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Letter to the Editors-in-Chief

Triple jeopardy: A case of Glanzmann's thrombasthenia with anti-GPIIb-IIIa antibodies and HPA incompatibility resulting in stillbirth

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1. Case report

Glanzmann's thrombasthenia (GT) is a rare, autosomal recessive genetic coagulopathy, in which platelets have a qualitative or quantitative defect of glycoprotein IIb-IIIa (GPIIb-IIIa) receptors due to mutations in any of the two genes, namely *ITGA2B* or *ITGB3*. The integrin αIIbβ3 (GPIIb-IIIa) is a large transmembrane receptor which consists of αIIb (GPIIb) and β3 (GPIIIa) subunits. This complex plays an important role in platelet aggregation during clot formation. Alloimmunization against human leukocyte antigen (HLA) and GPIIb-IIIa epitopes in GT patients is known due to multiple transfusions or during pregnancy which leads to refractoriness of platelets [1]. These maternal anti-GPIIb-IIIa antibodies may cross placenta and lead to destruction of fetal/neonatal platelets resulting in thrombocytopenia. Thus, pregnancy in these patients is complicated and is also a challenge for the clinicians, as there is a risk for both the mother and the fetus; there are no standard guidelines for the management.

1.1. Case

A 30 year old primigravida type-I GT patient married to a non-consanguineous partner visited Department of Hematology, KEM Hospital during her 29th week of gestation with vaginal bleed. She was diagnosed as GT at 12 years of age at our Institute. Menarche was attained at 13 years of age, wherein she had menorrhagia which was managed with platelet transfusions and anti-fibrinolytic agents like oral tranexamic acid. The patient gave a history of 8 units of platelet transfusion on three occasions, twice due to menorrhagia and once due to haematochezia. She was advised hormonal therapy in the form of low-dose estrogen and progesterone containing pills, which she took for 8 years to control excessive menorrhagia. She did not require any transfusion support subsequently, but discontinued hormonal therapy without medical advice. However, from the age of 16 years, she did not have any bleeding manifestations. This pregnancy was a natural conception and did not require any medical help.

The antepartum vaginal bleeding at 29 weeks was managed with only platelet transfusion and bleeding was completely controlled. Cost and availability of rFVIIa in our country restricted its use in this patient. Thus, a combination of rFVIIa and platelets are being used for managing pregnancy in women with platelet disorders.

Ultrasonography done at 21st and 31st week of gestation showed normal growth of fetus. However, during the 33rd week, USG anomaly scan showed the biparietal diameter (BPD) of 11 cm with hydrocephalus and extra-ventricular haemorrhage in the retro-cerebral region. This finding was confirmed by magnetic resonance imaging (MRI) too. After two days, USG guided cephalocentesis was performed to aspirate out the fluid. Post procedural BPD was found to be 8.1 cm and the fetal cardiac activity was normal. The patient had normal platelet count throughout her pregnancy.

The patient underwent a full term vaginal delivery at 36 weeks of gestation, but it was a stillbirth. Patient did not receive any anesthesia for delivery. She had received 1 dose of rFVIIa (4 mg) prior to delivery and 1 single donor platelet post-delivery as she had mild post-partum haemorrhage, which was controlled with above measures. No thromboprophylaxis or antifibrinolytics were used. Sequential therapy was used due to limited supply of rFVIIa. No clinical reaction to platelets was observed.

However, neonatal platelet count was not done. The management was done with rFVIIa (*NovoSeven®*) and platelets both prior and post-delivery. Patient was discharged on day 7 post-delivery.

The laboratory investigations (Table 1) revealed total absence of GPIIb-IIIa receptors in the mother, whereas her husband showed normal GPIIb-IIIa expression. The mutation analysis by Sangers sequencing of both *ITGA2B* and *ITGB3* of the patient showed a frame-shift mutation [c.1230_1230delC, cDNA.1262_1262delC (Y411Tfs*20)] due to a single base deletion in exon 13 of *ITGA2B* (Fig. 1C). With suspicion of fetal thrombocytopenia due to isoantibodies against GPIIb-IIIa, mixing platelet aggregation studies were done. A 1:2 mixture of normal platelets and patient plasma was incubated at 37 °C for 30 min (Test); normal platelets with normal plasma under similar conditions acted as a control. Platelet aggregation studies with adenosine-5-diphosphate (ADP: 6 μM) revealed inhibition of aggregation in the test sample (8% aggregation), which was not observed in the control sample (108% aggregation), indicating the presence of anti-platelet antibodies (Fig. 1A). These results were further confirmed by mixing studies and subsequent analysis of platelet receptors by flow cytometry. Platelets

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Table 1
Laboratory data of the patient and other family members.

	Normal control	Patient
Basic coagulation screening		
Bleeding time (BT) (min)	1–3	> 10
Prothrombin time (PT) (s)	13.4	12.4
Activated partial thromboplastin time (APTT) (s)	26.8	24.5
Thrombin time (TT) (s)	13.6	11.4
Platelet aggregation		
ADP (6 μM) (%)	96	0
Ristocetin (1.25 mg/mL) (%)	87	83
Collagen (4 μg/mL) (%)	83	0
Platelet receptors		
GPIb (%)	92.6	94.5
GPIX (%)	95.2	91.9
GPIIb-IIIa (%)	91.9	0
GP IIb-IIIa antibody detection		
Platelet aggregation with 6 μM ADP: mixing studies (1:2)		
Control platelets + control plasma (%)	108	
Control platelets + patient plasma (%)	8	
Platelet GPIIb-IIIa receptor studies: mixing studies (1:2)	Platelet number (n = 10,000)	Platelet number (n = 2000)
Control platelets + control plasma (%)	82.1	81.4
Control platelets + patient plasma (%)	59.6	28
HPA genotyping data		
	Father	**Mother (GT case)**
HPA-1	1a/1a	1a/1a
HPA-2	2a/2a	2a/2a
HPA-3	3a/3a	3a/3a
HPA-4	4a/4a	4a/4a
HPA-5	5a/5a	5a/5a
HPA-6	6a/6a	6a/6a
HPA-15	**15b/15b**	**15a/15a**
Mutation data		
GPIIb-IIIa	Wild type	Frameshift mutation in *ITGA2B* (exon13) → [c.1230_1230delC, cDNA.1262_1262delC (Y411Tfs*20)]

Abbreviations: ADP: adenosine-5-diphosphate, HPA: human platelets antigens, GP: glycoprotein.

from normal healthy controls (platelet count 2×10^3/μL and 5×10^3/μL) were incubated with both patient and control plasma for 30 min at 37 °C. After washing 3 times with PBS buffer, fluorescein isothiocyanate (FITC) labeled specific monoclonal antibody against platelet antigen-CD41 (CD41-PE, IgG1, κ, clone HIP8) was used to detect GPIIb-IIIa expression on these platelets. The test sample (normal platelets + patient plasma) showed only 28% GPIIb-IIIa expression as compared to 81.4% GPIIb-IIIa expression seen in the control sample (normal platelets + control plasma) (Fig. 1B) confirming the presence of GPIIb-IIIa antibodies in patient plasma. The couple was also screened for the commonly studied HPA 1 to 6 [2] along with HPA-15 which revealed HPA-15 incompatibility, wherein mother had a/a genotype and father had b/b genotype (Fig. 1D).

Pregnancy in GT involves a major risk for both mother as well as fetus. Maternal risk factors include early miscarriages, antepartum or postpartum haemorrhage; severe late postpartum bleeding can even occur up to 8 weeks after delivery. Fetus has a high risk for developing fetal or neonatal thrombocytopenia (F/NATP) due to iso-GPIIb-IIIa antibodies which may lead to disastrous bleeding manifestations. About 15–30% of GT patients become refractory to platelets due to multiple platelet transfusions and a considerable proportion develops antibodies against GPIIb-IIIa or HLA [1]. It may be caused either due to exposure of incompatible fetal platelet alloantigens to the mother's immune system leading to the development of antibodies or the mother may already have the iso-antibodies before pregnancy, or the iso-antibody titer may have increased during pregnancy after transfusion.

Fetal platelet antigens are expressed in normal levels in the 16th to 18th week of gestation [3,4] whereas IgG alloantibodies can reach the fetus as early as 13 weeks of gestation with the maximum transplacental transfer occurring in the third trimester leading to fetal thrombocytopenia and bleeding complications [5]. There are reports which show that presence of anti-HLA and anti-HPA antibodies can lead to NAIT in the fetus [6]. There are limited reports related to the presence of HPA antibodies along with anti-GPIIb-IIIa antibodies in GT patients and the management of pregnancy in such patients.

The mixing studies form the basis of antibody detection not only in hemostasis but in a wide range of clinical settings. In the present case, the inhibition of platelet aggregation by maternal plasma and not by control plasma only suggests that the patient has produced antibodies against GPIIb-IIIa; this was further confirmed by platelet receptor studies by flow cytometry. The limitation of our study is that HLA and HPA-15 antibodies were not checked; thus direct evidence for platelet-based antibody binding is still missing. Wijemanne et al. [7] reported a case wherein the GT patient was tested positive for anti-GPIIb-IIIa antibodies at 29th gestational week. The patient had a caesarean section with no ICH in baby. Thrombocytopenia was seen in the baby for which the baby was treated with immunoglobulin and platelet transfusion. Leticee N et al. [8] described a case of a GT patient (2nd gravida) having high titers of anti-GPIIb-IIIa and HLA antibodies; all the USG scans showed normal reports except in the 31st week wherein there was ICH leading to stillbirth.

The current gold standard test for the detection of platelet-specific antibodies is the monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay. This test is not routinely available and needs to be customized for a particular antigen. Thus, although the detection of these antibodies in all such patients, especially during pregnancy is of foremost importance, yet it is rarely done due to unavailability of these tests. However, platelet aggregation studies for

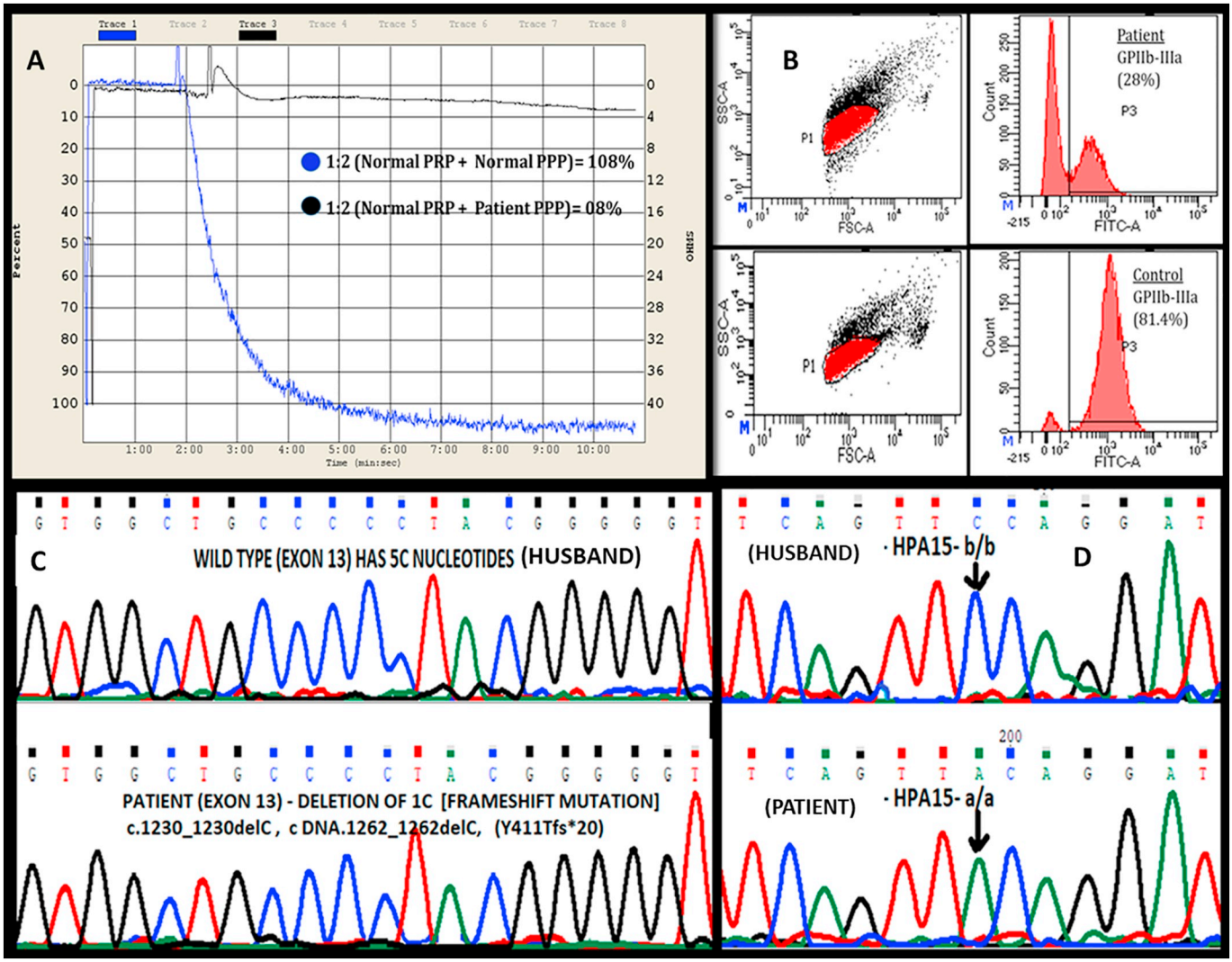

Fig. 1. A) Platelet aggregation studies showed inhibition in ADP aggregation when normal platelets were mixed with plasma of GT patient, whereas no inhibition was observed when mixed with control plasma.
B) Platelet receptor studies showed decrease in GPIIb-IIIa expression on platelets when normal platelets were mixed with plasma of GT patient, whereas no difference in the expression of GPIIb-IIIa was observed when mixed with normal plasma.
C) DNA sequencing of*ITGA2B*in GT patient revealed a frameshift mutation [c.1230_1230delC (Y411Tfs*20)] due to a single base deletion in exon 13.
D) Genotyping of HPA-15 by DNA sequencing in GT mother and husband revealed incompatibility, wherein GT mother and husband had a/a and b/b genotype.

screening of antibodies is simple and may be done. The flow cytometric technique, once standardized and validated could be performed to detect anti-GPIIb-IIIa antibodies. Even in our patient antibodies were never checked before or during her pregnancy.

Mother being diagnosed with GT and married to a normal partner, it is very unlikely that the fetus itself was a GT case. On later analysis, the mother was not only found to be strongly positive for anti-GPIIb-IIIa antibodies but also had HPA-15 incompatibility in family leading to HPA15 a/b child. It is known that transplacental transmission of these antibodies towards fetus can lead to NAIT [6]. Both these antibodies may lead to ICH and fetal loss. Also, GT patients with a deleterious mutation leading to inhibition of protein synthesis are on higher risk for anti-GPIIb-IIIa immunization [9]. A frameshift mutation was found in our case leading to early termination of the protein.

Thus, it is difficult to pinpoint whether it is solely the GPIIb-IIIa antibodies or only the HPA-15 incompatibility or the combination of both that led to ICH in fetus.

Thus, this is an interesting case wherein the patient is not only a congenital GT patient but also has isoantibodies against GPIIb-IIIa along with HPA-15 incompatibility in the family; thus a case of triple jeopardy which led to ICH and fetal loss. Difficulties in management of such pregnancies pose various diagnostic challenges including identification of antibodies, high treatment cost, and optimal time to start regimen and availability of a Fetal Medicine Specialist for monitoring the fetus through pregnancy. It must be noted that platelets given during pregnancy for vaginal bleeding in our patient potentially contributed to the increase in antibody titer and thus to further fetal complications. Transfusion with rFVIIa should thus be preferred, if available, before and during pregnancy to limit any further risk. Different approaches for peripartum and post-partum management of thrombocytopenia such as plasmapheresis, intravenous immunoglobulin, steroids or their combinations have been tried [10]. The prepartum management of thrombocytopenia includes IVIG at different doses ranging from 0.5 to 1 g/kg body weight per week with around 97.3% success rate [11]. There are various case series studying the role of addition of steroids with IVIG, compared with only IVIG, but none of them have proven that combination is effective than only IVIG in neonatal outcomes in the form of decreasing intracerebral bleed and mortality. D. Winkelhorst et al. [10] suggest that non-invasive treatment strategies are safe and effective options for the antenatal

management of pregnancies complicated by FNAIT, with a lower risk of severe complications compared with fetal blood sampling and/or intrauterine platelet transfusions. The gestational age at which to start antenatal IVIG treatment in FNAIT has however not been well defined. It is reasonable to consider the severity of the disease in previous pregnancies when making treatment decisions. For the neonate, the treatment includes IVIG 1 g/kg body weight for 2 days and may be repeated every 2 weeks till thrombocytopenia resolves. Bleeding neonate requires platelet transfusion along with IVIG, preferably the platelet donor should be the one who is negative for the implicated antigen. Here the best possible donor would be the mother, but the platelets should be plasma reduced to remove antibodies and irradiated [12].

Preconception counseling regarding possibility of fetal thrombocytopenia after screening for antibodies and therapeutic strategies needs to be discussed. Checking for platelet specific antibodies prior conception and after every 2–4 weeks of gestation during pregnancy by simple mixing platelet aggregation method and fetal monitoring for intracerebral bleed by ultrasound should become the standard of care in GT patients. If these antibodies are present in the mother prior or during pregnancy, besides ultrasonography (USG) monitoring, intravenous immunoglobulin therapy (IVIg) should be started as soon as possible, the standard schedule being once a week [10]. Neonates should be screened for thrombocytopenia and treated if affected; IVIg being the treatment of choice.

The take home message is that it is important to check the presence of platelet specific antibodies in all women with GT during pregnancy which will be helpful to plan the treatment and therefore will help decrease incidence of NAITP, ICH and fetal loss. For the same, a multidisciplinary care involving hematologist, obstetrician, fetal medicine specialist and a good laboratory for comprehensive investigations is required to ensure a successful outcome.

Declaration of competing interest

The authors declare no competing financial interests.

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The authors stated that they have no interests which might be perceived as posing a conflict or bias. SS conceived and designed the experiments. PS and AP performed the experiments and analyzed the data. PS and RP wrote the manuscript. The clinical evaluation was done by CS and SM. Critical revision of manuscript for important intellectual content and final approval was done by SS. This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

References

[1] B. Laursen, S.E. Jacobsen, E. Taaning, N. Grunnet, Transfusion problems in patients with Glanzmann's thrombasthenia, Eur J Haematol. 49 (1992) 143–144.
[2] B. Kulkarni, D. Mohanty, K. Ghosh, Frequency distribution of human platelet antigens in the Indian population, Transfus Med. 15 (2005) 119–124.
[3] C. Kaplan, C. Patereau, M.F. Remikoff-Etievant, J.Y. Muller, Y. Dumez, A. Kesseler, Antenatal PL^{A1} typing and detection of GP IIb-IIIa complex, Br J Haematol 60 (1985) 586–588.
[4] Y. Gruel, B. Boizard, F. Daffos, F. Forestier, J. Caen, J.L. Wautier, Determination of platelet antigens and glycoproteins in the human fetus, Blood 68 (1986) 488–492.
[5] F. Saji, Y. Samejima, S. Kamiura, M. Koyama, Dynamics of immunoglobulins at the feto-maternal interface, Reviews of Reproduction 4 (1999) 81–89.
[6] E. Brojer, A. Husebekk, M. Debska, M. Uhrynowska, K. Guz, A. Orzinska, R. Debski, K. Maslanka, Fetal/neonatal alloimmune thrombocytopenia: pathogenesis, diagnostics and prevention, Arch Immunol Ther Exp (Warsz) 64 (2016) 279–290.
[7] A. Wijemanne, I. Watt-Coote, S. Austin, Glanzmann thrombasthenia in pregnancy: optimising maternal and fetal outcomes, Obstetric Medicine 9 (2016) 169–170.
[8] N. Leticee, C. Kaplan, D. Lemery, Pregnancy in mother with Glanzmann's thrombasthenia and isoantibody against GPIIb–IIIa: is there a foetal risk? Eur J Obstet Gynecol Reprod Biol. 121 (2005) 139–142.
[9] M. Fiore, R. d'Oiron, X. Pillois, M.C. Alessi, Anti-αIIb β3 immunization in Glanzmann thrombasthenia: review of literature and treatment recommendations, Br J Haematol. 181 (2018) 173–182.
[10] D. Winkelhorst, M.F. Murphy, A. Greinacher, N. Shehata, T. Bakchoul, E. Massey, J. Baker, L. Lieberman, S. Tanael, H. Hume, D.M. Arnold, S. Baidya, G. Bertrand, J. Bussel, M. Kjaer, C. Kaplan, J. Kjeldsen-Kragh, D. Oepkes, G. Ryan, Antenatal management in fetal and neonatal alloimmune thrombocytopenia: a systematic review, Blood 129 (2017) 1538–1547.
[11] R. Rayment, S.J. Brunskill, P.W. Soothill, D.J. Roberts, J.B. Bussel, M.F. Murphy, Antenatal interventions for feto-maternal alloimmune thrombocytopenia, Cochrane Database Syst Rev. (5) (2011) CD004226.
[12] J.A. Peterson, J.G. McFarland, B.R. Curtis, R.H. Aster, Neonatal alloimmune thrombocytopenia: pathogenesis, diagnosis and management, British Journal of Haematology 161 (2013) 3–14.

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