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A RARE BLEEDING DISORDER: GLANZMANN'S THROMBASTHENIA: A SERIES OF THREE CASES

Pathology

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ABSTRACT

Background: Glanzmann's thrombasthenia (GT), is a rare bleeding disorder which affects the megakaryocytic lineage. It is characterized by lack of platelet aggregation, due to quantitative and/or qualitative abnormalities of αIIb-β3 integrin, which is a receptor that helps in aggregation of platelets at the site of any endothelial injury.

Cases: We present a series of 3 cases in young children. These cases were diagnosed on the basis of characteristic clinical features of Glanzmann thrombasthenia presenting with gingival bleed, petechial hemorrhage, epistaxis, injection site bleed. Laboratory parameters of prolonged bleeding time, abnormal clot retraction, absence of platelets clump on peripheral blood smear, platelet aggregometry and flow cytometry clinches the diagnosis. With accurate diagnosis and proper supportive care GT has a very good prognosis.

Conclusion: Initial recognition and knowledge about etiopathogenesis may be of benefit to pathologists, haematologists and physicians who deal with hemostatic disorders to plan safe and effective care.

KEYWORDS

Glanzmann's Thrombosthenia, Platelet Aggregation, Glycoprotein Complex GPIIB IIIa.

Introduction

Glanzmann thrombosthenia (GT) was documented by a German Pediatrician Dr. Edward Glanzmann in 1918. It was described as "hereditary hemorrhagic thrombasthenia (1). GT is a rare autosomal recessive bleeding disorder, characterised by deficiency or dysfunction of GPIIb and GPIIIa fibrinogen receptor leading to the failure of platelets to bind fibrinogen, retract a fibrin clot or aggregate after stimulation by physiological agonists such as adenosine diphosphate (ADP), thrombin, epinephrine or collagen alone or in combination (2). GT is associated with clinical variability, some presenting with bruising while others have frequent, severe and potentially fatal hemorrhages in the form of purpura, epistaxis, gingival hemorrhages and menorrhagia are nearly constant features, while GIT bleeding and hematuria is less common (3). Spontaneous, unprovoked bleeding, typical of disorders of coagulation and leading to hemarthroses or deep hematomas, is uncommon in GT patients.It is estimated that one in 1,000,000 individuals have GT, though the exact number is unknown (4). GT has an increased incidence in populations in whom marriage among close relatives is an accepted custom, such as south Indians, Iranians, Iraqi Jews, Palestinian and Jordanian Arabs, and French Gypsies as incidence of consanguinity is high in these communities (5). Due to the rarity and heterogeneity of inherited platelet abnormalities, at present no specific guideline/algorithm for diagnosis of GT is available in our institute. We are presenting a series of three cases of Glanzmann Thrombosthenia along with the algorithm for its laboratory diagnosis.

Clinical presentation

Case1: A 3-year old girl child presented with history of petechial hemorrhages on back and hands with epistaxis since 7 days. There was history of previous hospitalization for the same complaints 6 months before this episode. No diagnosis was made during previous admission, epistaxis was managed conservatively. ENT examination showed no anatomical abnormalities.

Case 2: A 4 month old boy presented with bleeding episode of 3 hrs after intramuscular DPT vaccination that he had taken 2 months before. He was referred from primary health centre to our tertiary care set up for the investigation of bleeding disorder. There were no complaints at the time of admission.

Case 3: A 3 years old female child reported with chief complaint of bleeding from oral cavity following injury to upper front teeth due to fall from bed 10 hours ago. Clinical examination revealed continuous gingival bleeding and subluxation of upper incisor teeth. There was a history of prolonged, continued bleeding from tongue bite while eating and hospitalization for the same at the age of two. There was history of frequent episodes of continuous and excessive bleeding followed by minor injury. General physical examination of patient revealed multiple ecchymotic patches and bruises on skin.

In all the above cases there was no history of skin rashes, fever, joint pain, lymphadenopathy, organomegaly and medications. There were no similar complaints in the family but history of consanguinity was present in first two cases. Clinical examination was completely normal.

Material and methods

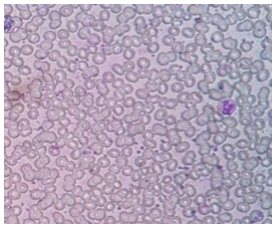
Blood samples for coagulation studies and platelet function test were collected in 3.8% sodium citrate vacutainers. EDTA samples were collected for platelet count and direct peripheral blood smears were prepared from finger prick. Clot retraction test was also done. In this test whole blood of patient and a normal control is taken in glass test tubes and are incubated at 37ºC in a waterbath.

Platelet aggregation studies were done on APACT- 4004 four channel thrombocyte function aggregometer by using Plasma rich platelets. It is widely accepted as the gold standard diagnostic tool for assessing platelet function. The principle of the machine is turbidometric technique. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by centrifuging whole blood at low spin (135g for 15 min) and high spin (1500g for 15 min) respectively. The instrument setting was done for 100% transmission by PPP and 0% transmission by PRP. Once the instrument has been zeroed, percent maximal aggregation was calculated after adding agonist (ADP-5µm, Collagen-10µg/ml and Ristocetin-1.5mg/ml) to PRP. With each batch of tests normal control was run. Flow cytometry was done on Beckman Coulter Navious three laser ten colours flow cytometer using CD41 PE and CD61 FITC.

Results

Laboratory investigations revealed the following findings (Table1). Hematological investigation revealed discrete platelets there was no clumping Fig no (1), the bleeding time was prolonged, coagulation parameters that is APTT and PT were normal. There was no clot retraction in all the three cases Fig no (2). Clots should normally be reduced by 50% of their original mass within 1 hour (6). Platelet aggregation studies showed the following finding as shown in Fig no (3,4,5).

Flow cytometry was positive for CD41 PE and CD61 FITC. Rest hematological investigations were completely normal.



Fig(1):Peripheral smear showing the discrete platelets

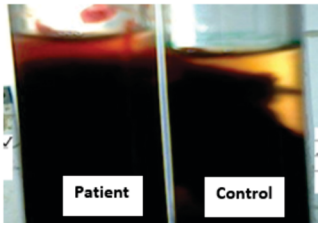


Fig (2): Clot Retraction test: Absent in Patient

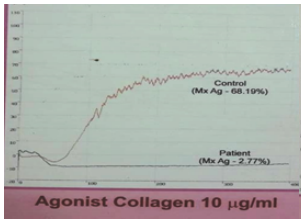


Fig (3): Agonist collagen – No aggregation

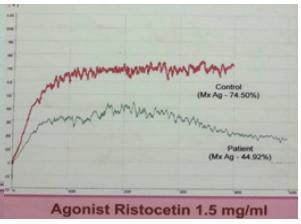


Fig (4): Agonist Ristocetin--- Normal response

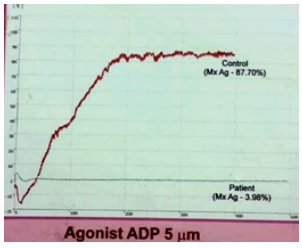


Fig (5): Agonist ADP – No aggregation

Table no1: Laboratory investigations of the three cases

Test	Case 1	Case 2	Case 3
Hemoglobin gm%	9.4 gm%	10.6 gm%	9.8 gm%
TLC	6500/cmm	11000/cmm	9000/cmm
Platelet count	1.74 lacs/cmm	3.08 lacs/cmm	2.10 lacs/cmm
Bleeding time by Ivy's method	Prolonged	Prolonged	Prolonged
Morphology of platelet on finger prick PBS	Discrete without aggregation of platelets	Discrete without aggregation of platelets	Discrete without aggregation of platelets
Coagulation profile - PT	Normal	Normal	Normal
APPT	Normal	Normal	Normal
Clot Retraction Test	No clot retraction	No clot retraction	No clot retraction
Flow Cytometry CD41 and CD61	4.31%- Type I	12.10% - Type II	Not done

Discussion

A clinical history of mucocutaneous bleeding often indicates various differential diagnoses in children, such as aplastic anemia, leukemia, coagulation disorder and platelet-derived bleeding diatheses. The laboratory evaluation of these disorders can range from simple to complex, but should initially include a thorough evaluation of the patient's medical history, concentrating on personal and familial bleeding disorders and all current medications. Inherited thrombocytopenias are eliminated by a normal platelet count. Leukemia can be ruled out by the absence of organomegaly clinically and blasts in the blood and bone marrow. Absence of deep hematomas and normal coagulation parameter exclude the possibility of coagulation disorder. Simple procedures, such as platelet count, peripheral blood smear, and a platelet function screening test, will often lead the investigator to more specific analyses (6) (Table 2).

Table no 2. Showing presentation of various platelet function disorders (Differential diagnosis) –

Condition	Pl. count	Pl. morphology	BT/CT	PT/PTTK	Clot retraction	Platelet Function Test			
						ADP	Collagen	Epinephrin	Ristocetin
Glazmann Thrombasthenia	N	Small & no aggregates	BT - Prolonged	N	Absent/D	Absent	Absent	Absent	N
Bernard Soulier syndrome	N	Giant Platelets	BT - Pronged	N	N	N	N	N	Abn
von Willebrand disease	N	N	BT - Prolonged	PTTK raised	N	N	N	N	Abn
Afibrinogenemia	N	N	BT– Prolonged	Raised	Absent	N/D	N/D	N/D	N/D

N-Normal, D-Decrease, BT- Bleeding Time, PT-Prothrombin time, PTTK-Partial prothrombin time with kaolin, Abn-Abnormal

Hemorrhagic symptoms occur only in patients homozygous for mutations causing GT; the heterozygous condition is mostly asymptomatic, even though these subjects have only a half-normal concentration of platelet αIIbβ3. The most common presentation in our case series was mucocutaneous bleeding in the form of epistaxis, gum bleeding and bleed at the site of injury. These findings are similar to other studies put forth by George et al and Sebastian et al (7,8). Other forms of clinical presentation includes menorrhagia, gastrointestinal bleed, hematuria, purpura (7,9,10). Hemarthrosis is rare in GT as seen in coagulation disorders. Mucocutaneous bleeding with absent platelet aggregation in response to all physiologic stimuli is pathognomonic for GT. Hematological findings show normal platelet morphology and count. Finger prick peripheral blood smear show scattered platelets, without clump formation. Prolonged bleeding time, normal prothrombin time and activated partial thromboplastin time and absent or decreased clot retraction supports the diagnosis of glanzmann thrombasthenia. In present case series all the three patients showed prolonged bleeding time, normal platelet count and abnormal clot retraction. This is well supported by other studies (8, 9, 11).

Absence of platelet aggregation in response to ADP, collagen, epinephrine, thrombin and normal ristocetin induced aggregation confirms the diagnosis. Ristocetin interacts with GpIb/Ixa receptors and Von Willebrand factor hence its response in GT is normal. This helps to distinguish GT from Bernard Soulier and Von Willebrand disease. In all the three patients in our study, there was decreased/ absent aggregation with ADP and collagen while response to Ristocetin was normal. This finding was consistent with other studies (7, 9, 12). Diagnosis is further confirmed by measuring the amounts of platelet glycolproteins GpIIb/IIIa on the platelet surface membrane using monoclonal antibodies by flow cytometry or immunoblot analysis (13).

Platelet aggregation defects specific to agonists imply abnormalities at various level, such as adenosine diphosphate (ADP) or collagen, imply abnormalities of their primary receptors or of signaling pathways. Defects in the second wave of aggregation to ADP or in the response to collagen can imply storage pool disease and an absence of the secretory stores of ADP in dense granules. Deficiencies in the platelet response to arachidonic acid can point either to an inherited abnormality in thromboxane A2 formation or a platelet function defect temporarily acquired through aspirin ingestion. GT is the only disease in which platelet aggregation is defective to all agonists, while absent clot retraction is another frequent characteristic (12). Defective response with ristocetin but not with other agonists is feature of Von Willebrand disease and Bernard Soulier syndrome.

Normal ristocetin-induced platelet agglutination and normal platelet size clearly rule out the Bernard-Soulier syndrome, a disorder of platelet adhesion. Normal coagulation parameters rule out clotting disorders that can also affect platelet function such as congenital afibrinogenemia and Von Willebrand disease. Another problem in diagnosing GT is to eliminate patients with acquired autoantibodies

that block aggregation, although these patients would often be thrombocytopenic (13). These antibodies can be detected immunologically by their binding to αIIbβ3 of control platelets during incubation with the patient's serum (12).

Glanzmann thrombasthenia can be classified according to the amount of glycoprotein IIb/IIIa: type I - 0% to 5% of normal; type II - 6% to 20% of normal; and variant disease - 50% to 100% of normal with abnormal fibrinogen binding. Type I is the most common; however, little correlation exists between the severity of disease and the subtypes (14). An acquired variant of GT has been reported in which autoantibodies against glycoprotein complex IIb/IIIa is formed, interfering the normal platelet function (12). Since the final common pathway of platelet aggregation is the expression of functionally active GPIIb/IIIa complex, and since ligand binding sites have been identified, the GPIIb/IIIa has become a target for development of new antithrombotic agents, benefit patients undergoing percutaneous coronary interventions. A patient with GT who developed a unique powerful antibody against GPIIb/IIIa following multiple transfusions appeared to be a potential source of a therapeutic agent (15). In general, the bleeding tendency in GT decreases with age. The rare co-existence of GT with other inherited diseases such as mild von Willebrand disease, may accentuate the clinical severity of bleeding (12).

Although GT can be a severe hemorrhagic disease, the prognosis is excellent with careful supportive care. Most adult patients are in good health and their disease has a limited effect on their daily lives. Death from hemorrhage in diagnosed patients is rare unless associated with trauma or other disease (*e.g.* cancer). In contrast, families often report deceased siblings on diagnosis of GT (12). Life-style advice and a continuous patient education program are crucial general measures to be implemented.

Bleeding in Glanzmann is refractory bleeding requires transfusion of normal platelets. HLA-matched platelets is the treatment of choice to prevent alloimmunization. Medical treatment includes antifibrinolytic agents inhibit fibrinolysis via inhibition of plasminogen activator substances.Administration of recombinant factor VIIa is an increasingly used therapeutic alternative (4, 12, 13, 14).

Carrier detection and genetic counseling have become an important and integrated part of comprehensive care of patients with GT.Carrier detection can be done by phenotypic analysis (flow cytometry and Western blot) and direct gene analysis (sequencing or restriction fragment length polymorphism)(5).

Conclusion

Multiple causes exist for platelet-derived bleeding diatheses. The laboratory evaluation include a thorough evaluation of the patient's clinical history, history of familial bleeding disorders and all current medications. With this as a starting point, the clinician and/or pathologist may find algorithms to guide investigations toward elucidating the underlying origin for platelet-derived bleeding.

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REFERENCES

1. Nurden AT, Pillois X, Wilcox DA. Glanzmann thrombasthenia: state of the art and future directions. Semin Thromb Hemost 2013; 39(6): 642–55.
2. Di Minno G, Coppola A, Di Minno MN, Poon MC. Glanzmann's thrombasthenia (defective platelet integrin αIIb-β3): proposals for management between evidence and open issues. Thromb Heamost 2009; 102(6): 1157–64.
3. Briet E, Vismans FJ, VanVoorthuisen AE. Renal embolisation in Glanzmann's thrombasthenia. Br Med J 1980; 281(6247):1039.
4. Tia S, Botsford A, SolhM. Glanzmann's thrombasthenia: pathogenesis, diagnosis, and current and emerging treatment options.J Blood Med 2015; 6:219–27.
5. Kannan M, Ahmad F, Yadav BY, et al.Carrier Detection in Glanzmann Thrombasthenia Comparison of Flow Cytometry and Western Blot With Respect to DNA Mutation. Am J Clin Pathol2008; 130: 93-98.
6. Marchant KK, Corcoran G. The Laboratory Diagnosis of Platelet Disorders. Arch Pathol Lab Med. 2002 Feb; 126(2): 133-46.
7. George JN, Caen J-P, Nurden AT. Glanzmann's thrombasthenia: the spectrum of clinical disease. Blood 1990; 75: 1383–95.
8. Sebastiano C, Bromberg M, Breen K, Hurford MT. Glanzmann's thrombasthenia: report of a case and review of the literature.Int J Clin Exp Pathol 2010; 3(4): 443–47.
9. Badhe BA, Jayanthi S, Dutta T. Clinical spectrum of Glanzmann thrombasthenia. Indian J Pathol Microbiol 2000; 43(3): 297-2.
10. Ali N, Moiz B, Shaikh U, Adil S, Rizvi B, Rahman Y. Diagnostic tool for Glanzmann Thrombasthenia Clinicopathologic spectrum. J Coll Physicians Surg Pak 2008; 18: 91-94.
11. Chediak J, Telfer MC, Vander Laan D, Maxey B, Cohen L. Cycles of agglutination and disagglutation induced by Ristocetin in thrombasthenic platelets. Br J Haematol 1979; 43:113-26.
12. Alan T Nurden.Glanzmann thrombasthenia. Orphanet J Rare Dis 2006; 1: 10.
13. Jhansi KP, Varghese P, Anand A. A case of Glanzmann's thrombasthenia type I in a primigravida treated with factor VIIa – a case report. J Obstet Gynecol India 2009; 59(6): 576-77.
14. Bellucci S, Caen J. Molecular basis of Glanzmann's Thrombasthenia and current strategies in treatment. Blood Rev 2002; 16: 193–2.
15. Seligsohn U. Glanzmann thrombasthenia: a model disease which paved the way to powerful therapeutic agents. Pathophysiol Haemost Thromb 2002; 32: 216–17.