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# Diagnostic Tool for Glanzmann's Thrombasthenia Clinicopathologic Spectrum

Natasha Ali, Bushra Moiz, Usman Shaikh, Salman Adil, Bushra Rizvi and Yasmeen Rahman

## ABSTRACT

**Objective:** To platelet aggregometry and describe the clinical spectrum of Glanzmann's thrombasthenia diagnosed by platelet aggregometry.

**Study Design:** A case-series.

**Place and Duration of Study:** This study was carried out at the clinical laboratories at the Aga Khan University Hospital, Karachi from January 2003 to January 2006.

**Patients and Methods:** All patients irrespective of age and gender presenting with bleeding symptoms and having normal platelet count were evaluated. Demographic details, relevant clinical history along with results of complete blood count, bleeding time and platelet aggregation studies were retrieved through computerized data base and evaluated for the diagnosis of Glanzmann's thrombasthenia.

**Results:** During the study period, 50 out of 2317 patients (2.2%) were diagnosed as Glanzmann's thrombasthenia by platelet aggregometry with male to female ratio of 0.85:1 and median age of 10.2 years (ranging from 3 months to 27 years). Common symptoms were epistaxis, oral and gingival bleed, bleeding from minor cuts and trauma that were observed in 46% of the patients; while 18%, 8% and 10% of them also complained of bruising, hematuria and bleeding per rectum respectively. Majority i.e. 86% had a bleeding time greater than 10 minutes. All patients had received blood or blood products for their bleeding episodes.

**Conclusion:** Platelet aggregometry is a useful diagnostic modality for the assessment of Glanzmann's thrombasthenia. The disorder presents with muco-cutaneous bleeding and was found to be a common cause of bleeding in our setup.

**Key words:** *Glanzmann's thrombasthenia. Platelet aggregation tests. Platelet function disorders. Bleeding disorders.*

## INTRODUCTION

Glanzmann's thrombasthenia (GT) is a rare bleeding diathesis, the mode of inheritance being autosomal recessive.<sup>1</sup> It affects the megakaryocyte lineage and is marked by prolonged bleeding time, normal platelet count and lack of platelet aggregation in response to all platelet agonists except Risocetin.<sup>1</sup> The disorder was named after its discoverer who recognized this disease in children from a Swiss Alps village in 1918.<sup>2</sup>

Although thrombasthenia is a rare disorder, it is being found with increasing frequency in some regions of the world where consanguinity is common, resulting in increased expression of autosomal traits.<sup>3</sup>

Quantitative or qualitative defect of integrin alpha II b beta 3 results in abnormal platelet aggregation as seen in GT resulting in mucocutaneous bleeding.<sup>3</sup> Platelet counts and functions that are not dependent on this receptor complex are normal in patients with such disorders. The varying degree of deficiency in this

platelet fibrinogen receptor gives rise to heterogeneity in the severity of bleeding problem. Thus, patients with GT can be classified into three types depending on their integrin alpha II b beta 3 levels that may be markedly low (< 5%) in type I, reduced (10-20%) in type II and normal but dysfunctional in variant type.<sup>4</sup> However, before integrin alpha II b beta 3 abnormalities were discovered, the original classification was based on platelet fibrinogen binding and clot retraction.<sup>5</sup>

Purpura, epistaxis, gingival haemorrhage<sup>6</sup> and menorrhagia<sup>7</sup> are the common manifestations in GT but they can also present with life-threatening bleeding, especially at the time of haemostatic challenges. Acquired thrombasthenia due to glycoprotein IIb-IIIa platelet antibodies has also been defined in a variety of conditions.<sup>8</sup>

The common and well-established tools for screening and diagnosing GT are platelet aggregation<sup>9</sup> and flow cytometry<sup>10</sup> respectively. More specialized tests (Western blotting, immunoprecipitation analysis and gene sequencing) are generally reserved for reference laboratories.<sup>11,12</sup>

The objective of the present study was to determine the common clinical manifestations and laboratory findings in patients with GT diagnosed through platelet aggregometry.

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## PATIENTS AND METHODS

This was a cross-sectional observational study conducted at the Aga Khan University Hospital, Karachi. All the patients referred to the clinical laboratory with suspected inherited platelet defects based on clinical history and baseline screening tests (normal platelet count and coagulation tests with elevated bleeding time) were evaluated from January 2003 till January 2006. Detailed clinical history was taken and physical examination was performed by a doctor and findings were recorded on a pre-designed proforma. Patients with thrombocytopenia or those who had received platelet transfusions, aspirin, non-steroidal anti inflammatory drugs and steroids, within ten days prior to the aggregation studies, were excluded.<sup>13</sup>

Complete blood count (CBC) was performed on EDTA blood through Coulter automated analyzer (Coulter Electronics, Fullerton, CA, USA) and peripheral film was also examined for each patient. Results of platelet count were available for all 50 patients, however, red cell and white cell parameters could be retrieved for only 26 patients from the computerized system. Bleeding time was done by Ivy's method and the population based reference range established in the laboratory was 1-6 minutes.

Platelet aggregation tests were performed on Chronolog Lumi Aggregometer (560 CA-Chronolog Corp, Havertown, PA, USA) with reconstituted ristocetin, adenosine 5-diphosphate (ADP), epinephrine barbate and native collagen fibrils type1 from equine tendons (Chrono-par® and chrono- lume® reagents) in the final concentration of 1.25 mg/ml, 10 µ M, and 2µ g/ml respectively. Reagent label and expiry dates were checked for all agonists before use. Twelve ml of patient's blood sample was collected in two separate tubes as follows: nine ml in 3.2% sodium citrate in 9:1 ratio and 3 ml in K3 EDTA tube. Citrated sample was centrifuged at 190 g for 15 minutes to prepare the platelet rich plasma (PRP) and upper 2/3rd was transferred to a separate tube while remaining sample was hard spin at 10,000 g for 10 minutes to obtain platelet poor plasma (PPP).<sup>14</sup> The platelet count in PRP was adjusted to 200-300 x 10<sup>9</sup> with the help of autologous PPP after it was allowed to stand at room temperature for 30 minutes. A sample from normal subjects was treated similarly to prepare platelet rich plasma and run simultaneously to serve as control. All the assays were done within three hours of sample collection and the manufacturer's instructions were strictly followed for conducting aggregation studies. The aggregation was considered abnormal if it showed less than 50% of increase in light transmission or absence of secondary wave of aggregation.<sup>14</sup>

## RESULTS

A total of 2317 patients with history of mucocutaneous

bleeding and suspected platelet function defects were analyzed during the study period. GT was diagnosed on the basis of platelet aggregometry when no or reduced responses to various agonists except ristocetin were observed. While on the contrary, those with only Ristocetin defective responses and normal von Willebrand assay were labeled as Bernard-Soulier syndrome. Glanzmann's thrombasthenia was the most common platelet disorder and was observed in 50 patients while Bernard-Soulier syndrome was diagnosed in 18 patients. The remaining 2249 patients showed normal platelet aggregometry. There was case of storage pool disease as secondary wave of aggregation to ADP and collagen was present in all of those patients.

There were 23 males (46%) and 27 females (54%). There was considerable heterogeneity in the age at which these patients presented ranging from birth to 24 years with median of 10.2 and 13 (26%) patients below the age of 5 years.

The most common clinical manifestations were bleeding from mouth, gingival bleeds, epistaxis and bleeding from minor cuts and trauma seen in 23(46%) patients. Nine patients (18%) presented with bruising alone while few others showed bleeding per rectum and hematuria (Table I). There were 8 females in the reproductive age group, of whom 4 had frequent complaints of menorrhagia. All 23 males were circumcised; however, 5 gave history of post circumcision bleeding.

**Table I:** Clinical details in our patients diagnosed as Glanzmann's thrombasthenia (n=50).

Clinical details in GT	
Age at the time of presentation	0-24 years (median 3.5 years)
Age at the time of diagnosis	0.4-27 years (median 10.2 years)
Sex	27 Females and 23 males
Epistaxis	18 patients
Petechiae/ecchymosis	14 patients
Gingival bleed	22 patients
Hematuria	04 patients
Rectal bleeding	05 patients
Post circumcision bleeding	05 patients
Menorrhagia	04 patients
Siblings with bleeding	14 patients
Consanguinity	07 patients

Fourteen patients (28%) gave positive family history of bleeding while first cousin marriages among the parents were observed in 7 patients (14%). History of red cell or platelet transfusion at least once was observed in all patients prior to diagnosis and, 27 (54%) had multiple transfusions depending on their bleeding episodes.

Hemograms of the 26 patients (19 children, 3 adult females and 4 adult males) showed that hemoglobin levels were reduced in children and females with

hypochromic microcytic indices (Table II). Leucocytosis was characteristically observed in children. Platelet counts of thrombasthenic patients (n= 50) ranged from 102 to 805 x 10<sup>9</sup>/l (mean 320) with subnormal values in 4 patients only. Examination of peripheral film did not reveal any abnormality in platelet morphology in the patients. Bleeding time was elevated to above 10 minutes in 43 patients (86%) while it was normal in 7 patients (14%). All patients with GT showed defective platelet aggregometry in response to the agonists used except ristocetin.

**Table II:** Hemogram, white cell count and bleeding time in our patients (n=26).

Laboratory tests	Results: Range (mean)		
	Adult males n=4	Adult females n=3	Children (<15) n=19
Hemoglobin g/d	10.2 -13.6 (12.1)	5.6-11.6 (8.0)	4.5- 11.9(7.7)
MCV fl	77.5-79.2 (82.3)	66.6-77.6 (73.5)	54.3-68.3(67)
MCH pg	23.3-28.8 (26.2)	20.8-26.7 (23.7)	15.3-26.7 (21)
Total white cell count x 10 <sup>9</sup>	5.6-12.5 (9.2)	4.4-10 (7.4)	5.6-32.3 (15.7)
MPV fl	8.4-13.2 (11)	9.7-9.9 (9.75)	6.8-17 (8.5)
Bleeding time (minutes)	>10	>10	2-10 (10)*

\* Median bleeding time is mentioned.

## DISCUSSION

Glanzmann's thrombasthenia is a rare platelet function disorder, which although has a worldwide distribution, is concentrated in those areas where consanguinity is common. Hence, ethnic groups like Iraqi Jews,<sup>15</sup> Northern Iranis<sup>16</sup> and Southern Indians<sup>17</sup> have shown high prevalence of this disorder. Similarly, in Pakistan where intermarriages among families are quite a common practice and accepted cultural norm, GT is not rare as shown. For the same reason, there was a positive family history of bleeding in significant proportion of those patients.

Thirteen patients were younger than 5 years at the age of diagnosis and this was similar to what has been reported previously.<sup>18</sup> The earlier diagnosis of GT is very crucial for proper management of the disease, hence, this platelet disorder should be considered in every young patient who presents with mucocutaneous bleeding or purpura, along with a normal platelet count and elevated bleeding time.

Epistaxis and gum bleeding were the commonest clinical manifestations in our experience and was observed in all age groups. This finding is supported by others as well.<sup>19</sup> Hematomas or hemarthroses are rare manifestations of the disease,<sup>20</sup> however, such soft tissue swelling were not seen in any of our patients. A frequent problem that afflicts thrombasthenic women is menorrhagia, which affects 25-30% of them at some stage during their reproductive age and even after in a few.<sup>21</sup> Correct diagnoses in such patients are essential

since menorrhagia itself is a debilitating condition. Women with Glanzmann's thrombasthenia in the childbearing age often complain of this symptom and patients included in this category of our study had similar complaints.

Post-circumcision bleeding can be a devastating problem in boys born with GT and it may require platelet transfusions.<sup>22</sup> Five of 23 circumscribed males in this series had similar complaints.

Of the 26 patients, 17 patients (2 females and 15 children) were anemic as revealed by their hemograms. All these patients had hypochromic microcytic anemia, which probably represent their chronic bleeds. This is a common finding in GT patients consistent with the severity of the disease. However, nutritional iron deficiency anemia<sup>23</sup> and beta thalassemia trait<sup>24</sup> are very common in our country and possibility of these as co-existing etiologies could not be excluded.

Most of the patients showed platelet and white cell counts within normal range, however, leucocytosis was observed in significant number of patients, especially children group, that may be due to active bleeding or infections. Bleeding time of more than 10 minutes was present in 43 patients (86%). Although bleeding time is an easily available tool for assessing *in vitro* platelet functions; its high operator dependency, invasiveness and lack of precision are the major obstacles in providing meaningful results. PFA 100 is a better test in this respect.<sup>25</sup> However, lack of this instrument in the set up restrained the researchers from such workup. Moreso, definitive diagnosis of GT requires flow cytometry and molecular studies like western blot and gene sequencing; unfortunately, such sophisticated technology is non-existent in Pakistan.

Majority of the patients had been transfused with red cells or platelets at least once before diagnosis was made. The triggers for these transfusions were anemia and bleeding episodes respectively. Over half of them received repeated transfusions for their recurring hemorrhage. Although management discussion was not the scope of our paper, HLA typed platelets to avoid iso immunization should be considered. For those developing refractoriness to subsequent platelet transfusions, recombinant factor VII is an available option.<sup>26</sup> However, none of these therapeutic options were used in those patients before diagnosis was made.

## CONCLUSION

Platelet aggregometry test is a useful adjunct for diagnosis of Glanzmann's thrombasthenia. There was a 2.2% frequency of thrombasthenia in the reported series. The main symptoms were cutaneous and mucosal bleeding. Majority of patients had normal platelet count with elevated bleeding time which forms the basis for screening for platelet dysfunction.

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