

Original Article**Classification of Glanzmann's Thrombasthenia patients on flow cytometry**Saira Gul¹, Moizza Sahar², Tooba Fateen³, Faheem Shahzad⁴, Ghulam Mustafa⁵, Ismat Ullah⁶**Abstract:**

Background: Glanzmann's Thrombasthenia (GT) is an autosomal recessive platelet disorder caused by mutations in the ITGA2B and ITGB3 genes, leading to partial or complete deficiency of the GPIIb/IIIa (CD41/CD61) complex on platelets, causing a quantitative or qualitative defects of platelet fibrinogen receptors α IIb β 3 glycoprotein complex. This results in abnormal platelet aggregation, diminished clot retraction and mild to severe bleeding episodes. Affected individuals suffer from lifelong moderate to severe bleeding, mostly mucocutaneous in nature. The present study was designed to characterize GT subtypes through quantitative flow cytometry

Material and Methods: A descriptive study was conducted on 46 GT patients attending Allama Iqbal medical college / Jinnah Hospital. After obtaining written informed consent, blood samples were taken, severity of bleeding was assessed by Glanzmann's Thrombasthenia Italian Team protocol (GLATIT) and expression of platelet integrin was determined by quantitative flow cytometry.

Results: On flow cytometry 20 patients were categorized as type I (43.5%), 07(15.2%) as type II and 19(41.3%) as type III according to the level of receptor deficiency.

Conclusion: Type I is the most common followed by type III then type II. Most cases were severe bleeders followed by mild then moderate bleeders. Initial yet important account of clinical and phenotypic characterization of GT in local patients, which may spark further studies to help molecular diagnosis, optimal disease management and genetic counselling-based prevention efforts.

Keywords: Glanzmann's Thrombasthenias; Inherited Platelet Disorder Platelet, Glycoprotein GPIIb-IIIa Complex;

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INTRODUCTION

Glanzmann Thrombasthenia (GT; MIM # 273800), first identified by Edward Glanzmann in 1918 as "hereditary hemorrhagic thrombasthenia"¹, is a rare autosomal recessive bleeding disorder with an estimated incidence of 1 in 1 million population.¹ It results in life-long,

moderate to severe mucocutaneous bleeding.

Key features include prolonged bleeding time, abnormal clot retraction, normal platelet count, and abnormal responses to ADP, epinephrine, and collagen, though aggregation with Risto cetin remains normal.² Clinical manifestations include epistaxis, gum bleeding, petechiae, purpura, easy bruising, prolonged bleeding from injuries, bleeding post-circumcision in males, and menorrhagia in females. The bleeding severity varies from minimal bruising to severe, potentially fatal hemorrhages, with over two-thirds of patients needing blood or platelet transfusions, underscoring the disorder's severity.¹ Bleeding severity was categorized into

¹ Demonstrator AMIC, Lahore

² PGR Pathology Dept AMIC, Lahore

³ Assistant Prof. Pathology Dept. AMIC, Lahore

^{4,5} Lecturer UHS, Lahore

⁶ MO Medicine Dept. AMIC

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mild (trauma-related), moderate (spontaneous but non-life-threatening), or severe (recurrent and

life-threatening, requiring transfusion) as defined by the GLATIT protocol.

Glanzmann Thrombasthenia (GT) is a rare bleeding disorder with a prevalence of about one in a million, more common in populations with high consanguinity, such as in the Middle East, Europe, and Pakistan, where it is the most prevalent platelet functional disorder.³ This study aims to classify GT patients in Pakistan using flow cytometry, providing insights into diagnostic and treatment variations in this underrepresented population. Flow cytometry is used to assess platelet integrin $\alpha\text{IIb}\beta 3$ (CD41/CD61) expression, crucial for platelet aggregation. It identifies severe $\alpha\text{IIb}\beta 3$ deficiencies in types I and II and rare variant forms like type III, making it the gold standard for GT diagnosis.^{2,4} Seven novel variants have been identified, highlighting the importance of flow cytometry in carrier detection, prenatal diagnosis, and reducing the GT burden.⁵

This study aims to classify Glanzmann's Thrombasthenia (GT) in 46 Pakistani patients using flow cytometry to identify diagnostic and treatment variations. By focusing on an underrepresented population, the research seeks to improve global understanding of GT and develop tailored diagnostic and therapeutic strategies. It examines the link between bleeding severity and surface receptor expression in these patients

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted at the Hematology Department of Allama Iqbal Medical College/Jinnah Hospital Lahore, spanning from December 2022 to December 2023, with ethical approval granted (ERB144/11/09-06-2023/S1). A total of 46 unrelated patients diagnosed with Glanzmann's Thrombasthenia (GT) were enrolled based on clinical and laboratory confirmation. The sample size was determined using the World Health Organization's (WHO) formula for health studies version 2.0.21.13

$$n = \frac{Z^2_{1-\alpha/2} P (1-P)}{d^2} \text{ with a 95\% confidence level}$$

($Z = 1.96$), an anticipated proportion of 3%, (prevalence of disease) and a margin of error of 5%.⁽³⁾ Convenient sampling was used to select patients, with inclusion criteria based on a history of mucocutaneous bleeding, prolonged bleeding time with normal platelet count and coagulation profile, and platelet aggregation failure when tested with ADP, epinephrine, and collagen, while showing a normal response to Risto cetin. Patients with acquired bleeding disorders, those on anticoagulant or antiplatelet therapy were excluded. Data on demographic details, bleeding history, transfusion requirements, and bleeding severity, were collected. Bleeding severity was categorized into mild (trauma-related), moderate (spontaneous but non-life-threatening), or severe (recurrent and life-threatening, requiring transfusion) as defined by the GLATIT protocol.

Flow cytometry was performed in the department of Immunology University of Health Sciences Lahore on BD FACS Caliber flow cytometer. Peripheral blood samples were collected from patients and controls after informed consent. Five milliliters of blood was drawn into EDTA vials for flow cytometric analysis. Expression of $\alpha\text{IIb}\beta 3$ integrin (CD41/CD61) was assessed using monoclonal antibodies against CD41 (GPIIb) and CD61 (GPIIIa). A forward vs. side scatter (FSC/SSC) dot plot was used to identify the platelet population (R1 region), followed by analysis of CD41 and CD61 markers. Patients were categorized into GT type I, II, or III based on the percentage of $\alpha\text{IIb}\beta 3$ expression:

- Type I: <5% $\alpha\text{IIb}\beta 3$ expression
- Type II: 5-20% expression
- Type III: >20% expression

Isotype controls were used to correct for non-specific binding.

Key outcome variables included GT subtype (flow cytometry), bleeding severity (GLATIT protocol), and hematological parameters (Hb, MCV, MCH, MCHC, platelet count). Descriptive statistics summarized continuous variables (mean \pm SD) and categorical variables (frequencies/percentages). One-way ANOVA test analyzed differences between GT subtypes and

clinical manifestations. Pearson or Spearman correlation assessed the association between α IIb β 3 expression and bleeding severity. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS software version. 20.

RESULTS

GT patients were enrolled based on following hematological data: the mean hemoglobin (Hb) level 9.8 ± 2.5 g/dL, with RBCs indices indicating chronic bleeding and iron deficiency anemia (MCV 69.6 ± 15.5 fl, MCH 21.9 ± 6.9 pg and MCHC 31.6 ± 2.2 g/dL). Bleeding time of 11 minutes was recorded in all patients, normal platelet count (median $314 \times 10^9/L$), and normal PT, and APTT values were in concordance with GT diagnosis. The demographic data according to the clinical assessment and history are provided in the table below:

Table.1: The Demographic Data of GT patients (n=46)

Clinical Detail of GT Patients	No. of Patients (n=46)	Percentages (%)	
Gender			
Male	27	58.7%	
Female	19	41.3%	
Cousin Marriage	44	95.70%	
Purpura	46	100%	
Bruises	46	100%	
Nose Bleed	43	93.50%	
Gum Bleed	35	76.10%	
Gastrointestinal Bleed	7	15.20%	
Hematuria	8	17.40%	
Menorrhagia	5	26.30%	
Family History of GT	26	56.50%	
Transfusion History	38	82.60%	
Severity of Bleeding	Mild	6	13.00%
	Moderate	2	4.30%
	Severe	38	82.60%

Samples were analyzed using FACSCalibur (BD Biosciences) Cell Quest pro software, where 10,000 events were sampled from each reaction. Quantitative flow cytometric analysis of platelet integrin GPIIb/IIIa (α IIb β 3) was performed on 46 GT patients, revealing all the three types of GT. The level of glycoprotein receptor were $< 5\%$ of normal for type I, 5-20 % of normal for type II and $> 20\%$ of normal for type III. The α IIb β 3 (GPIIb/IIIa or CD41/CD61) levels on the platelet surfaces were quantified, identifying 20 (43.5%) type 1 GT cases with a mean α IIb β 3 level of 1.78 ± 2.2 % of normal. Seven (15.2%) patients were diagnosed with type II GT with a mean α IIb β 3 levels of 12.39 ± 3.92 % and CD 41 $<$ CD 61. Nineteen (41.3%) patients were diagnosed with type III GT having a mean value α IIb β 3 $50 \pm 2\%$ of normal platelets with level of CD 41 $<$ CD 61 in all the patients with type 3 GT (represented in Figures 1, 2, 3 & 4).

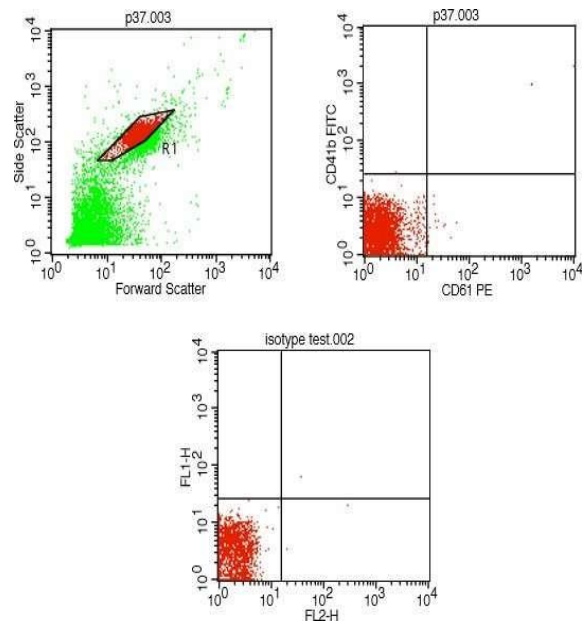
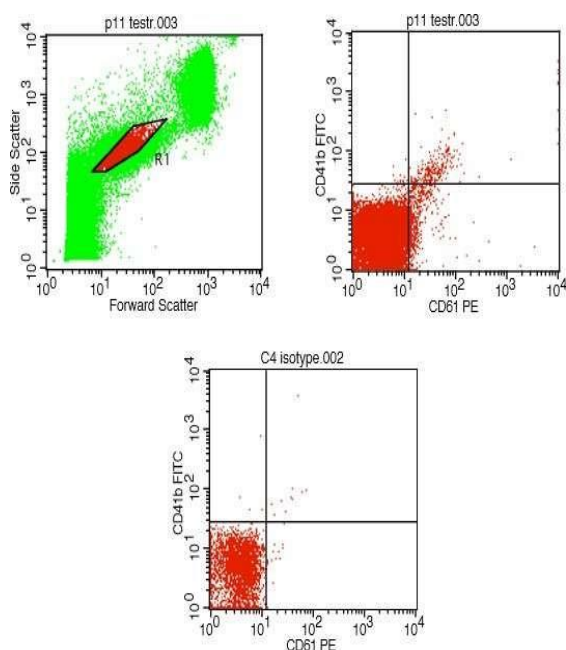
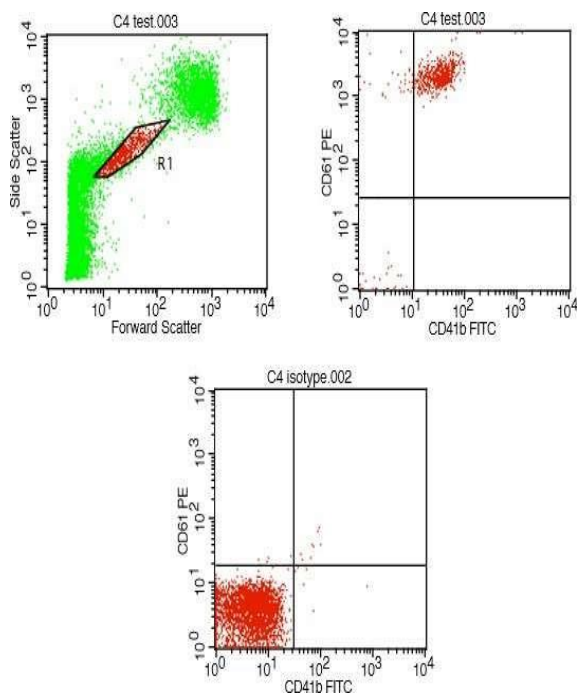


Figure 1:

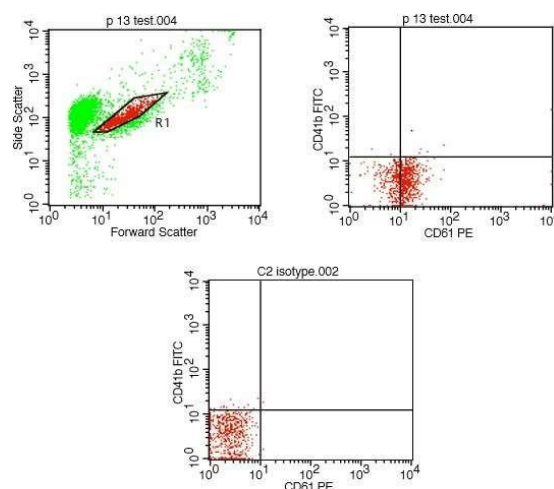
Representative flow cytometric dot plots of Glanzmann's Thrombasthenia (GT) patient and control: (a) R1 shows platelet gating based on physical properties in forward vs side scatter dot plot (b) normal CD41 and CD61 marker activity in a healthy control (indicating normal activity of GPIIb/IIIa on platelets (c) Isotype control (d) reduced CD41 and CD61 activity in a GT patient, indicating diminished GPIIb/IIIa activity on platelets.

**Figure 2:**

The dot blot from a GT patient, platelets showed no activity of CD41 or CD61, indicating the absence of GPIIb- IIIa complex, which is consistent with type I GT

**Figure 3:**

The dot plot from a type II GT patient shows activity of CD 41 (0.35%) and CD 61 (13.65%). The patients had a family history of GT and consanguinity, but no history transfusions.

**Figure 4:**

In the type III GT patients, platelets showing activity of CD 41 (0.12%) and CD 61 (77397 %). The patients had a family history of GT, consanguinity, and history of transfusions.

Table-2: Association of Age and Laboratory Parameters with the types of GT

Lab Parameters	GT Type 1 (n = 20) Mean ± SD Median (IQR)	GT Type 2 (n = 07) Mean ± SD Median (IQR)	GT Type 3 (n = 19) Mean ± SD Median (IQR)	P-value
Age (years)	8.05 ± 6.5	17.0 ± 13.4	10.37 ± 4.3	0.024
Hb (g/dL)	10.2 ± 2.6	10.3 ± 2.2	9.3 ± 2.5	0.464
Platelets (x10 ³ /μL)	316.4 ± 110.8	288.5 ± 81.8	340 ± 122.3	0.55
Bleeding Time (Minutes)	11.60 ± 2	11.8 ± 1.1	11.3 ± 1.2	0.636
CD 41 Expression (%)	0.62 ± 0.97	0.67 ± 0.42	0.46 ± 0.6	0.751
CD 61 Expression (%)	1.16 ± 1.5	11.72 ± 3.5	49.62 ± 19.4	0.00
CD 41/61 Combine Expression (%)	1.95 ± 1.3	13.25 ± 4.24	50.74 ± 19.1	0.00

Association of age and laboratory parameters with the types of GT (Table 2) showed that only the age of the patients, expression of CD61 and combined CD41/ CD61 levels were found to be statistically significant. *P*-value <0.05.

Table: 3 Association of Glanzmann's Thrombasthenia (GT) type on Flow cytometry with Severity of Bleeding as per GLATIT score

GT Type	Severity of Bleeding			<i>P-Value</i>
	Mild	Moderate	Severe	
GT Type I (n= 20)	03	0	17	0.549
GT Type II (n = 07)	01	0	06	
GT Type III (n = 19)	02	02	15	

The association of clinical presentation, as per the GLATIT bleeding score, with the type of GT on flow cytometry is given in Table 3. It reveals that the majority of the patients were classified as severe bleeders among all the three types of GT, with no statistically significant correlation found between the type of GT and the severity of bleeding

DISCUSSION

Glanzmann's thrombasthenia (GT; MIM# 273800) is a rare autosomal recessive platelet disorder, with an incidence of approximately 1 in 1 million, but higher in regions like Pakistan where consanguinity is common. This study involved 46 GT patients (27 males, 58.7%, and 19 females, 41.3%), reflecting a male predominance similar to Iranian reports.⁶ The median age of patients was 9 years, consistent with studies from Turkey.⁴ USA⁷ and Iraq,⁸ and 65% were under 10 years old, aligning with Indian data.⁹ High consanguinity (95.7%) was noted, mirroring Iranian studies.^{4,10} Family history of bleeding was present in 56.5% of cases, slightly higher

than previous Pakistani studies,¹¹ with 43.5% lacking a family history, suggesting possible new mutations.¹² Common symptoms included epistaxis (93.5%), gum bleeding (76%), hematuria (17.4%), gastrointestinal bleeding (15.2%), and menorrhagia (26.5%), consistent with findings from the USA and China.^{13,14} Severity was classified as 13% mild, 4.3% moderate, and 82.6% severe.^{5,15} with most severe cases occurring in those under 10 years old. Over 84% required transfusions,^{6,16} highlighting the disorder's severity.

Hematological parameters showed a mean Hb of 9.8 ± 2.5 g/dL, with RBC indices indicating chronic bleeding and iron deficiency anemia, consistent with studies in Pakistan and India.^{1,17} All patients had prolonged bleeding time (11 minutes), a sign of poor platelet aggregation, though bleeding time is less specific than PFA-100.¹⁸ Platelet count, PT, and APTT were normal, which is characteristic of GT.¹⁹

Flow cytometry classified 20 patients (43.5%) as type I GT with a mean α IIb β 3 level of $1.78 \pm 2.2\%$ of normal, 7 patients (15.2%) as type II with a mean α IIb β 3 level of $12.39 \pm 3.92\%$, and 19 patients (41.3%) as type III with a mean α IIb β 3 level of $50 \pm 2\%$ of normal. CD41 was lower than CD61 in all type III cases, consistent with previous studies.²⁰ Type I GT was most common, followed by type III, as noted in India and Iraq.⁸ The study found no significant correlation between GT type and bleeding severity, aligning with previous research.^{7,21} Flow cytometry of GPIIb/IIIa in relatives may aid in detecting carrier status.²²

The study highlights the need for a thorough assessment of disease-causing mutations in GT patients from highly consanguineous Pakistani populations. This approach could provide new insights into α IIb β 3 integrin biology and enhance therapeutic options, including gene therapy, through the identification and characterization of novel mutations.

CONCLUSION

The novelty of this study lies in its focus on the Pakistani population, where consanguinity increases the prevalence of autosomal recessive disorders like Glanzmann Thrombasthenia (GT). As the first local study to use flow cytometry for GT classification, it provides essential baseline data for genetic counseling, explores GT subtype-bleeding severity correlations, and suggests improved management strategies.

CONFLICT OF INTEREST

None

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AUTHOR'S CONTRIBUTIONS:

SG: Manuscript Writing

MS: Date Collection

TF: Supervision

FS: Manuscript Editing

GM: Thesis Working

IU: Manuscript Writing

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