INTRODUCTION

Glanzmann’s thrombasthenia, an inherited platelet function disorder, was first described by a German pediatrician Dr. Glanzmann in 1918,\(^1\) inherits in a classic Mendelian autosomal recessive fashion.\(^2\) The prevalence of the GT is estimated to be 1 in 1,000,000,\(^3\) being higher in areas with high consanguinity.\(^4\) It is caused by the quantitative or qualitative deficiency of the platelet glycoprotein (GP) IIb-IIIa.\(^5\) The common clinical manifestations are epistaxis, gum bleeding, and menorrhagia.\(^6\) Three types of GT are described: patients with severe $\alpha_{IIb}\beta_3$ deficiency (expression level < 5%) are classified as having type I GT, moderate deficiency (10% - 20%) is classified as type II GT, and higher expression (> 20%) with a dysfunctional $\alpha_{IIb}\beta_3$ is classified as the variant form.\(^7\) Essential diagnostic features are: normal platelet count and morphology, greatly prolonged bleeding time, absence of platelet aggregation in response to ADP, collagen, epinephrine, thrombin and to all aggregating agents which ultimately depend on fibrinogen binding to platelets for this effect.\(^8\) The aggregation response to high-dose Ristocetin is usually normal.\(^9\) In flow cytometric analysis, absence or greatly decreased levels of CD41 and CD61 and normal levels of CD42 are consistent with a diagnosis of GT.\(^10\) Flow cytometry, however, may not recognize variant cases expressing functionally abnormal $\alpha_{IIb}\beta_3$ platelet aggregation studies; and genetic analysis are preferred for these patients.\(^11\)

The objective of this study was to evaluate common presenting complaints, and laboratory findings including platelet aggregometry for making diagnosis in Glanzmann thrombasthenia patients.

METHODOLOGY

This descriptive study was carried out at the Department of Hematology and Transfusion Medicine, The Children Hospital and Institute of Child Health, Lahore, from January 2006 till Dec 2013. A total of 796 patients presenting with mucocutaneous bleeding of either gender and different age groups were evaluated by platelet aggregometry. The results of all the platelet aggregometry studies were analysed retrospectively and the prevalence of Glanzmann thrombasthenia

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evaluated. Medical records of all the patients having GT were further scrutinised, retrospectively.

The diagnosis of GT was based on a normal platelet count and morphology, a greatly prolonged bleeding time, absence of platelet aggregation in response to ADP, collagen, and normal or decreased to Ristocetin. History including age of the patient, gender, sibling and family history, consanguineous marriage of parents, sign and symptoms, sites of bleeding, and the severity of bleeding were noted.

For each patient, bleeding severity was graded according to the information obtained from questionnaire and examination of the patient. GT patients were clinically classified as mild, moderate, or severe bleeders, in accordance with the ISTH bleeding assessment tool. Those who bled only after trauma or surgery or had minor symptoms, were classified as mild bleeders, those with history of spontaneous or life-threatening hemorrhages were classified as moderate bleeders, and those who had repeated episodes requiring blood or platelet transfusions were classified as severe bleeders.

Baseline screening tests for inherited platelet function disorders, i.e. platelet count, coagulation tests and bleeding time were performed. Detailed clinical history and physical examination findings were recorded on a pre-designed proforma. Blood sample for CBC was taken in EDTA vial. Complete blood count (CBC), including platelet count, was performed on Sysmex Kx 2100 automated analyser. Assessment of platelet morphology was done by peripheral smear examination for each sample.

Bleeding time was determined by Ivy's method and results were reported with established reference range of 2-9 minutes. Patients with suggestive history and screening tests were evaluated by platelet aggregometry. Blood was drawn from a forearm venipuncture with minimal venous occlusion in citrated vials for coagulation and platelet aggregation test. The blood samples were processed within 2 hours of collection. Coagulation tests were performed manually.

The citrated blood was centrifuged at 1000 - 1200 rpm for 7 - 8 minutes to obtain platelet-rich plasma and collected in separate tube. The remaining sample was centrifuged at 3500 rpm to obtain platelet-poor plasma (PPP). Platelet count of PRP was adjusted between 200 - 400 x 10^9/L and allowed to stand at room temperature for 30 minutes.

Platelet aggregation was performed by turbidometric technique on Chronolog aggregometer by adding 10 µm/ml Adenosine diphosphate (ADP), 1.25 mg/ml Ristocetin, collagen 2 µg/ml, and Epinephrine 10 µm/ml in 250 µL PRP in separate cuvettes. Platelet aggregation was recorded as the percentage change in light transmission after 3 minutes (maximal platelet aggregation percentage). Samples from healthy volunteers were treated similarly to prepare PRP and PPP, and ran simultaneously to serve as controls. The percentage of aggregation was compared with controls and the normal reference ranges provided by the company.

The data was collected in a well-designed proforma and analysed using Statistical Package for Social Sciences (SPSS) version 16. Frequency and percentage, and mean and standard deviation of different variables, were determined.

RESULTS

Among the 796 patients, 163 (20.4%) patients of Glanzmann's thrombasthenia were diagnosed. It was the most common platelet disorder observed in patients with mucocutaneous bleeding and normal platelet count and coagulation profile. Ninety-two (56.4%) patients were males and 71 (43.55%) females with male to female ratio of 1.2:1. All patients were Punjabis. A positive history of first-degree consanguinity was observed in 106 (65%). In 83% families, there was a positive family history in siblings and/or other family members. The mean age of the patients was 7 ±2.5 years. The oldest was 35 years (referred to Children Hospital for investigation of menorrhagia and platelet aggregation studies as it is not available in any Government sector hospital in Lahore) and youngest patient was 3 months old. Forty (24.5%) patients were below the age of 5 years. The age of onset of symptoms varies from first day of life to 12 years. In 86 (52.7%) cases, symptoms appeared within one year of life.

The common clinical presentations included epistaxis in 102 (62.5%), gum bleeding in 92 (56.4%), easy bruising in 125 (76.6%), and increased bleeding after minor trauma in 77 (47.2%) patients. Hematuria was reported in 13 (8%) and 12 (7.36%) cases of GIT bleeding (Figure 1). There was history of intracranial bleeding in 2 patients only. None of the patients presented with hemarthrosis and hematomas causing musculoskeletal handicaps. There were 20 females in reproductive age group, and 14 (70%) of them presented with menorrhagia. Among the 92 males, 40 (43.4%) gave history of increased post-circumcision bleeding. Patients were graded according to severity at the time of presentation. Details of severity of disease, according to age groups, are shown in Table I. Thirty-six (22%) mild, 77 (47.23%) moderate, and 50 (30.06%) were severe bleeders. Twenty-six severe bleeders were below 5 years of age.

Complete blood count of all patients showed Haemoglobin in range of 4.0 - 12.5g/dl (mean 7.4 ±4.3 g/dl), and platelet count ranged from 150 to 540 x 10^9/L (mean 310 ±84). Total leukocyte count was mildly raised...
Examination of peripheral film showed normal platelet morphology. All patients had normal PT and APTT. One hundred and fifty (92%) patients had a prolonged bleeding time, more than 9 minutes. One hundred and six (65%) patients gave history of transfusions.

GT was diagnosed on the basis of platelet aggregometry when no or reduced responses to various agonists including, ADP, collagen, epinephrine. While most patients had normal responses to aggregation with Ristocetin, but 9.2% (n=15) patients showed markedly reduced aggregation in response to Ristocetin also.

### DISCUSSION

Glanzmann’s thrombasthenia is the most commonly encountered inherited platelet function disorder affecting the megakaryocytic lineage and characterised by lack of platelet aggregation.12 Patients have a lifelong hemorrhagic syndrome, typically characterised by episodes of spontaneous mucocutaneous bleeding.13 Platelets fail to aggregate in response to stimuli because they lack or have non-functional αIIbβ3 integrin (formerly known as GP IIb-IIIa). Resting normal platelets are suspected to have αIIbβ3 in a bent conformation. When platelets are stimulated, the integrin straightens in parallel to the exposure of determinants essential for the binding of fibrinogen or other soluble adhesive proteins.14 The spectrum of clinical presentation and complications in patients with GT appears to be wide. Although thrombasthenia is a rare disorder, its occurrence is increased in some regions of the world where intracommunity marriage and consanguinity are common, resulting in increased expression of autosomal recessive traits.15 Consanguinity is reported in 65% of these patients.

Age at diagnosis varies; but most cases are diagnosed at an early age,16 as in this study. Various studies done in Pakistan and abroad have shown that GT is a disease of children and young adults with majority of patients being under 20 years.17 The age of patients in this study ranged from 3 months to 35 years; 72% patients were below 10 years of age and 97.5% were below 20 years. The age of onset of symptoms varies from first day of life to 12 years. In 86 (52.7%), symptoms appeared within one year of life. A slight female predominance is reported;3 but in this study, 56% were male patients.

The common clinical presentations included easy bruising, epistaxis, gum bleeding, and menorrhagia in this study which are similar to worldwide reports.18-20 Post-circumcisional bleeding occurred in 40 patients and 65% of cases required transfusion. Life-endangering symptoms are much rarer than in inherited coagulation disorders; and mortality was negligible in this series, despite relatively poor resources for management, especially in rural areas. Two patients died because of intra-cranial haemorrhage due to fall. Bleeding episodes such as hemarthroses and hematomas causing musculoskeletal handicaps, typical of hemophilia, are rare.21 At the time of presentation, 22% mild, 77 (47.23%) moderate, and 50 (30.06%) severe bleeders were found. Majority of these patients (47.23%) were between 5 to 10 years of age. Out of 50 severe bleeders, 26 were under 5 years of age. Sixty-five percent patients gave history of transfusions including whole blood, packed red blood cells, and platelet transfusion.

Prolonged bleeding time (BT) is an indication of delay in formation of primary hemostatic plug due to defective platelet aggregation. Hence, 92% patients had prolonged BT. The BT has poor reproducibility, sensitivity and specificity, as well as being invasive; for these reasons, it is not recommended.22 Use of PFA-100 system can replace BT tests for GT to increase sensitivity of test.1 PFA-100 provides an optional screening test, but this must be interpreted with caution and in the context of the clinical background, as the test is neither diagnostic nor sensitive for mild platelet disorders. The PFA measures the closure time when blood is passed through collagen-based filters under high shear stress. Blood from GT patients fail to plug the filters, resulting in prolonged closure time.

All patients showed decreased or absent aggregation with ADP, collagen, and epinephrine. This finding was consistent with the other studies.3,16 These agonists...
activate platelets through GP Ib-IIIa complex, and in GT patients they fail to induce platelet aggregation. Ristocetin interacts with platelets through GP Ibα and Von Willebrand factor and does not require GP Ib-IIIa complex to cause platelet aggregation; and hence, GT patients usually show normal aggregation with Ristocetin. It is important to diagnose GT and differentiate it from other inherited bleeding disorders and similar platelet function abnormalities. In this study, the classical laboratory features for its diagnosis were demonstrated in all patients and unusual findings were also seen; unlike the typical aggregation pattern in GT, Ristocetin-induced platelet aggregation was also reduced in some patients.

Analysis of platelet GP by flowcytometry should be part of the laboratory investigations for diagnosis of GT. Due to unavailability of this test in Pakistan at the time of study, the authors did not perform flowcytometry in these patients.

Routine hematological screening should be mandatory in children with positive family history of bruising and bleeding as a predictor for Inherited bleeding disorder. Extensive collaborated studies are needed to predict the true incidence of GT in Pakistan. Physicians should pay attention to families with a positive history of mucocutaneous bleeding and in selected cases should propose hematological consultation.

CONCLUSION
Glanzmann's thrombastothenia is a rare bleeding disorder but there was a substantial number of patients (20.4%) in this study, because of the trend of consanguineous marriages. This study shows a larger number of GT patients from Punjab, Pakistan. GT can present from early age to adulthood.

REFERENCES