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Glanzmann thrombasthenia and Bernard–Soulier syndrome in south Iran

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Summary Glanzmann thrombasthenia (GT) and Bernard–Soulier syndrome (BSS) are two rare inherited disorders of platelet function. In this study, we report the demographic, clinical and biological characteristics of 23 patients with GT and of seven patients with BSS from southern Iran who had been followed for many years but fully characterized only recently, when platelet aggregation tests and flow cytometric studies became available for the first time in the country. We found a high prevalence of both diseases that can be explained by the high rate of consanguineous marriages in south Iran. Patients affected by GT and BSS suffer mainly from mucocutaneous bleedings causing anemia and transfusion requirements.

Keywords Bernard-Soulier syndrome, Glanzmann thrombasthenia

Introduction

Patients suffering from inherited defects of platelet function are characterized by a lifelong clinical history of mucocutaneous bleeding, usually not very severe but nevertheless able to cause anemia and transfusion requirements. Among these disorders Glanzmann thrombasthenia (GT) (Bellucci & Caen, 2002) and the Bernard–Soulier syndrome (BSS) (Lopez *et al.*, 1998) are very rare, but particularly important for their severity and their biological characteristics. GT, an autosomal recessive disorder, is caused by the quantitative and/or qualitative deficiency of the platelet fibrinogen receptor, the glycoprotein (Gp)IIb/IIIa. In these patients, platelet aggregation is severely impaired and even relatively small challenges of hemostasis may cause important bleeding symptoms. Based upon the degree of GpIIb/IIIa expression GT is classified as type I (<5% of GpIIb/IIIa expressed), type II (GpIIb/IIIa expression levels between 5 and 20%) and type III (normal expression of a dysfunctional GpIIb/IIIa). BSS, characterized like GT by an autosomal recessive pattern of inheritance, is caused by the deficiency of one of the proteins of the GpIb/IX/V complex, usually GpIb. This complex is responsible for platelet adhesion to the proteins of the subendothelial matrix, in particular to von Willebrand factor, following an endothelial lesion. Accordingly in these patients platelet adhesion is grossly defective, while platelet aggregation is normal, with the exception of that induced by ristocetin. BSS is typically associated with macrothrombocytopenia. To make an accurate diagnosis of GT and BSS, platelet aggregation and flow cytometric studies are needed, so that when these facilities became available at our center, we chose to better characterize previously identified patients with a history of mucocutaneous bleeding associated with a prolonged bleeding time, a normal prothrombin time (PT) and activated partial thromboplastin time (APTT) and a negative laboratory screening for von Willebrand disease. We diagnosed 23 patients with GT from 17 different families and seven patients with BSS from four different families, indicating that both diseases are relatively frequent in the south of Iran, which has a population of approximately 5 million. We report herewith their clinical and laboratory data, focusing on the bleeding history and transfusion requirements.

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Materials and methods

Patients selection

In the last few years, 43 patients with a diagnosis of inherited disorders of platelet function had been identified and followed up at the Dastghieybe Hospital of Shiraz Medical University. The following tests were carried out in our patients: PT, APTT, complete blood count (CBC), morphological examination of a blood smear, skin bleeding time (BT), clot retraction, von Willebrand factor antigen and ristocetin cofactor activity assay. Three patients were excluded from further laboratory testing after the diagnoses of immune thrombocytopenic purpura (two) or of von Willebrand disease (one) were made. In the remaining 40 patients, platelet aggregation studies and flow cytometry were performed as detailed below.

Blood sampling and preparation

Blood samples were collected from patients and healthy individuals (taken as controls) who had no antiplatelet medication for at least 2 weeks. All individuals gave informed consent; the sampling from the children was carried out with the permission of the parents. To minimize platelet activation blood was obtained from an antecubital vein through a 19-gauge (G) needle for adults and a 22- to 23-G needle for children. The first 2 ml of blood were discarded; 9 ml of blood were collected into two separate tubes, one containing 0.129 M sodium citrate for preparation of platelet rich plasma (PRP) and another containing 7.5% K_3 EDTA for CBC and flow cytometry in whole blood. Citrated blood samples were centrifuged at 190 *g* for 15 min at room temperature to obtain PRP. Platelet poor plasma (PPP) was immediately prepared from the remaining citrated blood by centrifugation at 10 000 *g* for 10 min. PRPs from citrated blood samples that appeared macrothrombocytopenic on observation of the corresponding blood smears were prepared by means of 4 h sedimentation at room temperature of acid–citrate–dextrose anticoagulated blood.

Platelet aggregation and flow cytometric studies

For platelet aggregation an optical instrument (Packs-4; Helena Laboratories, St. Leu, France) was used; PRP was allowed to stand for 30 min at room temperature, before platelet number was adjusted to 200 000–300 000/μl by addition of autologous PPP. Before performing the test PRP was warmed at 37 °C. To calibrate the aggregometer 0% light transmission was set with each PRP and 100% transmission with the corresponding autologous PPP. High concentration of ADP (final concentration 20 μM), collagen (10 μg/ml), arachidonic acid (500 μg/ml) and ristocetin (1.5 mg/ml) were used as aggregating agents. Platelet aggregation was considered abnormal if it was reversible or if the maximum amplitude was <50% of normal control. For flow cytometry, the antibodies used in the study were fluorescein isothiocyanate (FITC)-labeled mouse IgG1 (used to set background fluorescence levels), FITC-labeled mouse antihuman CD41 and FITC-labeled mouse antihuman CD42 (Becton-Dickinson, San Jose, CA, USA). Whole blood or PRP samples (5 μl) were diluted 1 : 10 in phosphate buffered saline (PBS) and then incubated for 30 min in the dark with the corresponding antibody. The reaction was then stopped adding 500 μl of PBS and the specimens were immediately analyzed with a FACS-Calibur flow cytometer (Becton-Dickinson) following the manufacturer's instructions.

Results

Forty patients with normal PT and APTT but a prolonged BT and a history of bleeding suggesting the presence of a defect of primary hemostasis underwent additional laboratory testing. Based on the criteria detailed below 23 patients were diagnosed with GT and seven with BSS; in four patients, we found more subtle defects of platelet aggregation that are currently being further investigated while in six no aggregation defects were found in spite of a slightly prolonged skin BT (range 7–10 min, with normal values <7 min).

Glanzmann thrombasthenia

Twenty-three patients from 16 different families were diagnosed with GT. Consanguinity among parents was present in all but one patient. Diagnosis was made in patients who had a normal platelet count, severely impaired platelet aggregation with high concentrations of ADP, collagen and arachidonic acid but normal aggregation with ristocetin. On flow cytometry 19 patients were diagnosed type I GT (<5% of GpIIb/IIIa expression) and four type II GT (GpIIb/IIIa expression between 5% and 20%). GpIb expression was normal in all patients.

Median age at first symptoms was 2 years; >50% of patients (Table 1) were diagnosed because of epistaxis, other common presentations being petechiae, ecchymosis and postcircumcision bleedings. From the first bleeding symptom to the moment of the collection of these data patients had been followed by our center for a median of 12 years (range 3–22 years); median actual age of the

	GT		BSS	
	Presentation	Follow-up	Presentation	Follow-up
Epistaxis	55	86	28	57
Petechiae and/or ecchymosis	14	32	0	29
Postcircumcision bleeding	14	20	0	0
Gum bleeding	5	50	15	28
Gastrointestinal bleeding	0	18	0	42
Bleeding after dental extraction	0	0	15	0
Muscle hematoma	9	33	0	0
Postvaccination bleeding	0	0	42	0

Table 1. Percentage of patients affected by Glanzmann thrombasthenia (GT) or Bernard–Soulier syndrome (BSS) who presented at least once, at presentation or follow-up, a given bleeding symptom

patients was 14 years. During this period almost all the patients (86%) had at least one episode of epistaxis. Mucocutaneous bleedings other than epistaxis, mostly gum bleeding (50%) and petechiae (32%), were other common symptoms. Median hemoglobin level was 10.3 g/dl (range 6.5–16.4) and MCV 70.5 fl (range 57.7–84.5 fl), with other laboratory signs of iron deficiency such as low values of serum ferritin. Almost all the patients (83%) had been transfused at least once and 60% more than five times during the follow-up period.

BSS

Bernar–Soulier syndrome was diagnosed in seven individuals from four different families. All the patients had consanguineous (first or second degree) parents. Diagnosis was made on patients who had normal platelet aggregation to ADP, collagen and arachidonic acid but a severely defective response to large concentrations of ristocetin (1.5 mg/ml). Flow cytometry revealed that GpIb expression was in six cases <2% compared with the expression in a control, in the remaining patient it was 12% (normal limits 40% or more).

In these patients, platelet count was always low (median 35 000, range 16 000–63 000 platelets/μl). When blood smears were analyzed macrothrombocytopenia was a constant feature. Median hemoglobin levels were 12.2 g/dl (range 9.5–16.8 g/dl) and MCV 76 fl (range 65.5–83.9 fl), with frequent laboratory signs of iron deficiency.

Median age at first symptom was 15 months; the most common presentations were postinjection or vaccination bleeding, epistaxis (28%), gum bleeding (15%) and post-dental extraction bleeding (15%). Patients had a median follow-up of 15 years (median age of patients at the time of examination was 16 years); epistaxis (57% of patients), gastrointestinal bleedings (42%), petechiae (29%), and gum bleedings (28%) were frequent symptoms. Three patients had been transfused more than five times, two at least once while the remaining two never required platelet transfusion.

Discussion

Glanzmann thrombasthenia and BSS are extremely rare disorders, but are noted to be more common in such ethnic groups as Iraqi Jews, where the prevalence of GT is almost 1 : 80 000 (Seligsohn & Rososhansky, 1984), and in regions where consanguineous marriages are common such as the south of India (Khanduri *et al.*, 1981) and northern Iran (Toogeh *et al.*, 2004). Even BSS has been almost exclusively reported in the offspring of consanguineous parents (Lopez *et al.*, 1998). Our findings are remarkable for the high prevalence of GT and BSS relative to the general population of south Iran. Assuming that we were able to identify all the affected patients an approximate prevalence of 1 : 200 000 for GT and 1 : 600 000 for BSS can be estimated.

We reviewed the clinic and hospital records of the patients before and after referral to our specialized center in order to describe the natural history of these conditions in terms of bleeding symptoms and transfusion requirements. The morbidity associated with GT was significant, because two-thirds of patients came to our observation due to bleeding complications before the age of five and above 80% of the patients with GT were transfused at least once to control bleeding. This observation is consistent with what had been previously reported for GT (George, Caen & Nurden, 1990; Toogeh *et al.*, 2004). Moreover, in this group of patients the median hemoglobin level was 10.3 g/dl, probably indicative of the presence of unadverted chronic bleeding. The vast majority of GT patients were type I, in accordance with previous reports (Kannan *et al.*, 2003), only four patients being diagnosed with type II. No patients had type III. BSS was also associated with significant bleeding problems that required medical attention early in life, with a median age of presentation at

approximately 1 year. In almost half of the patients, transfusion requirements were substantial.

In conclusion, we have reported the first complete laboratory-based investigation of a large cohort of south Iranian patients with GT or BSS, showing that both the disorders are associated with a significant bleeding diathesis. Earlier diagnosis of these patients would be helpful for their clinical management; therefore, these diagnoses should be aggressively sought in the offspring of consanguineous marriages presenting with a suggestive bleeding history.

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References

Bellucci S. & Caen J. (2002) Molecular basis of Glanzmann's thrombasthenia and current strategies in treatment. *Blood Reviews* **16,** 193–202.

George J.N., Caen J.P. & Nurden A.T. (1990) Glanzmann's thrombasthenia: the spectrum of clinical disease. *Blood* **75,** 1383–1395.

Kannan M., Ahmed R.P., Jain P., Kumar R., Choudhry V.P. & Saxena R. (2003) Type I Glanzmann thrombasthenia: most common subtypes in north Indians. *American Journal of Hematology* **74,** 139–141.

Khanduri U., Pulimood R., Sudarsanam A., Carman R.H., Jadhav M. & Pereira S. (1981) Glanzmann's thrombasthenia. A review and report of 42 cases from south India. *Thrombosis and Haemostasis* **23,** 717–721.

Lopez J.A., Andrews R.K., Afshar-Kharghan V. & Berndt M.C. (1998) Bernard–Soulier syndrome. *Blood* **15,** 4397–4418.

Seligsohn U. & Rososhansky S. (1984) A Glanzmann's thrombasthenia cluster among Iraqi Jews in Israel. *Thrombosis and Haemostasis* **29,** 230–231.

Toogeh G., Sharifian R., Lak M., Safaee R., Artoni A. & Peyvandi F. (2004) Presentation and pattern of symptoms in 382 patients with Glanzmann thrombasthenia in Iran. *American Journal of Hematology* **77,** 198–199.