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RESEARCH ARTICLE

Glanzmann's Thrombasthenia in Iraq

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Abstract:
Aim: To study the clinical presentation of patients with *Glanzmann's* thrombasthenia (GT) and to correlate their clinical presentation and laboratory findings.
Method: This is prospective study was carried out during the period 1stof January 2013 to end of December 2013 in the National Center of Hematology NCH, Baghdad/Iraq. Forty eight patients were enrolled in the study who were diagnosed as *Glanzmann's* thrombasthenia, medical history and clinical data were recorded, with the basic laboratory parameters includes: complete blood count (CBC), peripheral blood smear (PBS), prothrombin time(PT), activated partial thromoboplastin time (APTT), bleeding time (BT) and platelet aggregometry.
Result: Forty eight patients were diagnosed in one year as GT, below 10 years were (68.75%), while below 20 years (79.16%), with male: female ratio 1.08:1. Regarding residency most of the patients were from Baghdad (39.58%), the second region was middle Euphrates (18.75%). From North of Iraq (16.66%), the last from the west and the east region each had equal percent (8.33%).Arabic patients were the majority (95.83%) and (4.16%) were Kurdish. The consanguineous marriages were noticed in most of the families (93.75%). Mucocutaneous bleedings were the most common type of bleeding which includes ecchymosis or petechiae then epistaxis followed by gum bleeding, menorrhagia was found in all females in the reproductive age group. The significant finding in laboratory test was prolonged bleeding time seen in (97.91%), platelet aggregation study was performed for all patients, all of them showed absent or impaired aggregation with ADP, collagen, and epinephrine while disaggregation with high dose of ristocetin except in one whodisplay normal aggregation pattern with high dose of ristocetin. Hence most of our patients presented with the classical symptoms and laboratory findings of GT however some had unusual findings.
Conclusion: GT cannot be regarded as rare disease in Iraq, mostly seen in Arab ethnic group. Another study is recommended with longer time to identify the true incidence of GT in Iraq. Although basic hemostatic screening tests and platelet aggregometry are sufficient to diagnose the majority of patients, yet some may require more sophisticated tests like flow cytometry for glycoprotein (GP) IIb-IIIa and cytogenetic study.

Key Words: *Glanzmann's thrombasthenia, consanguineous marriage, platelet aggregation.*

Introduction

Glanzmann's thrombasthenia is a hereditary disease, was first described by a German pediatrician Dr. Glanzmann in 1918. He designated it as "hereditary hemorrhagic thrombasthenia (1). This disease is inherited as autosomal recessive pattern and is regarded as the most common inherited abnormality of platelets. Its prevalence is high in areas with custom of consanguineous marriage (2). It is extremely rare except in populations (such as Jordan, India, Saudi Arabia and Iraqi-Jews and Arabs living in occupied Palestine, where consanguinity is common (3). This disorder characterized by a quantitative or qualitative abnormality of platelet glycoprotein (GP), GPIIb -IIIa receptors(3). Impaired platelet aggregation is the hallmark of GT,it has beenclassified into three types:

*Type 1 (severe): GPIIb/IIIa<5% of normal levels with no platelet aggregation, no clot retraction.
*Type 2 (moderate): GPIIb/IIIa10-20% of normal level with no platelet aggregation, residual clot retraction.
*Type 3 (mild): Normal levels of receptors, but GPIIb/IIIa with dysfunctional action withno or very abnormal platelet aggregation, and clot retraction variably affected (4).

There are more than one hundred genetic defects have been found ranging from point mutations, small deletions and inversions occurring with even distribution on both αIIb and β3 genes. These defects lead to disruption

of GPIIb-IIIa synthesis receptor and/or function (5). Molecular biology techniques have been shown to diagnose heterozygote carriers who are asymptomatic (3). The prognosis of GT is reported to be a severe disease but with generally good survival (6).

Materials and Methods

This study is a prospective study was carried out during the period from 1st of January 2013 to end of December 2013 (one year) in the National Center of Hematology in Baghdad/ Iraq. Clinical data included: age, gender, type of bleeding, family history of bleeding and consanguineous marriages. Laboratory investigations included hemostatic screening tests: complete blood count (CBC) by Hemolyzer 5 (Analyticon), peripheral blood smear (PBS) stained with Leishmen stain, prothrombin time (PT) normal value N.V. (13-16 seconds) and activated partial thromoboplastin time (APTT) N.V. (30-40 seconds) by using semi-automated bench top Hemostasis coagulation analyzer (Diagnostica Stago, model: ST ART, France), bleeding time (BT) N.V. (1-10 sec). Platelet aggregation studies with adenosine diphosphate (ADP) 10 µM/ml, collagen 2 µg/ml, epinephrine 10 µM and ristocetin induced platelet aggregation (RIPA) 1-1.5 mg/ml were done on Light transmission aggregometry LTA [Platelet Aggregation Profiler (PAP-E8), Bio/DATA corporation, USA].

Peripheral blood samples were collected from each patient. The blood (10 ml) was collected by venipuncture, and it was drawn into three tubes; the first was EDTA tube for CBC and Blood picture, the other tubes were Sodium Citrate (1.1M), to perform the coagulation tests and platelet aggregation studies. The blood samples were processed within 3 hours.

Platelet aggregometry was determined through measuring OD (Optical Density) of mixed platelet rich plasma (PRP) after adding the agonist to aggregometer cell. Most aggregometers can be standardized and calibrated by putting patients'PRP in the holder place of cell which indicates 0% of light transition; and putting platelet poor plasma (PPP) in it, 100% of light transition occurs. Increase in light transition from 0% to 100% in recording curve or digital displayer reflects that aggregometer is reading. When the agonist is added to PRP, platelets start aggregating which results in the rise of light transition. This light is recorded and used as platelet activation standard, the percentage of aggregation was compared with controls (normal individual) and the normal reference ranges provided from the company. This study was approved by ethical committee of the National Center of Hematology. Finally the results were statistically analyzed by computer programmed statistical package of social sciences (SPSS) version 21.

Results

Forty eight patients were diagnosed in one year as GT during the current study, with a normal platelet count and morphology, normal PT and APTT, prolonged bleeding time (BT), and absent platelet aggregation to ADP, epinephrine, and collagen.

Age range from two months to 57 years, with the mean age of the patients 28.58 years, below 10 years of age were 33 patients (68.75%), while below 20 years were 38 patients (79.16%), as shown in Table-1. The youngest was two months old female while the oldest female was 39 years old and the oldest male patient was 57 years old.

There were 25 male and 23 females, with male: female ratio 1.08:1.

Regarding residency most of the patients were from the capital of Iraq Baghdad 19 patients (39.58%), the second region was middle Euphrates which includes Karbala, Najaf and Babylon had 13 patients (18.75%). From North of Iraq we had 8 patients (16.66%) from Kirkuk and Salah Al-Deen, from the west (Al-Anbar) and the east region (Wasit) each had 4 patients with equal percent (8.33%), as illustrated in Table-2, Figure-1.

Forty six patients (95.83%) were Arabic and only 2 patients (4.16%) were Kurdish, which represent the ethnic group distribution.

The consanguineous marriages in parents were noticed in most of the families 45 patients out of 48 (93.75%), 64% of them the parents were first cousins; while 36% they were second cousins; and for the remaining 3 patients (6.25%), there was no apparent relationship.

A positive family history in siblings and/or other family members was found in 41 patients (85.41%), while a negative family history was seen in 7 patients (14.58%).

Regarding the type of bleeding most of the patients had mucocutaneous bleedings; the commonest was ecchymosis or petechiae (83.33%) then epistaxis (54.16%) followed by gum bleeding (47.91%) and prolonged bleeding from the trauma site (45.83%).Menorrhagia was found in all females in the reproductive age group, 8 females ranging from (12-39) years (100%), hence menorrhagia was the most common problem bothering females with their parents, no one of the females were married. Amore serious bleeding was observed less: melena in 6 patients (12.5%), hematemesis and hematuria were found in 2 patients (4.16%). Hemarthrosis was found in only one female patient 30

years old (2.08%), as shown in (Table-3) ,Figure -2.All 48 patients had a normal platelet count and morphology, normal PT and APTT as was expected in GT. Forty seven patients had a prolonged BT (97.91%), whereas 1 patient showed a normal BT (2.08%) (Table-4). Platelet aggregation study was performed for all patients, all of them showed absent or impaired aggregation with ADP, collagen, and epinephrine while disaggregation with high dose of ristocetin (Figure-3), except in one whodisplay normal aggregation pattern with high dose of ristocetin (Figure-4), while Figure-5 showed platelet aggregation pattern of normal aggregation with high dose of Ristocetin (1.2mg/ml) in control- 1- and disaggregation pattern in 47 patients -2-.

Table-1Distribution of the patients according to the age groups (n= 48).

Age Group	No. of patient	Percentage
≥ 1 year	3	6.25%
1-10 year	30	62.50%
11-20 year	8	16.66%
≤ 20 year	7	14.58%

Table-2: Residency of 48 G.T patients.

Residency	Patient No.	Percentage
Middle of Iraq: Baghdad	19	39.58%
Middle Euphrates: Najaf	4	18.75%
Middle Euphrates: Karbala	4	
Middle Euphrates: Babylon	5	
North of Iraq: Kirkuk	3	16.66%
North of Iraq: Salah Alden	5	
West of Iraq Al an bar	4	8.33%
East of Iraq Wasit	4	8.33%

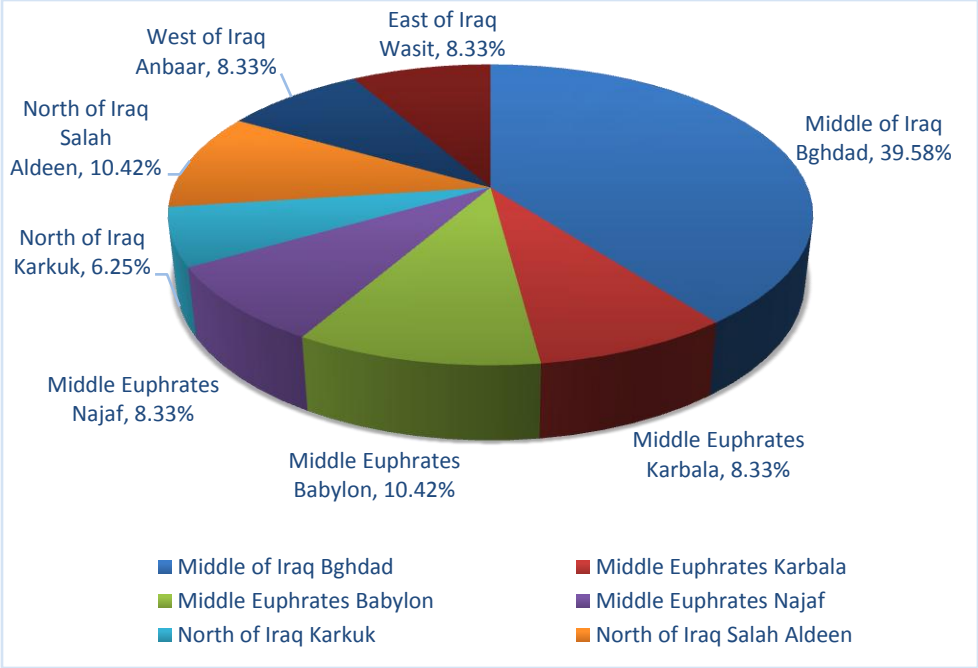


Figure -1

Table 3: Spectrum of clinical presentation in the patients (n=48).

Type of bleeding	No. ofpatients*	Percentage
Ecchymosis or Petechiae	40	83.33%
Epistaxis	26	54.16%
Gum bleeding	23	47.91%
Prolonged bleeding from trauma site	22	45.83%
Menorrhagia	9	100%
Melena	6	12.50%
Hematemesis	2	4.16%
Haematuria	2	4.16%
Hemarthrosis	1	2.08%

*The total number is greater than 48 because most of the patients had more than one bleeding symptom.

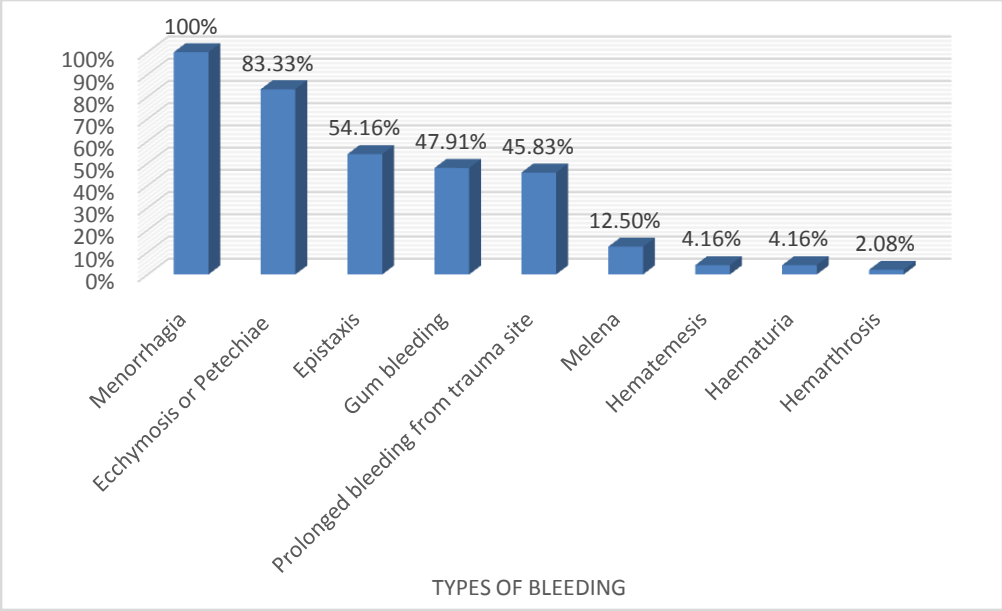
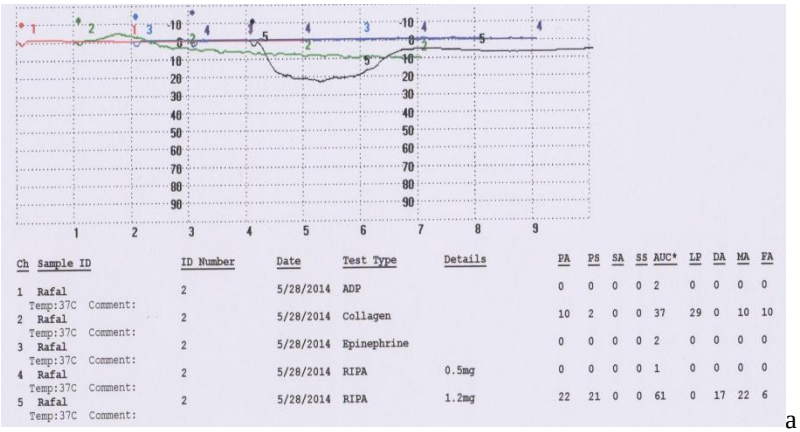


Figure-2

Table 4: Hemostatic screening tests in the patients (n= 48).

Test	Result	Percentage
Bleeding Time	Normal: 1	2.08%
	Prolonged: 47	97.91%
Platelet count	Normal: 48	100%
PT and APTT	Normal: 48	100%

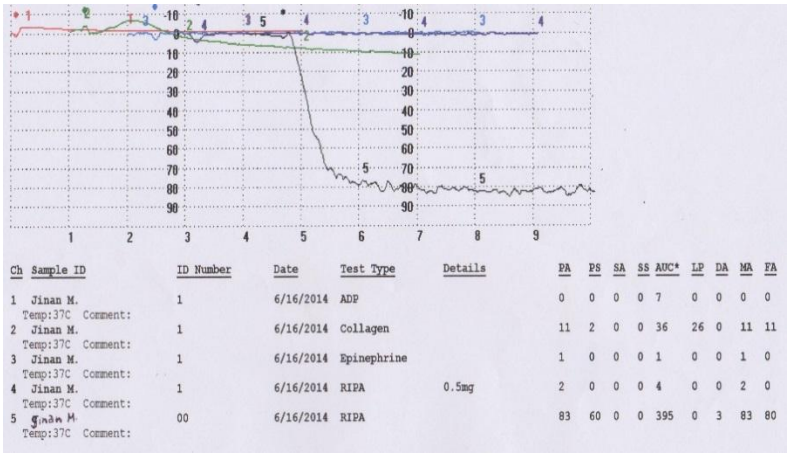


a

Ch	Sample ID	ID Number	Date	Test Type	Details	PA	PS	SA	SS	AUC*	LP	DA	MA	FA
1	c Temp:37C Comment:	0	5/29/2014	ADP		69	49	0	0	301	0	0	69	68
2	c Temp:37C Comment:	0	5/29/2014	Collagen		70	49	0	0	334	34	0	70	68
3	c Temp:37C Comment:	0	5/29/2014	Epinephrine		16	10	55	9	257	0	0	71	70
4	c Temp:37C Comment:	0	5/29/2014	RIPA		2	0	0	0	6	0	0	2	0
5	c Temp:37C Comment:	0	5/29/2014	RIPA		25	19	49	35	338	0	0	74	72

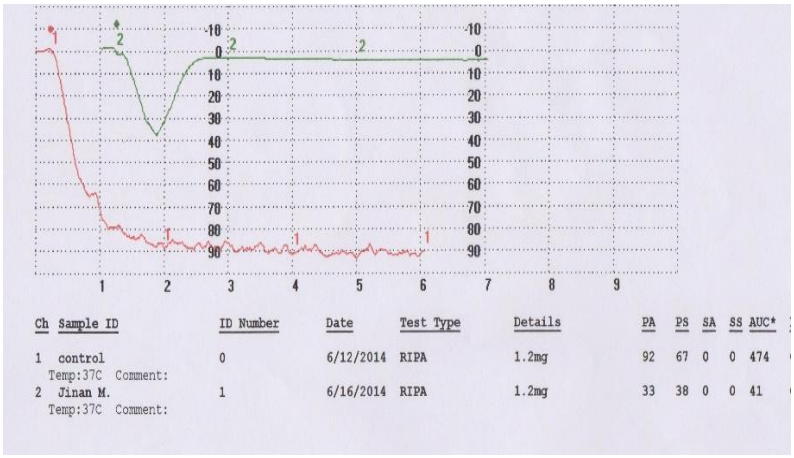
b

Figure-3: Platelet aggregation pattern with ADP, Collagen, Epinephrine and Ristocetin in G T patient (a) and control (b)



Ch	Sample ID	ID Number	Date	Test Type	Details	PA	PS	SA	SS	AUC*	LP	DA	MA	FA
1	Jinan M. Temp:37C Comment:	1	6/16/2014	ADP		0	0	0	0	7	0	0	0	0
2	Jinan M. Temp:37C Comment:	1	6/16/2014	Collagen		11	2	0	0	36	26	0	11	11
3	Jinan M. Temp:37C Comment:	1	6/16/2014	Epinephrine		1	0	0	0	1	0	0	1	0
4	Jinan M. Temp:37C Comment:	1	6/16/2014	RIPA	0.5mg	2	0	0	0	4	0	0	2	0
5	Jinan M. Temp:37C Comment:	00	6/16/2014	RIPA		83	60	0	0	395	0	3	83	80

Figure-4: Platelet aggregation pattern in GT showing normal aggregation with Ristocetin but decreased aggregation with other agonists



Ch	Sample ID	ID Number	Date	Test Type	Details	PA	PS	SA	SS	AUC*
1	control Temp:37C Comment:	0	6/12/2014	RIPA	1.2mg	92	67	0	0	474
2	Jinan M. Temp:37C Comment:	1	6/16/2014	RIPA	1.2mg	33	38	0	0	41

Figure-5: Platelet aggregation pattern showing normal aggregation with high dose of Ristocetin (1.2mg/ml) in control -1- and disaggregation pattern in 47 patients -2-.

Discussion

This study reflects the diversity of the clinical features of GT and that it is not rare in Iraq. It is clear that GT is not only prevalent in Iraqi- Jews patients (3), GT was found in forty six Arab patients (95.83%) and 2 patients (4.16%) were Kurdish. During this study with one year duration, forty eight cases were diagnosed, the study done in India, which had reported 42 cases of GT in 14-years period from June 1966 to June 1980 (7). While the largest series of 382 patients was investigated in Iran in a retrospective study 1996-2001 (8). A study was conducted retrospectively in Saudi Arabia over 20 year's diagnosed 31 cases (9). The explanation of this large number of cases of GT diagnosed in one year in our study is that the platelet aggregation test was newly introduced in our center and in Iraq that is why most of the cases suspected to have GT were referred from 8 out of 14 governorates in Iraq, although patients from six other governorates, three in the north and three in the south were not included in this study since patients were not referred because of the availability of diagnostic tests there and those governorates are far from our center. Various studies done have shown that GT is a disease of children and young adults with majority of patients being less than 20 years. (2, 6, 10). In our study 79.16% were below 20 years of age also .in the current study the patients ranged from two months to 57 years. The male to female ratio was 1.08:1 in this study, just like other studies done in India and other parts of the world. (6, 10) However, Badhe*et al*. in their study have shown markedly high incidence of GT in females (2).

Consanguineous marriage between parents has been found to be very important risk factor for GT. So the incidence was found high in populations in whom marriage among close relatives is common, like Iran which showed that the consanguinity was 87%, 64%of parents were first cousin and 23% of parents were second cousin and the remaining 13% there were no obvious relationship (8). In our study the consanguineous marriages in parents were higher ,noticed in most of the families 45 patients out of 48 (93.75%), 64% of them the parents were first cousins; while 36% they were second cousins; and for the remaining (6.25%), there was no apparent relationship. While in Saudi Arabia still high percent of consanguinity (84%) but lower than that of Iraq as showed in our study (9). In India consanguineous marriage between parents had been found in 71% (7), whereas in Pakistan the incidence was only 14% (10).A review of 177 patients with GT (of which 113 were literature reports and 64 were seen at the *HôpitalLariboisière*, Paris) 2006, only 12 were from the United States, while 55 patients were from Israel and Jordan, and 42 were from South India. In certain ethnic groups, such as South Indian Hindus, Iraqi Jews, French gypsies and Jordanian nomadic tribes, thrombasthenia may actually bea common hereditary hemorrhagic disorder (11).

The clinical presentations of patients with GT was wide and mostly presented with mucocutaneous bleeding, mainly ecchymosis or petechiae, epistaxis and gum bleeding, this fact was found in other studies (8, 9), so as menorrhagia which was found in all females in the reproductive age group, 9 females ranging from (12-39) years. Our study has illustrated many patients with varying severity and clinical presentations, even presented with more serious gastrointestinal bleeding melena in 12.5% and hematemesis in 4.16%, so as hematuria 4.16%. Even haemarthosis, which is rare presentation, mostly found in sever hemophilic patients, was found in one GT female 2.08%, those results were found in other studies (10), but a higher number of patients with haemarthosis found in Saudi Arabia (13%) (9).Yet life threatening bleeding such as central nervous system bleeding was not encountered, and no fatality was noticed in this study, similar finding was recorded by G. Toogeh (8).

In the current study the classical laboratory diagnostic features of GT were illustrated in all patients, but unexpected findings were also found. Only one patient had normal BT, this was initially thought to be a technical error in the performance of BT, but the BT was repeatedly normal for this patient. This might be a variant form of GT and needs confirmation by flow cytomertry by analyzing the GP IIb-IIIa expression.

A study in Saudi Arabia also had one out of 31 patients with normal BT (9). On the contrary Badhe*et al*. had shown bleeding time more than 10 minutes in 100% of their patients (2). Prolonged BT is an indication of delay in formation of primary hemostatic plug due to defective platelet aggregation. Although BT is an easily available diagnostic lab test for assessing platelet function but the major obstacle its high technical dependency and it is not a précis test to provide a meaningful results (10). The use of PFA-100 system can replace BT tests for GTdiagnosis, and it is used in many hematological centers nowadays. The PFA-100 measures the closure time when blood is passed through collagen-based filters under high shear stressblood from GT patients fail to plug the filters, resulting in prolonged closure time (1).

The classical pattern of platelet aggregation in GT is absent or reduced aggregation with agonists (ADP, collagen, epinephrine) except ristocetin which is typically normal, however the aggregation pattern of the platelets induced by ristocetin (RIPA) was not seen in this study except in one patient, while forty seven patients demonstrated disaggregation pattern, which may suggest a new variant of GT. This finding was supported by Layla B. from Saudi Arabia also had 13 patients out of 31 patients showed disaggregation to ristocetin induced platelet aggregation (9). Although typically ristocetin interacts with platelets through GP Ibα and Von Willebrand factor and does not require

GPIIb-IIIa complex to cause platelet aggregation and hence classically GT patients show normal aggregation with ristocetin (1). Flow cytomertry is needed to confirm the diagnosis of such cases and to distinguish the subtypes.

Conclusion

GT cannot be regarded as rare disease in Iraq, and it is found mainly in Arab ethnic group. GT has varying severity and clinical presentations mainly mucocutaneous bleedings with menorrhagia in all females; it is diagnosed at an early age. Another study is needed with longer time with collaboration with the national registry of the hereditary bleeding diseases in Iraq to identify the true incidence of GT in Iraq. The use of monoclonal antibodies and flow cytometry to quantitate GP IIb-IIIa is recommended to confirm the diagnosis of GT and also identifying the subtypes of the disease. Genetic study as well is recommended to clarify the mutations giving rise to GT in our population.

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