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Glanzmann's Thrombasthenia, Correlation by Flow, Platelet Aggregometry and Platelet Function Assay (PFA)

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Objective: To evaluate Glanzmann's Thrombasthenia (GT) as an inherited bleeding disorder, its age and gender distribution.

Design: A Retrospective Study.

Setting: King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Method: Patients were tested for Glanzmann's Thrombasthenia by platelet immunophenotyping from January 2005 to December 2009. The results of platelet immunophenotyping were compared with PFA and platelet aggregometry.

Result: Thirty-three (77.3%) were type I, 7 (13.6%) were type II and 4 (9.1%) were type III. GT was mainly found in females and in pediatric age group. There was a good correlation between flow cytometry study and aggregation study.

Conclusion: GT is a common bleeding disorder in a community where consanguineous marriage is common.

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Glanzmann's thrombasthenia (GT) is an inherited bleeding disorder which was described in 1918 by Dr. Glanzmann as bleeding tendency with normal platelet count and prolonged bleeding time; it was called hereditary hemorrhagic thrombasthenia[1,2]. It is an autosomal recessive bleeding disorder, which is common in consanguineous marriage due to mutation of platelet glycoprotein. Glycoprotein IIb/IIIa leads to loss of GP receptor function, detected as abnormality in platelet aggregation, adhesion and clot retraction. Patients present as epistaxis, menorrhagia, gum bleeding, bruises, ecchymosis and petechia. Laboratory investigations reveal normal platelet count, but abnormal clot retraction, high PFA, absent response to all agonists except ristocetin induced aggregation by platelet aggregometry and decreased or absent platelet glycoprotein by flow cytometry.

Type I GT is severe; it is less than 5% of normal GPIIb/IIIa; type II is less severe with 10%-20% of normal glycoprotein IIb/IIIa and type III has normal level of GPIIb-IIIa, but with dysfunction.

The aim of this study is to correlate the three types of GT diagnosis between flow cytometry, platelet aggregation and platelet function assay PFA.

METHOD

Forty-four patients were diagnosed as GT by platelet immunophenotyping from January 2005 to December 2009. The results of platelet immunophenotyping were compared with PFA and platelet aggregometry.

Flow cytometry analysis (FACS) cases for platelet membrane glycoprotein, using monoclonal antibodies (CD41, CD61, CD42a and CD42b) for GT were reviewed.

Platelet aggregometry on laser 4X is used to diagnose GT with aggregating agents at varying concentration of Arachidonic acid, Adenosine Diphosphate, Collagen and Ristocetin.

PFA-100, Platelet function assay measures the closure time (CT) of platelet adhesion and aggregation with a cartridge system, coated with collagen/epinephrine (COL/EPI) as the primary cartridge or collagen/ADP (COL/ADP) as the secondary cartridge.

Cases tested by flow cytometry were included. However, all the cases which showed intact expression of platelet glycoprotein, normal platelet aggregation and normal PFA were excluded.

RESULT

Forty-four GT cases were diagnosed from 2005 to 2009. Nineteen (43.2%) were males and 25 (56.8%) were females. Eight (18.2%) were ≥14 years and 36 (81.8%) were <14 years. The youngest was 4 weeks and the eldest was 40 years.

The result revealed that 33 (77.3%) were type I GT, 7 (13.6%) were type III and were 4 (9.1%) were type III, see table 1. Three of the familial cases were type I, one was type II and one was type III.

Table 1: Total GT Cases Tested by Flow Cytometry

	Number and Percentage
GT1	34 (77.3)
GT2	6 (13.6)
GT3	4 (9.1)
Total	**44**

There was a good correlation between flow cytometry study and aggregation study, see table 2. The twenty-four cases which were tested by flow cytometry and platelet aggregation showed a very high correlation. All the 32 cases which were tested by flow cytometry and PFA showed a very good correlation, see table 3. PFA, flow cytometry and aggregometry showed dependable correlation in GT if combined.

Table 2: GT Cases by Flow Cytometry and Aggregation

Flow	Aggregation
GT1	15 (34.1%)
GT2	5 (11.4%)
GT3	4 (9.1%
Total	24 (54.9%)

*Only 24 out of 44 were tested by Flow Cytometry and Aggregation

Table 3: GT Cases by Flow Cytometry and PFA

Flow	PFA
GT1	23 (52.3%)
GT2	5 (11.4%)
GT3	4 (9.1%)
Total	32 (72.7%)

*Only 32 out of 44 were tested by Flow Cytometry and PFA

DISCUSSION

In hereditary bleeding disorder, the medical and family history of the patient is very important for initial diagnosis of GT. The bleeding history and physical examination of the nature of bleeding is essential because in GT: purpura, petechiae and easy bruising are considered to be common[3]. Laboratory investigations are used to confirm the diagnosis of GT.

In our study, 44 cases of GT showed slight female predominance with familial inheritance. Flow cytometry is the primary diagnostic test for GT type I and type II, which would reveal deficiency in glycoprotein antibodies. Flow cytometry have a good correlation with platelet aggregometry and PFA.

Type III GT is not easy to diagnose due to normal expression of the glycoprotiens, by cytometry, therefore PFA and aggregometry are to be considered important.

A study in Saudi Arabia revealed that 12 cases of GT were among 34 inherited bleeding disorders[4]. Another study in Saudi Arabia showed 18 GT cases among 168 cases of hereditary bleeding disorders[1].

In another study, sixteen Saudi patients presented at an age of less than 15 years, the age range was from birth to 14 years. Positive history of first degree consanguinity was observed in 93% of the patients[5]. It showed, however, a slight male predominance (56%), while in our study there is female predominance; however, this finding is insignificant due to the recessive inheritance pattern of the disease[5].

Thirty-one patients were diagnosed in another study in 2005 in Saudi Arabia and have been described as the third common hereditary bleeding disorder[1].

In our study, the diagnosis is limited to PFA, flow cytometry and platelet aggregometry; however, molecular testing was not performed, which is the most dependable confirmatory test. At the present time, 38 mutations in GPIIb and 25 mutations in GPIIIa have been recorded[6,7].

Mutations causing GT affect either ITGA2B or ITGB3. GT is an autosomal recessive disease; patients are mostly compound heterozygotes for ITGA2B or ITGB3 mutations. ITGA2B gene encodes for the aIIb subunit, whereas ITGB3 gene encodes for b3[2,8].

CONCLUSION

Type I GT is the most common in KSA and females are affected predominantly. There is good correlation between flow cytometry and platelet aggregation.

Type III variant GT is difficult to exclude by flow cytometry only. We recommend that flow cytometry is to be used as a screening test for GT cases. However, if the flow cytometry result is normal, platelet aggregation is recommended. Further study is recommended to correlate clinical severity and type of GT.

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