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BERNARD – SOULIER SYNDROME: A FLOW CYTOMETRIC ANALYSIS OF MEMBRANE GP – Ib EXPRESSION*

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SUMMARY: Yeşilipek MA, Karadoğan İ, Ündar L. (Departments of Pediatrics and Internal Medicine, Akdeniz University Faculty of Medicine, Antalya, Turkey). Bernard-Soulier syndrome: a flow cytometric analysis of membrane GP-Ib expression. Turk J Pediatr 1996; 38: 375-379.

A case of Bernard-Soulier syndrome in a five-year-old female is presented. The diagnosis was confirmed by flow cytometric analysis of glycoprotein Ib (CD 42b) in addition to the patient's classic laboratory findings such as prolonged bleeding time, mild thrombocytopenia, large platelets and failure of platelet aggregation with ristocetin. Her parents and sibling had normal coagulation tests and CD 42b levels. It is emphasized that flow cytometric analysis is useful in the confirmation of congenital platelet function defects. *Key words: platelet function disorders, Bernard-Soulier syndrome, flow cytometric analysis.*

Bernard-Soulier syndrome is a rare autosomal-recessive bleeding disorder characterized by mild thrombocytopenia, large platelets and prolonged bleeding time[1]. The underlying molecular defect is deficiency of membrane glycoprotein Ib/IX complex (CD42). This complex is the receptor for von Willebrand factor (vWF) and is necessary for normal platelet adhesion to the subendothelium[2].

We present a five-year-old girl diagnosed with Bernard-Soulier syndrome with classical laboratory abnormalities. Flow cytometric analysis of the patient's platelets showed a lack of membrane glycoprotein Ib (CD42b) expression.

Case Report

A five-year-old female was admitted to Akdeniz University's Pediatric Hematology Department with epistaxis. She had a history of a hematemesis attack two years previously, following recurrent epistaxis episodes. She had also suffered from oral mucosal bleeding and severe post-traumatic ecchymosis. There was second-degree consanguinity between her parents.

Her physical examination was normal except for a few ecchymoses on the lower extremities. Laboratory studies revealed a hemoglobin level of 11.3 g/dl, WBC

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6 x 10^9/L and platelets 65x 10^9/L. Large platelets were seen on the peripheral smear (Fig. 1). Prothrombin and partial thromboplastin times were normal. The bleeding time (Ivy method) was 13 minutes.

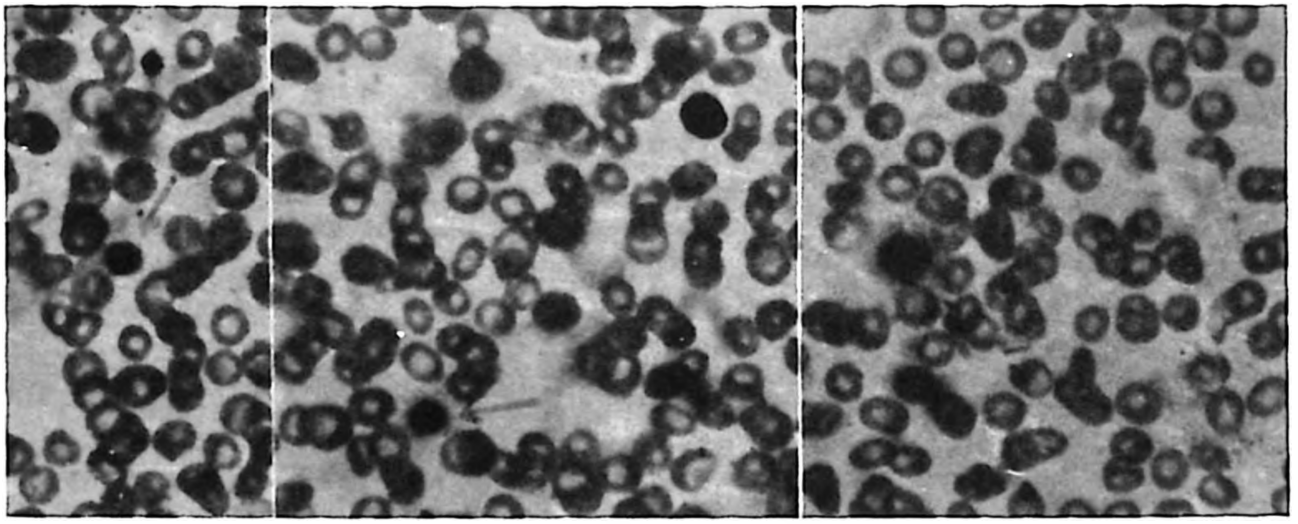

Fig. 1: Large platelets on peripheral smear of the patient.

With a presumptive diagnosis of Bernard-Soulier syndrome, platelet aggregation studies were performed. Because of the absence of ristocetin-induced platelet aggregation, a flow cytometric analysis for platelet membrane glycoprotein Ib expression was done.

Her epistaxis was controlled with local compression. She was discharged without any platelet transfusions, as she had no serious bleeding problems.

Material and Methods

Platelet Aggregation Studies: Adenosine diphosphate, collagen and ristocetin were used as aggregation-inducers (Chrono-Par reagents, Chrono-Log Corp., PA, USA). Platelet aggregation was studied using the whole-blood method (Chrono-Log Whole-Blood Aggro-Meter, model number 530-VS, Chrono-Log Corp., PA, USA).

Platelet Preparation for Flow Cytometric Analysis: Platelet-rich plasmas of the patient, her parents and sibling were prepared, and platelet counts were adjusted to 50 x 10^9/L with phosphate-buffered saline ethilene diamine tetra-acetate (PBS-EDTA).

Flow Cytometric Analysis: The samples were analyzed in a Coulter EPICS Profile II flow cytometer (Coulter Electronics Ltd, Florida, USA). The setting of a platelet gate was based on light scatter data (forward vs. side scatter).

Mouse monoclonal antibody to human platelet glycoprotein IIIa (CD61-FITC) (Serotec, UK) was used to confirm the identification of platelets. At least 100,000 events were acquired in this gate. A positive event, i.e., expression of glycoprotein

Ib (CD42b-FITC) (Immunotech S.A., France), was defined as one exhibiting a fluorescence intensity greater than the isotypic control. The negative marker was set and held at this level for each individual subject. The percentage of CD42b-positive platelets was defined as the percentage of gated events with a fluorescence intensity greater than the negative marker.

Results

ADP- and collagen-induced platelet aggregations were normal, while ristocetin-induced platelet aggregation was absent. This defect could not be corrected by the addition of normal plasma, indicating that the defect was in the platelets themselves. Her parents and sibling had normal ristocetin-induced platelet aggregation. Events within the acquisition gate were more than 95 percent for anti-glycoprotein IIIa binding for all members of the family, including the patient.

Her parents and sibling had normal CD42b expression (93%, 93.5% and 92.7%). In contrast, the patient's platelets showed lack of CD42b expression.

Discussion

Bernard-Soulier syndrome is a rare, autosomal bleeding disorder clinically characterized by prolonged bleeding time, normal clotting retraction and mild thrombocytopenia with large and morphologically abnormal platelets, and the absence of platelet membrane glycoprotein Ib/IX complex (CD42)[1]. Glycoprotein Ib is a major platelet receptor protein concerned with vWF binding, platelet aggregation, and platelet adhesion, and it is required for normal hemostasis. Therefore, deficiency of this vWF receptor on the surface of Bernard-Soulier platelets prevents the formation of an initial layer of adherent platelets on the subendothelial matrix, and leads directly to the hemorrhagic manifestations of the syndrome[3].

Platelets provide for primary hemostasis by forming a hemostatic plug at sites of vascular damage. Normal platelet function can be divided into four phases: adhesion, aggregation, secretion and expression of procoagulant activity. Platelet adhesion initiates plug formation as platelets adhere to the connective tissue at the edges of a wound within seconds after vascular damage. Platelet adhesion requires the presence of subendothelial von Willebrand factor and a specific platelet receptor, the glycoprotein Ib/IX complex. Following initial adhesion, platelets aggregate to complete the formation of a hemostatic plug[2].

The platelet count is usually mildly depressed in these patients[1,3]. We had consistently determined platelet counts as low as 65,000-80,000/mm^3, but fortunately the patient did not need a platelet transfusion. The peripheral blood film revealed the presence of giant platelet forms with mean diameters of 5 to 6 micrometers[1]. Our patient had some platelets with large diameters (Fig. 1).

The key to the diagnosis of Bernard-Soulier syndrome is detection of the failure of platelets to agglutinate in response to the antibiotic ristocetin. In contrast to von Willebrand disease, this abnormality cannot be corrected by the addition of normal plasma, indicating that the defect is in the platelets themselves. Aggregation of Bernard-Soulier platelets induced by agonists such as ADP, collagen, thrombin, and epinephrine is normal[1].

Confirmation of a suspected diagnosis of Bernard-Soulier syndrome may be accomplished by confirming that glycoprotein Ib is absent from the platelet membrane, by using immunocytochemical or immunofluorescence methods[4-7]. We used flow cytometric analysis to detect this defect and confirmed the absence of glycoprotein Ib (CD42b). Some authors reported that heterozygous parents and offspring of patients with Bernard-Soulier syndrome have approximately half of the normal levels of glycoprotein Ib/IX and normal platelet function[1,8]. However we found normal CD42b levels in our patient's parents and sibling, and they did not have any sign of platelet dysfunction. Glanzmann thrombasthenia is an another platelet function disorder with similar clinical findings. Congenital absence of glycoprotein IIb/IIIa leads to fibrinogen-mediated aggregation abnormalities in these patients. It may differ from Bernard-Soulier syndrome by demonstrating the absence of glycoprotein IIIb/IIIa, and a lack of platelets to aggregate with ADP or collagen[1]. In our patient, in addition to normal aggregation of platelets with ADP and collagen, normal platelet membrane expression of glycoprotein IIIa (CD61) ruled out Glanzmann thrombasthenia.

A patient with Bernard-Soulier syndrome may be misdiagnosed with chronic immune thrombocytopenia. This defect has generally been noticed when thrombocytopenia persists in spite of steroid therapy. Moreover, antibodies to glycoprotein Ib have been observed in a small number of patients with idiopathic thrombocytopenic purpura[1]. However, large platelets, such as those seen in Bernard-Soulier syndrome, have not been observed in these cases.

In conclusion, flow cytometric analysis is very useful for studying platelet-associated immunoglobulin and platelet activation. It has also been a promising tool in the diagnosis or confirmation of congenital platelet defects via the demonstration of glycoprotein Ib/IX and glycoprotein IIb/IIIa complexes.

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