Antibodies against human platelet alloantigens and human leucocyte antigen class 1 in Saudi Arabian multiparous women and multi-transfused patients

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ABSTRACT

الأهداف: الكشف عن الأجسام المضادة لصفائح الدموية ومستضدات لكرويات الدم البيضاء لدى مرضى نقل الدم المتكرر والنساء ذوات الحمل المتكرر.

الطريقة: اجريت الدراسة خلال 2013م على 50 امرأة من ذوات الحمل التكرر من عيادات أمراض النساء والتوليد ولم ينقل لهن الدم من قبل. بالإضافة إلى 50 مريضاً من عيادة أمراض أورام الدم نقلت لهم صفائح دموية عدة مرات في مستشفى الملك خالد الجامعي بالرياض، المملكة العربية السعودية.

النتائج: نسبة التحصين ضد الصفائح الدموية بين النساء ذوات الحمل المتكرر كانت 76% كالتالي: 16% ضد مستضدات كرويات الدم البيضاء، 8% ضد مستضدات الصفائح الدموية و 52% ضد كل من المستضدات الكروية البيضاوية والصفائح الدموية. 42% كانت نسبة التحصين في مرضى نقل الدم المتكرر، 2% ضد مستضدات لكرويات الدم البيضاء، 22%ضد مستضدات الصفائح الدموية و 18% ضد كل من المستضدات الكروية البيضاوية والصفائح الدموية. نسبة التحصين ضد الصفائح الدموية تناسبت طردياً مع عدد مرات الحمل ولكن لم يتم التوصل إلى أي علاقة بين نسبة التحصين وعدد مرات نقل الصفائح الدموية.

الخاتمة: حدوث التحصين ضد الصفائح الدموية هو أمر شائع لدى مرضى نقل الدم المتكرر والنساء ذوات الحمل المتكرر. مما يشجع البحث عن مدى المضاعفات المحتملة في الجنين والأطفال حديثي الولادة النساء ذوات الحمل المتكرر وكيفية منع حدوثها.

Objectives: To determine the frequency of alloimmunization against human platelet antigens (HPAs) and human leucocyte antigen class 1 (HLA1) in multiparous women and multi-transfused patients.

Methods: This prospective study was conducted between January and August 2013, on 50 multiparous women with no history of previous blood transfusion recruited from the Obstetrics and Gynecology Clinic, and 50 patients, who received multiple platelet transfusions, recruited from the Hematology/Oncology Ward, King Khalid University Hospital, Riyadh, Saudi Arabia.

Results: The frequency of alloimmunization among multiparous pregnant women was 76%, as follows: 16% against HLA1 only, 8% against HPAs only, 52% against both HPAs and HLA1 antigens. In multi-transfused patients, the rate of alloimmunization was 42% as follows: 2% against HLA1 only, 22% against HPAs only, 18% against both HPAs and HLA1 antigens. The frequency of alloimmunization increases with the number of pregnancies, but not with the number of platelet transfusions.

Conclusion: Alloimmunization against HPAs and HLA1 is very common among Saudi multiparous women and multi-transfused patients, which encourages the search for the extent of the possible complications in the fetus and newborn and in multitransfused patients and how to prevent their occurrence.

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Alloimmunization against human platelet antigens (HPAs) and human leucocyte antigens class 1 (HLA1) results in the development of platelet reactive antibodies, which occur mostly in multi-transfused

patients,[1] and as a result of pregnancy.[2] Detection of these antibodies and recognizing their specificities will help in safeguarding effective transfusion therapy as well as the prediction of the severity of thrombocytopenia, feto-maternal allo-immune thrombocytopenia (FMAIT), and its management. Another problem associated with these antibodies is passive alloimmune thrombocytopenia in recipients of blood collected from female blood donors immunized as a result of previous pregnancies.[3] The detection of these antibodies in such recipients may also falsely indicate the production of platelet specific antibodies.[4] In hemato-oncology patients receiving multiple transfusions, the production of these antiplatelet antibodies will result in shortening the survival of donated platelets and render the patient refractory to platelet transfusions.[5,6] Information on these areas is lacking in our population and, in view of the genetic variations that exist within and between ethnic groups and the current practice of random selection and transfusion of platelet products, it is of interest to find out the extent of alloimmunization to these antigens. Therefore, the main aim of this study is to determine the frequency of antibodies to HPAs and HLA1 in multiparous women and multi-transfused patients from Saudi Arabia.

Methods. This prospective study was conducted between January and August 2013 on 50 multiparous pregnant women recruited from the Obstetrics and Gynecology Outpatient Clinic, King Khalid University Hospital, Riyadh, Saudi Arabia. Their mean age was 34.8 years (SD ± 5.9; range: 17-45 years). The inclusion criterion was history of multiple pregnancies (range: 3-10 pregnancies), exclusion criterion was no history of previous blood transfusion. Fifty multi-transfused patients were also recruited from the Hematology/Oncology Ward, King Khalid University Hospital. Riyadh, Saudi Arabia. Forty-two percent were females, and 58% were males. Their mean age was 42.7 years (SD ± 21.4; range: 16-78 years). They were suffering an assortment of hemato-oncology disorders (hematologic malignancies: n=40; solid tumors: n=6; bleeding disorders n=4). The inclusion criterion was history of multiple platelet transfusions (range: 2-124 random leuco depleted units), the pregnancy history of the female patients was not available at the time of inclusion in the study.

Informed consent was obtained from each subject, after receiving approval for study from the Institutional Review Board (IRB), College of Medicine, King Saud University, Riyadh, Saudi Arabia. The study was conducted according to the Helsinki declaration. Electronic database of PubMed was used as a source to find related articles and researches.

Ten ml of blood was collected in ethylenediamine tetraacetic acid (EDTA), mixed gently, and transported immediately to the blood bank where the plasma was separated and stored in aliquots in a -80°C freezer until testing. The stored plasma was tested for antibodies against HPAs and HLA1 using commercial kits (PAKPLUS®solid-phase enzyme linked immunosorbent assay (ELISA) (GTI Diagnostics, Hologic Gen-Probe Incorporated, San Diego, CA, USA). This assay kit detects antibodies against HLA1 antigens and to epitopes on platelet glycoproteins GpIIb/IIIa, Ib/IX, Ia/IIa, and IV. According to the manufacturer´s instructions, test and positive and negative control plasmas were diluted using specimen diluent (phosphate buffered saline solution containing bovine albumin and mouse serum as well as 0.1% sodium azide) and then dispensed into the wells according to their locations in the working sheet. The plate was sealed and incubated for 35 mins in a 37°C water bath. Then the plate was washed 4 times using concentrated wash solution 10x (tris aminomethane buffered solution containing sodium chloride and tween 20.1% sodium azide) and diluted conjugate (alkaline phosphatase conjugated goat affinity purified antibody to human immunoglobulins), and dispensed to all wells except the blanks. The plate was sealed and incubated for 35 mins in a 37°C water bath. The plate was washed 4 times and diluted PNPP solution (p-nitrophenyl phosphate substrate) was dispensed to all wells except the blanks. The plate was left in the dark for 30 mins at room temperature. The reaction was stopped by adding the appropriate stopping solution. The absorbance of each well was measured at 405nm using an ELISA reader, and the results were calculated and interpreted according to the manufacturers' instructions. The HPA genotyping was not available during testing.

Obtained data was analyzed with the IBM SPSS Statistics for Windows, Version 19.0. (IBM Corp., Armonk, New York, USA). Fisher's exact test and Chi-square test were employed to compare between multi-transfused and the pregnant group for the nominal variables: HLA1, GpIV, and GpIIb/IIIa (HPA-

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1a/1a, - 3a/3a, - 4a). Statistical significance was set at a p-value less than 0.05.

Results. Out of 50 studied multiparous women 76% were alloimmunized against platelet specific antigens and HLA1 antigen, as follows: 16% against HLA1 only, 8% against HPAs only, and 52% against both HPAs and HLA1 antigens. Further analysis of the data was undertaken to find out the relationship between the number of pregnancies and platelet alloimmunization. The women were divided arbitrarily into 2 groups: the first includes women who had 3 to 5 pregnancies and the second group included those who had ≥6 pregnancies (**Table 1**). It is clear that the rate of alloimmunization against GpIa/IIa (-5a/5a) and GpIV antibodies increases with the increase in the number of pregnancies. As to the order of frequencies of alloimmunization, HLA1 alloimmunization was the highest at 68%, followed by GpIIb/IIIa (HPA-1a/1a, -3a/3a, and -4a) and GpIV with similar prevalence value (32% and 30.6%). Twenty eight percent of the women carried alloantibodies against GpIa/IIa (HPA -5b/5b), and 20% against GpIIb/IIIa (HPA- 1b/1b, -3b/3b, and -4a). Alloantibodies against GpIb/IX and GpIa/IIa (HPA-5a/5a) had the lowest prevalence rates at 10% and 8%, respectively (**Table 2**).

Forty-two percent of the multi-transfused patients were alloimmunized against platelet specific antigens and HLA1 antigen, as follows: 2% against HLA1 only, 22% against HPAs only, and 18% against both HPAs and HLA1 antigens. There was also a statistically significant difference in the prevalence of HPA and HLA1 alloimmunization among our multi-transfused patients. Forty percent of the patients were alloimmunized against HPAs, while HLA1 alloimmunization was detected in 20% of the patients (p<0.0001). In addition, GpIV alloimmunization was noted in 26% of the patients; 16% produced alloantibodies against GpIIb/IIIa (HPA -1b/1b, - 3b/3b, and -4a), and 12% against GpIb/IX. Alloimmunization against GpIIb/IIIa (HPA-1a/1a, -3a/3a, and - 4a) and GpIa/IIa (HPA -5a/5a) alloimmunization was equally prevalent among the patients (6%), whereas GpIa/IIa (HPA -5b/5b) had the lowest prevalence rate (4%) (**Table 2**). To find out the effect of the number of random platelet transfusions on platelet alloimmunization, we divided the patients into 2 groups; those who received ≤15 platelet transfusions and the second group includes those who received >15 transfusions. No significant association was noted between the numbers of transfusions received with development of antibodies.

Table 1 - Prevalence of alloimmunization against glycoprotein-GpIa/IIa (HPA-5a/5a) and glycoprotein GpIV in 50 multiparous women, and its relation to the number of pregnancies.

Alloimmunization	Number of pregnancies		*P*-value
	3 - 5	≥6	
Alloimmunization against GpIa/IIa (HPA 5a/5a)	0	4 (16.7)	0.046
Alloimmunization against Gp IV*	2 (7.7)	13 (56.5)	<0.0001

*relationship between the number of pregnancies and the rate of alloimmunization of the other human platelet antigens (HPA) systems was found to be statistically insignificant. Gp - glycoproteins.
Data are expressed as number and percentage (%)

Discussion. Alloimmunization against platelet antigens is a known complication of platelet therapy and, like immunization to red cell antigens, occurs more frequently with the increase in the number of exposures to the immunizing antigens, which is met frequently in multi-transfused patients,[1,7] and as a result of repeated pregnancy.[2] It should also be born in mind that the distribution of blood cell antigens is not uniform within and between populations; therefore, the current local practice of transfusing patients with randomly selected platelet products exposes the recipient to the transfusion of genetically incompatible platelets and increases the risk of alloimmunization to platelet antigens. Saudis have a distinct distribution of HPA systems frequencies as follows: 60% HPA-1 (a+, b-), 65% HPA-2 (a+, b-), 81% HPA-3 (a+, b-), 96% HPA-4 (a+, b-), and 72% HPA-5 (a+, b-).[8]

A careful search of the literature failed to identify any study in this area. This prospective study investigates the production of antibodies against HPAs and HLA1 in Saudi multiparous women and multi-transfused patients, and presents representative data on antiplatelet alloantibodies that are potential immune risks to platelet

Table 2 - Antiplatelet alloantibodies in multiparous women (n=50) and multi-transfused patients (n=50).

Subjects	GpIIb/IIIa (HPA -1a/1a, -3a/3a, & -4a)	GpIIb/IIIa (HPA -1b/1b, -3b/3b, & -4a)	GpIa/IIa (HPA -5b/5b)	GpIa/IIa (HPA -5a/5a)	GpIb/IX (HPA -2)	GpIV	HLA1
Multiparous women	16 (32)	10 (20)	14 (28)	4 (8)	5 (10)	15 (30.6)	34 (68)
Multi-transfused patients	3 (6)	8 (16)	2 (4)	3 (6)	6 (12)	13 (26.0)	10 (20)

Data are expressed as number and percentage (%). HPA - human platelet antigens, Gp - glycoproteins

therapy. Our major finding is the high prevalence rate of the alloantibodies against HPAs and HLA1 in Saudi multiparous pregnant women and multi-transfused patients. Seventy-six percent of multiparous women were alloimmunized against platelet specific antigens and HLA1 antigen, 52% were alloimmunized to both HLA1 and HPAs, 16% to HLA1 alloantibodies alone, and 8% against HPA. Our figures for HLA1 (16%) are similar to reports in multiparous women from other populations (**Table 3**).

We noted with much interest that the HPA-5b/5b alloimmunization was detected in one third (28%) of multiparous women. These alloantibodies are of the IgG type that can readily cross the placenta to the fetal circulation and carry the potential risk of causing FMAIT.[2,9,10] Although there is no local data on the scale for this complication, many published studies have shown that 80-90% of cases in Caucasians are due to anti-HPA-1a, and 5-15% to anti-HPA-5b and the remainder due to other HPA alloantibodies; the involvement of HLA1 in FMAIT received less attention.[11] Similar figures were reported elsewhere.[12-14] The similarity of the findings of the current study with respect to alloimmunization against HPA-1a and HPA-5b, with earlier reports, confirms further the need to identify the extent of FMAIT in our local population, with simultaneous determination of both the genotype of HPAs in the parents and fetus as well as the newborn and the antibodies against HPA in maternal blood. We also found that the frequency of both types of alloantibodies increases with the increase in the number of pregnancies, which agrees with many previous reports.[4,15]

Information on alloantibodies against HPAs is also very relevant in transfusion medicine as female blood donors with a history of multiple pregnancies, are commonly immunized against HPA-5b.[4] Blood collected from such donors will pass the respective alloantibody to recipients, who in turn, become transient passively immunized against HPAs, but also they are at great risk for developing thrombocytopenia. On the other hand, transfusion-dependent patients are also liable to the development of alloantibodies; as a result, multiple exposure to HPAs and that could jeopardize the efficacy of platelet therapy (so-called refractoriness to platelet therapy).[1,7]

The frequency of alloantibodies against HPAs and HLA1 in the sera of multi-transfused hemato-oncologic patients is also high at 42% (2% against HLA1 alone, and 22% against HPAs, and 18% against both HLA1 and HPAs). The rate of alloimmunization compares favorably with reports in many earlier publications (**Table 4**). The 20% HLA1 alloimmunization is also close to the range reported in other studies (**Table 5**).

A closer look at the results has shown that the most frequent alloimmunization was against GpIV (26%), which is similar in magnitude to the most frequent alloimmunization rate to GpIV (30%) and GpIIb/IIIa (HPA -1a/1a, -3a/3a, -4a) (32%), and GpIa/IIa (HPA -5b/5b) (28%), among multiparous women. We also observed a statistically significant difference in the HPA-5b/5b alloimmunization between multiparous pregnant women (28%) and multi-transfused patients (4%) (p=0.001). Anti-HPA-5b alloantibody was reported to be the most common platelet specific

Table 3 - Prevalence of human leucocyte antigen-1 (HLA1) alloimmunization among multiparous women in different populations.

Country/references	HLA1 alloimmunization among multiparous women %	Parity range
Saudi Arabia [present study]	16.0	3-10
Nigeria[15]	22.0	2-11
Korea[19]	18.5	-
South India[20]	13.4	1-8
Caucasians[21]	18.7	-
America[22]	21.6	-
Venezuela[23]	9.7	>2

Table 4 - Prevalence of platelet alloimmunization among multi-transfused patients in Saudi Arabia (present study), and other countries.

Country	Platelet alloimmunization among multi transfused patients %	Transfusion range
Saudi Arabia [present study]	42.0	2-124
America[24]	52.0	-
Greece[25]	51.9	10-287
Brazil[26]	56.0	2-184
Austria[27]	54.0	-

Table 5 - Prevalence of human leucocyte antigen 1 (HLA1) alloimmunization among multi-transfused patients in Saudi Arabia (present study), and other countries.

Country	HLA1 alloimmunization among multi transfused patients %
Saudi Arabia [present study]	20
Thailand[28]	20
India[29]	21
Brazil[26]	19
America[30]	18
Chile[31]	17
Canada[32]	26
Australia[33]	32

antibody induced by pregnancy.[4,15-17] We observed ethnic differences in alloimmunization against HPAs where the frequent immunizing alloantibody among Saudi's is against GpIV while in Indians it is the GP1b/IX antibody. It is noteworthy that the prevalence of HLA1 alloimmunization in multiparous women (68%) was phenomenally higher than that in multi-transfused patients (20%; $p<0.0001$). This can be attributed to the multiparity effect on HLA1 alloimmunization among our multiparous women. In our study, 42% of the multi-transfused patients were females (21/50) while males represent 58% (29/50). It must also be added that we could not trace the gender of the source of the transfused platelet transfusions. Although previous studies reported an increase in the frequency of alloimmunization as the number of transfusions increases,[6] we failed to confirm these observations in our cohort. The wide prevalence of platelet alloimmunization in multi-transfused patients obtained in this study along with its possible clinical consequences, especially refractoriness to platelet transfusion and its close association with patient survival,[18] emphasizes the importance of pre-transfusion platelet antibody screening of such patients as well as the establishment of stocks of HPAs and HLA typed platelet units of platelet concentrate, so that the needy patients should receive units that lack any antigens corresponding to the antibodies detected in their blood. This will not only safeguard the best platelet increment, but will also economize the excessive consumption of the precious platelet therapeutic components.

The findings of current study, small as it is, also confirmed the high prevalence of antibodies against platelet antigens in multiparous women. This observation, if confirmed in larger studies, can be of use to clinicians as a non-invasive predictive indicator of the severity of FMAIT.[2,9,10] The findings of this study will raise awareness of physicians prescribing platelet therapy to the possible occurrence of transfusion related acute lung injury (TRALI) arising as a complication of transfusing platelet products donated by multiparous women, particularly to patients who require frequent platelet therapy.

In conclusion, our data confirm the high frequency of alloantibodies to HPA and HLA1 in Saudi multi-transfused patients as well as multiparous women. In multi-transfused patients, the findings highlight the need for the reconsideration of the random selection of platelet products and resort to platelet cross-matching to guarantee the safety and efficacy of platelet therapy. As to alloimmunization of pregnant women by HPAs inherited by the fetus from the father, at this moment there is not much that could be carried out to prevent this process. However, the high frequency rate of the alloimmunization uncovered in the present study should encourage future research involving a larger number of study subjects to ascertain the extent of the possible complications, whether platelet refractoriness in multi-transfused patients, and/or thrombocytopenia and its consequences in the fetus and newborn.

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