	+	–	–	–	"
VIII	+	+	+	+	+	+	+	–	–	–	"
IX	+	+	+	+	+	+	+	–	–	–	"
X											

– No aggregation
\+ 10–30 % aggregation
++ >30% aggregation

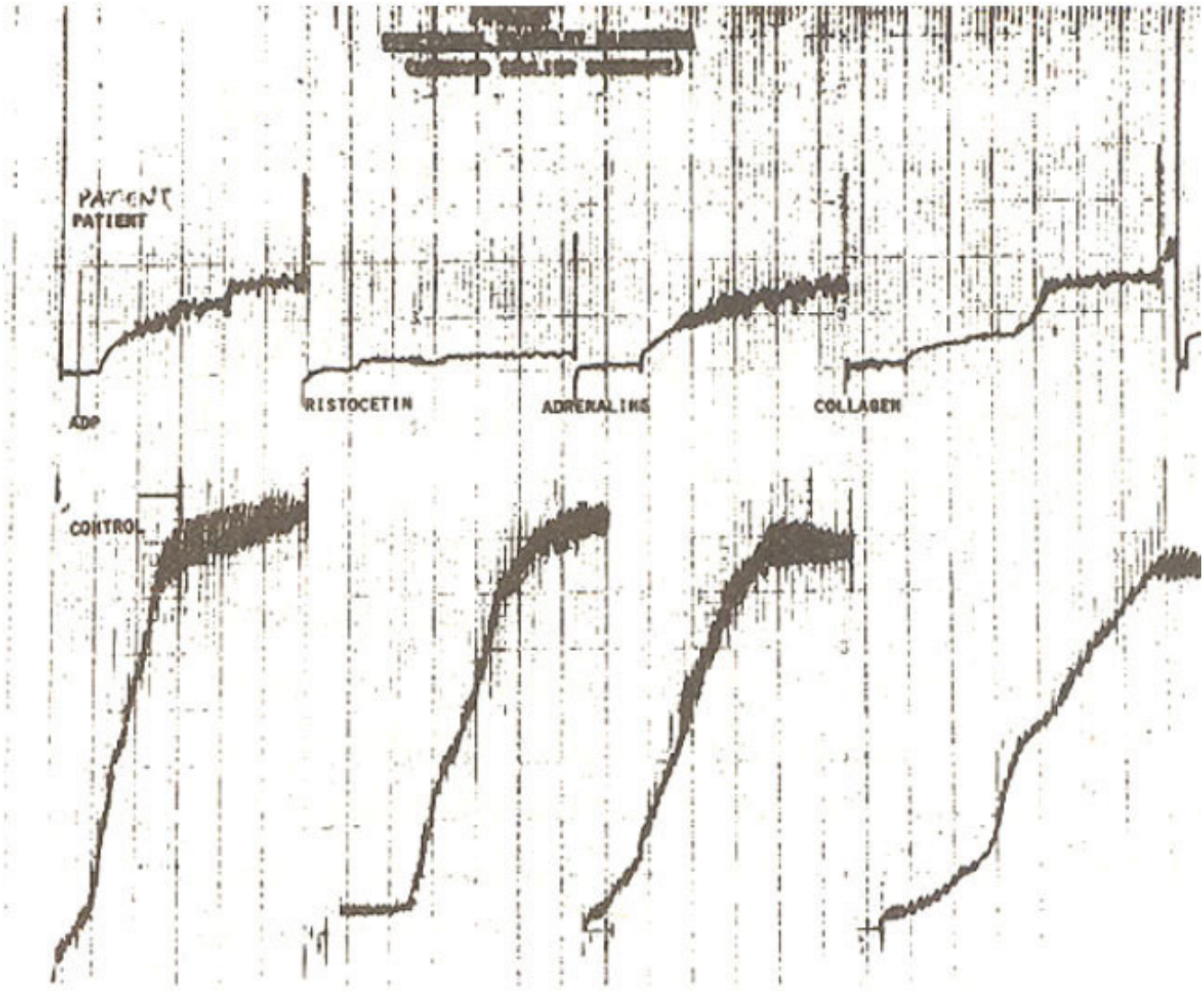

Figure 3. Functional platelet disorders (Bernard soulier syndrome.

These cases were labelled as BSS. 04 cases of BSS belonged to one family (Figure 1).

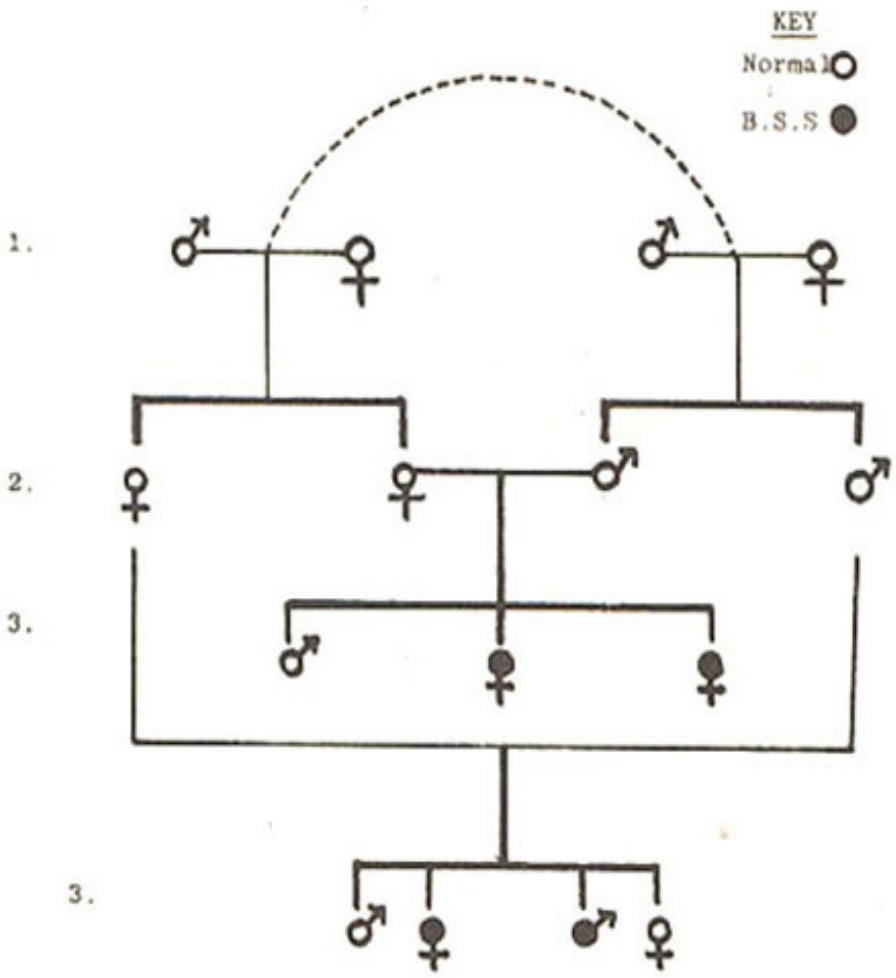

Figure 1. Family Tree of Bernard soulier syndrome case No.VI to IX.

The fifth case (Case X) did not show consanguinity. Bone mar-rows of all the 10 patients showed increased or normal megakaryocytes.

DISCUSSION

Qualitative platelet defects, in contrast to the quantitative defects, are a rare group of disorders which are being diagnosed more frequently since platelet aggregometers have become available. The present series describe 10 cases seen during a short period of one year which indicates that the disorders may be more prevalent in our country than expected. The clinical and haematological picture of these cases does not differ from the cases reported in literature.

The pattern of autosomal recessive inheritance and cànsanguinity was found in these cases as has been reported in other studies[3]

In this study 02 cases of BSS were siblings and other 02 cases were their first cousins. Their parents and other brothers and sisters were normal in coagulation studies and aggregation patterns. The presence of giant platelets with normal or decreased platelet count and normal number and morphology of megakaryocytes in bone marrow, with bleeding out of proportion to platelet count, are other characteristics which were in accordance with the findings reported in literature.[4]

The characteristic aggregation patterns observed in thrombasthenia and Bernard soulier syndrome confirm the diagnosis of these defects. The pattern of abnormal aggregation with ristocetin is found both in cases of BSS and Von Wile-brand's disease but the addition of normal plasma or VW factor corrects the defective aggregation in VWD but not in BSS. This confirms the finding that both a plasma factor and normal platelet membrane (Glycoprotein lb-is) are required for nstbcetin to be effective.[4]
The PCI in BSS is typically abnormal. According to some workers such platelets, due to scanty content of lb and is glycoproteins, don't accelerate the formation of plasma fibrin and are thus unable to act with plasma factors V. VIII and XI although such platelets have a normal content of platelet factor[3].
In thrombasthemc cases, defective clot retraction and complete failure to aggregate with any. concentration of ADP, collagen, adrenaline and normal aggregation with ristocetin, as observed in our cases, is in accordance with other reported cases (Hardisty, 1983). This is in conformity with the observation that the platelets in thrombasthenia lack fibrinogen receptors which are normally carried on the membrane glycopro. teins, GPI1b and III. The absence of these proteins with their receptors prevents platelet aggregation by reagents like ADP which exposes these receptors in normal individuals.
In conclusion it can be stated that functional platelet defects are not as infrequent in Pakistan as has hitherto been thought. Consequently necessary investigations to exclude these defects must be carried out in all patients who have a typical history but give a normal coagulation profile and a prolonged bleeding time with normal or moderately reduced platelet count abnormal platelet morphology.

REFERENCES

1. Hardisty, R.M. Hereditary disorders of platelet function. Clin. Haematol., 1983; 12: 153.
2. Dacie, J.V. and Lewis, S.M. Practical haematology. 6th ed. Edinburgh, Churchill Livingstone, 1984.
3. Menezes, S. and Parashar, S.K. Bernard Soulier syndrome. J. Assoc. Physicians India, 1980; 28: 487.
4. Pizzuto, J., Turn, C., de la Paz Reyna, M., Ballestesos, L., Morales, M.R. and Gonzabz Angulo, A. Haemostatic defects in the Bernard Soulier syndrome. Presentation of one case and literature review. Arch. Invest. Med. (Mex), 1981;12: 193.