Diagnosis of Bernard Soulier Syndrome and Glanzmann's Thrombasthenia in Iraqi Patients

Lina Mussa Kadhim[1], Muthana Ibrahim Malak[2], Safa A Faraj[3]

[1]Assistant Lecturer, [2]Assistant Professor at Wasit University, College of Science, Biology Department, [3]Assistant Professor at Wasit University, College of Medicine, MD Pediatrics Hema-Oncologist

Abstract

Background: Bernard–Soulier syndrome (BSS) and Glanzmann Thrombasthenia (GT) are rare inherited platelet function disorders, defined by a permanent history of mucocutaneous bleeding. The objective of this project was to identify biological and clinical characteristics of BSS and GT patients. **Methodology:** This study included 52 patients with bleeding disorders, the clinical, haematological and demographic features of patients were determined and the level of GpIIb/IIIa and GPIb/IX was assessed. **Results:** From a total of 52 patient, 20 were diagnosis with BSS (9 females and 11 males), their age range from 6 to 42 year and 23 diagnoses with GT (10 females and 13 males), their age range from 4 to 50 year. Epistaxis, easy bruising, menorrhagia and ecchymosis were the most frequent symptoms. Prolonged bleeding time (BT), normal PT and PTT were seen in all cases. Variable thrombocytopenia, large platelets with decreased GPIb/IX level were seen in BSS patients, while the level of GPIIb/IIIa was decreased in GT cases. **Conclusion:** Since BSS and GT are infrequent disorders diagnosis may be postponed, both diseases are combined with crucial bleeding tendency, therefore earlier detection would have been important for patients management.

Keywords: *Inherited platelet function disorders, Bernard–Soulier syndrome, Glanzmann Thrombasthenia.*

Introduction

Inherited platelet function disorders (IPFDs) are a heterogeneous group of bleeding disorders caused by defects of soluble agonist receptors, adhesive protein receptors, platelet granules and membrane phospholipids[1]. Inherited platelet disorders may change the number of circulating platelets as well as their function, cause mucocutaneous bleeding signs with varying intensity, generally bruise easily, epistaxis, ecchymosis and gingival bleeding[2]. Among IPFDs Glanzmann thrombasthenia (GT) and Bernard Soulier syndrome (BSS) are exceedingly rare, but their intensity and biological features make them especially important[3]. Bernard-Soulier syndrome is autosomal recessive bleeding disease resulting from a qualitative and/or quantitative defect in the GPIb/IX/V platelet receptor, it is prevalent in male and female with 1:1 ratio[4,5]. It is estimated to occur in 1 per million individuals however, due to under recognition and misdiagnosis the prevalence may be much higher than the estimated[6,7]. The typical BSS manifestation begins at birth and continues throughout life. Thrombocytopenia, giant platelets, prolonged BT and low/ absence of GP1b/V/IX expression are main indicators in BSS cases [8]. GPIb/IX/V is platelets restricted receptor bind with von Willebrand factor (vWF) to initiate platelet adhesion at site of damage, this interaction will transmit signals to the cytoplasm of platelet to trigger a series of events which lead to creation of clot[9]. GPIb/IX/V formed by the association of four proteins related to the leucine rich motif (LRM) family[10]. The GPIb/IX/V subunits are encoded by GPIBA, GPIBB, GP5 and GP9 genes which located at chromosomes 17p12, 22q11.2, 3q29 and 3q21 respectively[11]. Diverse mutations in genes encodes for GPIb/IX/V subunits impaired synthesis and expression of the receptor on the platelet surface and consequently cause BSS[12].

Corresponding author:
Lina Mussa Kadhim
email: leenmoosa99@gmail.com

Glanzmann's thrombasthenia (GT) is a rare autosomal recessive bleeding disease, in which the platelets have defective or low levels of GPIIb/IIIa, the diagnostic features of GT include normal count and shape of platelets, prolonged bleeding time, irregular clot retraction, lack of platelet aggregation in response to arachidonic acid, collagen and ADP, as well as GPIIb/IIIa defective or deficiency [13].

GPIIb/IIIa is a large heterodimeric cell transmembrane complex, express in large amounts in the platelet plasma membrane consist of a larger αIIb subunit linked non-covalently to a smaller β3 subunit [14]. Activation of αIIbβ3 and binding of soluble ligands (primarily fibrinogen) are critical for platelet aggregation. αIIb and β3 subunits are encoded by ITGA2B and ITGB3 genes which are closely situated at chromosome 17q21.31-32[15]. GT mutation screening reveals a wide diversity of nonsense and missense mutations involving small insertion, deletion, frameshifts and splice site defect that occur through the 45 exons of ITGB3 and ITGA2B genes results in a qualitative or quantitative defect in the GPIIb/IIIa complex [16,17].

Patients and Methods

Patients

This study included 52 patients suffering from bleeding complication and 36 apparently healthy individuals without bleeding symptoms (control group).

Hematological examination

Five ml of blood were collected from each patient and control, the collected blood samples were divided in two tubes, one of which containing sodium citrate for PT and PTT test and another containing EDTA for CBC and ELISA. Duke's method was used for detection the BT and blood smears were prepared and stained with Leishman's stain to examine platelets morphology.

Evaluation of platelet glycoproteins

The level of GPIb-IX and GPIIb/IIIa glycoproteins was assessed in cases and control group with commercial ELISA kits (Bioassay Technology/ China) by Human Reader Systems, (Germany). The microtiter plate has been pre-coated with antibody (capture antibody). The platelet surface antigens in the sample are added and bind to the immobilized capture antibodies, then the antigens bound to the immobilized antibodies are sandwiched with an enzyme-labeled antibodies for color development. The color develops in proportion to the glycoprotein amount, absorbance was measured at 450 nm.

Statistical Analysis

The statistical analysis was carried out using SPSS version 26, parametric variables were presented as mean ± standard deviation (SD). For all tests P-value less than 0.05 was considered as statistically significant.

Results

The study included 52 patients (26 females and 26 males) have normal PT and PTT, but a history of hemorrhage and prolonged bleeding time that shows evidence of a primary hemostasis defect.

The patients' mean age was (17.56±10.9, mean ±SD) year ranged from (1-61)year. The main clinical manifestations in patients were epistaxis, easy bruising, menorrhagia and ecchymosis. The consanguineous marriages in parents were reported in 84% (n= 44) of patients and a majority of patients have positive family history of bleeding. Statistical analyses have shown that there was a considerable differences in BT ,Hb, platelet count and MPV comparing between patients and the control group and the results of the PT, PTT and WBC were within the normal range, and non-significant difference from control (P higher than 0.05), table(1).

Depending on the hematological examination and level of platelets surface glycoproteins, 20 patients were diagnosed with BSS and 23 with GT. Variable thrombocytopenia, large platelets and decreased GPIb/IX level were seen in BSS patients, while the level of GPIIb/IIIa was decreased in GT cases, the basic characteristics of patients are shown in tables (2).

Table No.(1): Correlation between patients and control regarding laboratory data

Item	Patients (Mean±SD)	Control (Mean±SD)	P value
Age/ years	17.56±10.9	18.5±9.2	0.8
Hb g/dl	9.5±1.6	12.9±1.7	0.001*
WBC	8.8±2.7	8.6±2.3	0.69
Platelet	166±73.6	260±59.8	0.001*
MPV fL	12.4±2.2	9.5±1.8	0.001*
PT sec	11.9±1.3	12±1.4	0.6
PTT sec	32.7±3.1	32.6±3	0.9
Bleeding time /min	12±2.3	5±1.5	0.001*

*= Significant difference (P value ≤ 0.05

Table No.(2): Demographic data in patients groups and control

Diagnosis	N. of p.	Age in years (Mean±SD)	Sex		Consequent state%	
			Male	Female	Positive	Negative
Glanzmanns	23	18±13.2	13	10	58.5	41.4
Bernard soulier	20	18.4±9.4	11	9	60%	40%
Thromasthenia	9	12.5±4.5	2	7	55.5%	44.4%
control	36	18.5±9.2	19	17	80.5%	19.4%

Bernard Soulier syndrome group

BSS was diagnosed in twenty patients (11 males and 9 females), the mean of age was (18.4±9.4) ranged from 6 to 42 year. All hematological parameters showed non-significant differences (P higher than 0.05) when compared between patients groups except platelet count and MPV, in BSS group platelet count was always low with median(111±40) and the MPV was elevated (14.1±1.4) range from 10.5 to 16.8 fL.

The bleeding time was prolonged (12.7±2.3), median hemoglobin level was (9.5±1.6) g/ dl, PT, PPT and WBC within normal range, table (3). Peripheral

blood smear analyzed showed the presence of larger platelets and the GPIb/IX expression was reduced, while the GPIIb-IIIa level found to be no statistically significant difference from control group as indicated in table(4).

Table No.(3): Correlation between patients groups regarding laboratory data

Parameter	BSS (Mean±SD)	GT (Mean±SD)	Unclassified thrombasthenia Mean±SD	P value
Hb g/dl	9.5±1.6	9.5±1.7	9.3±1.5	0.9
WBC	8.2±2.9	9.2±2.2	10±3.5	0.2
Platelet 10 e3/µL	111±40	225±62	148±33	0.001*
MPV fL	14.1±1.4	11±1.9	11.1±1.4	0.001*
PT sec	11.7±1.3	12.2±1.4	11.6±1.3	0.4
PTT sec	33±2.9	32.2±3	32±3	0.9
Bleeding time /min	12.7±2.3	11.3±2.2	12.1±2.2	0.1

*= Significant difference (P value ≤ 0.05)

Table No.(4): Correlation between BSS group and control regarding Elisa test results

Glycoprotein	BSS (Mean±SD)	Control (Mean±SD)	P value
Alpha	.993±1.7040	2.77±3.122	0.006*
Beta	2219.1±1476.2	3867.6±1891.9	0.001*
GP9	191.50±238.8	401.55±447.4	0.055
GPII/IIIa	22.3±13.7	19.8±5.6	0.44

*= Significant difference (P value ≤ 0.05)

Glanzmann thrombasthenia

Twenty-three patients were diagnosed with GT(10 females and13 males) the mean of age was (18±13.2) ranged from 4 to 50 year. GT patients had a normal platelet morphology and count (225±62), prolonged bleeding time (11.3±2.2 min), PT and APTT within normal range, hemoglobin level was reduced (9.5±1.7 g/ dl),table (3). The expression of integrin GPII/IIIa was reduced whereas the level of GPIbα, GPIbβ and GPIX found to be no statistically significant difference from control as shown in table(5).

Table No.(5): Correlation between GT group and control regarding Elisa test results

Glycoprotein	Glanzmann (Mean±SD)	Control (Mean±SD)	P value
Alpha	2.2 ±2.5	2.77 ± 3.12	0.52
Beta	3478±1965	3867.6±1891.9	0.44
GP9	354.65 ± 347.8	401.55 ± 447.4	0.6
GPII/IIIa	12.6 ± 8.2	19.8 ± 5.6	0.001*

*= Significant difference (P value ≤ 0.05)

The remaining nine patients with unclassified thrombasthenia had a normal platelet morphology, prolonged bleeding time (12.1±2.2), hemoglobin level was reduced (9.3±1.5 g/ dl),table (3) and the expression of GPIb/IX and GPII/IIIa glycoproteins found to be non-considerable difference compared with control(P value higher than 0.05).

Discussion

The demographic and clinical characteristics of 52 patients with bleeding manifestation were examined. The consanguineous marriages in parents were reported in 84% of patients and a majority of patients have positive family history of bleeding. Toogeh *et al*., (2004) demonstrated that the family history with consanguinity marriage would definitely increase the probability of having children with IPDs. So the occurrence was found to be high in populations where marriage between the close relative is common, such as Jordan, Iran, Pakistan, India and Saudi Arabia [18].

Twenty patients diagnosis with BSS(11 males and 9 females) with mean age (18.4±9.4) ranged from 6 to 42 year. The appearance of markedly larger platelets on peripheral blood smear, low platelet count and reduce the expression of GPIb-IX expression support the diagnosis of BSS in these group. Similar to previous studies in the literature, we found a relatively equal gender distribution and bleeding symptoms are usually evident from early childhood. The GPIb-IX-V receptor defect is combined with abnormal platelet structure and function. The prolonged bleeding time in BSS is most likely due to GPIb-IX-V defect, thrombocytopenia and reduced thrombin creation[19]. The GPIb–IX–V receptor is important for development of proplatelet due to its participate in the dynamic reorientation of the underlying microtubular cytoskeleton which is needed to enable MKs to expand the proplatelet[20]. The interaction between GPIbα subunit and filamin A adjusts platelet size, morphology and ligand adhesion, so diminished this association can lead to the development of giant platelets[12].

GT was diagnosed in twenty-three patients(10 females and 13 males) the mean of age was (18±13.2) ranged from 4 to 50 year, this study has shown slight male predominance (56%). Some previous reports show slight female predominance in which revealed 53% in 113 patients and 66% in 64 patients[21]. This is not significant as GT is an autosomal recessive disease. The results of laboratory investigations showed normal platelet count and morphology, PTT and PT are within normal limit, BT was prolonged with an average of 11.3 min. The analysis of platelet glycoproteins revealed reduce in the level of GPIIb/IIIa. In the 1970s, Nurden and Caen established that GT patients had specific defects in the composition of platelet membrane glycoproteins, the platelets have defective or reduced levels of GPIIb/IIIa[22]. Physiologically this platelet complex binds numerous adhesive plasma proteins and this enables attachment and aggregation of platelets to ensure thrombus development at vascular injury sites[23]. Impaired binding of GpIIb/IIIa with fibrinogen or other adhesive proteins that bind platelets result in no cross-linked bridges between platelets which lead to defect in platelet aggregation and reduced retraction of clot and BT is considerably prolonged in GT cases [24].

Conclusion

The diagnosis of BSS and GT may be postponed due to their rarity, many cases with inherited thrombocytopenias misdiagnosed with immune thrombocytopenia or less frequently myelodysplastic syndrome, exposing patients to unsuccessful and harmful treatments, so earlier diagnosis might be beneficial for their medical management and must be specifically sought in the consanguineous family offspring that have a history of bleeding.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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