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Original Article

Correlation of Platelet Receptor Expression in Glanzmann Thrombasthenia with Clinical Presentation and Haematological Parameters

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ABSTRACT

Objective: To correlate platelet receptor expression with clinico-haematological parameters in patients of Glanzmann Thrombasthenia.
Study Design: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS), Rawalpindi Pakistan, from Jan 2023 to Jan 2024.
Methodology: Patients of Glanzmann thrombasthenia (GT) diagnosed by light transmission aggregometry were evaluated for the expression of CD 41 and CD 61 on platelet surface by flow cytometry. Data was assessed using BD FACS DIVA software. The correlation between the haematological parameters, clinical presentation based on bleeding assessment tool (BAT), and GT subtypes based on flow cytometric analysis was assessed.
Results: A total of 73 patients of GT were included in this study with mean age of 5.24±1.86 years. There were 31 males (42.47%) and 42 females (63.01%). Out of these patients, 46(63.01%) were classified as Type I (CD41/CD61 was absent or less than 5%). 21(28.77%), as Type II (5-25% CD41/CD61) and 06(8.22%) as Type III (CD41/CD61 greater than 25%). BAT score showed a significant, strong and negative correlation with platelet receptor expression (r=0.607, p<0.001) showing higher scores in Type I GT with median score of 9 (IQR 8-11) as compared to Type II with median score of 8 (IQR 7-9) and Type III with median score of 6 (IQR 5-7). Epistaxis was the most common clinical feature.
Conclusion: Type I Glanzmann Thrombasthenia (GT) was found to be the most common in our population. Bleeding Assessment Tool score had significant correlation with platelet receptor expression and GT Types with lowered scores in Type III as compared to Types I and II.
Keywords: Bleeding Assessment Tool, Flow Cytometry, Glanzmann Thrombasthenia.

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INTRODUCTION

In 1918, Eduard Glanzmann, a Swiss physician, was the first to describe Glanzmann thrombasthenia (GT), a kind of purpura in which patients had prolonged bleeding time, no or little clot retraction, however platelet count and size were normal.[1] GT is uncommon, with an incidence of one in a million, yet it is more common in regions where consanguineous marriage is prevalent.[2] Hallmark of disease is the failure of platelets to bind fibrinogen and form aggregates when various physiological agonists such as collagen, adenosine diphosphate (ADP), thrombin or epinephrine are present.

Clinical evaluation, complete blood counts, peripheral blood films with platelet morphology, and several diagnostic modalities like light transmission aggregometry and flow cytometry are all required for the diagnosis of the disease. Platelet function studies by light transmission aggregometry is gold standard for diagnosis of GT.[3] The pathognomonic feature of this condition is platelet aggregation in the presence of ristocetin, but not in the presence of other physiological agonists.[4,5]

Flow cytometry is done to quantify platelet receptor expression in GT by using monoclonal antibodies against CD41 which is a marker for αIIb domain of integrin αIIbβ3 receptor and CD61 for β3 domain of the receptor. This allows the classification of Type I GT with <5% expression, Type II GT with 5–25% expression and Type III or "variant" GT in which there is a functional defect in αIIbβ3 integrin with normal or near normal expression (25% to 100%).[5-9] Although genetic testing is not cost-effective and has minimal influence on patient management, it is performed for confirmation in equivocal cases with strong clinical suspicion to find DNA mutations in ITGA2B (aIIb) and ITGB3 (B3) on chromosomes 17.[10]

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Our study was conducted so that clinicians in resource-constrained settings can assess the severity of GT using BAT score and complete blood counts.

METHODOLOGY

This cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi Pakistan, between January 2023 to January 2024 for evaluation of bleeding diathesis. The study protocol was approved by the institutional ethics committee (Certificate No. FC-HEM22-16/READ-RB/23/2476 Dated 15 Apr 2023).

Inclusion Criteria: All patients diagnosed with Glanzmann thrombasthenia by light transmission aggregometry, irrespective of age or gender, were included.

Exclusion Criteria: Patients with acquired bleeding diatheses and those on regular antiplatelet drugs were excluded.

Sample size was calculated using WHO calculator taking reported probability of Glanzmann Thrombasthenia as 5%.[11] This came out to be 73. Informed consent was taken from participants, or their parents in case of minors. Non-probability consecutive sampling was used to recruit participants.

Diagnosis of patients of Glanzmann Thrombasthenia (GT) was established as per diagnostic algorithm starting with bleeding history using bleeding assessment tool score, platelet counts using automated haematology analyzer and peripheral blood film for confirmation of platelet counts and platelet morphology, abnormal bleeding time with normal PT and APTT, normal response to ristocetin and no response to collagen, ADP and epinephrine on light transmission aggregometry. Flow cytometry of these cases was done for CD41 and CD61 monoclonal antibodies and remaining hematological parameters of complete blood picture were also recorded.

Bleeding assessment tool (BAT) was first introduced by International Society of Thrombosis and Hemostasis (ISTH) as a comprehensive tool to assess severity of bleeding in patients with suspected bleeding disorders.[12] It includes 14 different bleeding parameters with grading of each parameter. It is employed as a preliminary screening method to identify heritable bleeding disorders. The BAT score's normal reference ranges are 0–3 for adult males, 0–5 for females, and 0–2 for the pediatric age group (less than 18 years). It has also been established that patients with bleeding disorders tend to have higher BAT score as compared to normal population.

A routine haemogram/ complete blood count was done on a seven-part haematology cell analyzer (Sysmex XN 3000). Peripheral blood films were stained using Leishman stain and examined for manual platelet counts and morphology. Duke's method was used to perform bleeding time test (normal range 2-7 min). Light transmission aggregometry was done on Chrono-Log Model 700 using platelet rich plasma (PRP) of patients. Concentration of agonists like ADP was 2 × 10−5 M AA at 500 µg/ml, epinephrine at 1.0 × 10−4 M and ristocetin at 2 mg/ml.

Blood samples (3 ml) of patients were collected for flow cytometry analysis in EDTA tubes. Platelet count was maintained at approximately 1.5-2 × 109/l in three tubes. Monoclonal antibodies CD41 (FITC/Per CP-Cy-5.5) and CD61 were added in the first tube and CD42a (FITC) and CD42b (PE-A) in the second tube. Controls were run to test samples using BD Biosciences antibodies. Before samples were run on the flow cytometer (BD FACS Canto-6 color), they were incubated for 20 minutes. BD FACS Diva software was used for all analysis and interpretation. Using the unstained tube serving as a negative reference, the flow cytometry results were presented as percentages measuring positive cells above a predetermined threshold. Additionally, adjustments were made for fluorochrome spectrum overlap.

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 26. Mean and standard deviation were calculated for quantitative variables including age, CD41 and CD61 levels, Hb, Platelet count, TLC. Median/IQR was calculated for BAT score, MCV and MCH. Qualitative variables like gender, clinical features, GT Type were recorded in terms of frequency and percentages. Qualitative variables like clinical features were compared across GT Types using the Chi square test, while quantitative variables were compared using ANOVA for Hb, platelet count and TLC. Non-parametric Kruskal-Wallis test was used for age of presentation, MCV and MCH. Correlation analysis was done using Pearson's correlation (r) to measure strength of association of platelet receptor expression with clinical phenoType evaluated as BAT score and haematological parameters. Paired t-test was used to compare means of CD41 and CD61 levels. A p-value of<0.05 was considered significant.

RESULTS

A total of 73 patients were examined in our study which included 31 males (42.47%) and 42 females (63.01%). Out of all cases, 46 patients (63.01%) were classified as Type I (CD41/CD61 was absent or less than 5%), 21 patients (28.77%) were classified as Type II (5-25% CD41/CD61) and 06 patients (8.22%) as Type III or GT Variants (CD41/CD61 greater than 25%).

Table-I shows mean age and common clinical signs and symptoms among patients with different Types of GT. Age of presentation showed a statistically significant association between Types of GT (p<0.001). Epistaxis was found to be the most common symptom in 56.16%±11.38% followed by gum bleed in 46.57%±11.45%, menorrhagia in 35.71%±10.98% females and petechiae in 32.87%±14.49%. No significant association was seen between clinical features and GT Types (p>0.05) except for petechiae (p=0.019).

Table-I: Age of Presentation and Clinical Features in different Glanzmann Thrombasthenia Types (n=73)

Ser No.	Variable		Type I GT (n=46) n(%)	Type II GT (n=21) n(%)	Type III GT (n=06) n(%)	Total (n=73) n(%)	p-value
1.	Age (years) Mean±SD		4.12±0.30	7.03±0.311	7.83±1.88	5.24±1.86	<0.001
2.	Clinical Features	Epistaxis	25(54.32%)	13(61.90%)	03(50%)	41(56.16%)	0.804
3.		Gum Bleed	19(41.31%)	11(52.38%)	04(66.67%)	34(46.57%)	0.412
4.		Petechiae	12(26.09%)	07(33.33%)	05(83.33%)	24(32.87%)	0.019
5.		GI Bleed	03(6.54%)	01(4.76%)	0	04(5.47%)	0.570
6.		Hemarthrosis	02(4.34%)	0	0	02(2.73%)	0.547
7.		Menorrhagia	10(21.74%)	03(14.28%)	02(33.33%)	15/42(35.71%)	0.576

Figure shows scatter plot for correlation of BAT score of patients corresponding to their percentage receptor expression in different Types of GT. BAT score showed significant, strong and negative correlation with platelet receptor expression (Pearson's correlation coefficient, r=0.607, p<0.001), showing higher scores in Type I GT with median score of 9 (IQR 8-11) as compared to Type II with median score of 8 (IQR 7-9) and Type III with median score of 6 (IQR 5-7).

Table-II shows mean values of haematological parameters in different Types of GT. Mean haemoglobin was found to be 9.39±0.25 g/dL, mean platelet count was 202±8.52 x 109/L with normal platelet morphology and mean TLC was 7.96±0.29 x 109/L.

No significant association was seen between haematological parameters and Types of GT (p-value>0.05).

Table-III shows results of correlation analysis of platelet receptor expression with haematological parameters. No significant correlation of platelet receptor expression was established with haematological parameters (p-value>0.05), due to presentation of patients at different ages or stages of disease progression and possible confounding factors like iron deficiency anemia as many patients had microcytic hypochromic anemia.

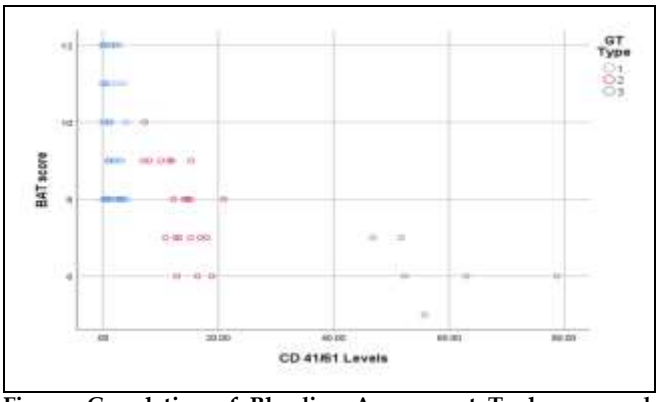

Figure: Correlation of Bleeding Assessment Tool score and platelet receptor expression in Glanzmann Thrombasthenia (n=73)

Table-II: Haematological parameters in Glanzmann Thrombasthenia Types (n=73)

Ser No.	Parameter	Type I GT (n=46)	Type II GT (n=21)	Type III GT (n=06)	p-value
1.	Haemoglobin (g/dL)	9.34±1.08	9.43±1.09	9.62±1.07	0.822
2.	Platelet count (x 109/L)	206.59±35.65	191.57±39.56	203.33±29.40	0.298
3.	TLC (x 109/L)	7.70±1.28	8.34±0.85	8.62±1.76	0.060
4.	MCV (fL) Median (IQR)	70.32 (69.91-81.68)	70.35 (69.75-81.13)	80.50 (78.50-82.62)	0.211
5.	MCH (pg) Median (IQR)	26.25 (21.22-28.23)	24.57 (20.41-29.55)	27.26 (21.78-31.74)	0.478

Table-III: Correlation of Platelet Receptor Expression with haematological Parameters (n=73)

Ser No.	Parameter	Pearson's correlation coefficient (r)	p-value
1.	Haemoglobin	0.063	0.595
2.	Platelet count	0.017	0.884
3.	TLC	0.210	0.075
4.	MCV	0.181	0.125
5.	MCH	0.079	0.509

Table-IV: Mean CD41 and CD61 levels in Glanzmann Thrombasthenia (n=73)

Ser No.	GT Type	Mean CD41 levels (Mean±SD)	Mean CD61 levels (Mean±SD)	p-value
1.	Type I (n=46)	2.43±4.31	15.97±26.36	0.001
2.	Type II (n=21)	13.48±3.70	22.39±20.25	0.050
3.	Type III (n=06)	58.01±11.45	76.14±19.71	0.047
4.	Total (n=73)	10.18±16.02	22.76±29.02	<0.001

Table-IV shows mean CD41 and CD61 levels in Types of GT. Mean CD41 levels were found to be

significantly lower as compared to mean CD61 levels in all cases of GT (p<0.001).

DISCUSSION

The objective of this study was to evaluate platelet receptor expression in GT cases of Pakistani population and correlate it with BAT score. Out of a total of 73, 46 patients (63.01%) were classified as Type I, 21 patients (28.77%) were classified as Type II and 06 patients (8.22%) as Type III or GT variants. Mutreja *et al.,* conducted a similar study in India and reported 24(47%) as Type I, six (11.8%) patients as Type II (5-20% CD41/CD61) and 21(41.2%) as Type III.[13] Farsinejad *et al.,* reported similar results in Iran.[14] Kannan *et al.,* and Canault *et al.,* also reported Type I GT as most common.[15,16]

Patients with Type I GT presented at an earlier mean age of 4.1±0.30 years due to severe clinical manifestation of disease as compared to Type II GT presenting at a mean age of 7.0±0.311 years and Type III GT presenting at mean age of 7.83±1.88 years. Mutreja *et al.,* reported that out of all patients of GT, thirty-three (64.7%) patients were diagnosed at an early age of less than six years, 16(31.4%) between the age of 6 and 16 years and only two (3.9%) patients above 16 years.[13] Mathews *et al.,* reported mean age of diagnosis of 1 year and 15% of GT patients presenting above age of 14 years.[17]

BAT score showed significant correlation with platelet receptor expression and GT Types in our study (r=0.607, p-value<0.001) with median score of 9 (IQR 11-8), as compared to Type II with median score of 8 (IQR 9-7) and Type III with median score of 6 (IQR 7-5.75). Mutreja *et al.,* used WHO bleeding assessment scale instead of BAT score for correlating bleeding severity with GT subtypes based on percentage receptor expression. No significant correlation was reported between bleeding scores and GT subtypes I and II in that study, however Type III GT variants showed significantly lower bleeding scores (WHO score III) with (p-value<0.034).[13] Earlier studies have not reported any significant correlation between the bleeding severity and the GT subTypes.[15-18] This can be attributed to the use of less comprehensive tools for quantification of bleeding severity in comparison with BAT as it includes 14 parameters for scoring and its use is validated by ISTH and BSH in heritable platelet function disorders.[19] BAT score in GT subtypes varies in relation with CD41 and CD61 expression.

A significant proportion of patients presented with microcytic hypochromic anemia (45%±10.45%), which can be attributed to age at presentation as all cases were children having bleeding episodes as well. Toogeh *et al.,* reported that in 832 total patients of GT, Mean Corpuscular Volume (MCV) was less than 80 fL in 66.8% of patients, with the possibility of iron deficiency as a cause of anemia.[20]

Epistaxis and petechial hemorrhage were the most common complaints in our study. Whereas, menorrhagia was the most common complaint in female patients who were in their pubertal years. These results were coherent with other reports in literature.[20,21]

Lower CD41 mean levels in comparison to CD61 were in concordance with other studies who reported similar results attributing this to spuriously false increase in CD61 expression due to adherence of platelets to fragments of white blood cells. It was corrected by excluding debris and WBCs from gated population.[13,14] One study that used western blot analysis also reported decreased expression of GPIIb as compared to GPIIIa.[14] Specific molecular mutations may be responsible for this heterogenous expression for which larger studies with molecular analysis are needed to be done in our population. Mutations in ITGA2B and ITGB3 genes including novel mutations have been reported in Pakistani population.[22,23]

LIMITATONS OF THE STUDY

Molecular studies of the patients were not done to find out molecular landscape of GT in our population and correlate it with GT subtypes and disease severity. Antiplatelet monoclonal antibodies like PAC-1 that recognize activation induced conformational epitope of GPIIb/IIIa receptor can be used to recognize activated GPIIb/IIIa complex as it will differentiate actual GT variants with functional abnormality from acquired GT. However, this test was not performed in our study.

CONCLUSION

Type I Glanzmann Thrombasthenia (GT) was found to be the most common in our population. Bleeding Assessment Tool score had significant correlation with platelet receptor expression and GT Types with lowered scores in Type III as compared to Types I and II.

Conflict of Interest: None.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AC & MB: Data acquisition, data analysis, critical review, approval of the final version to be published.

HSM & MH: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

RM, AK, AK & SSS: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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