ORIGINAL ARTICLE

Prevalence of Anti-HLA Antibodies in Multiply Transfused Patients with Hematologic Malignancy

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Background: Human leukocyte antigen (HLA) alloimmunization can be induced by exposure to non-self HLA molecules, such as foreign tissue during transplantation, pregnancy, and blood products. Antibodies against HLA lead to serious complications in patients receiving blood transfusions.

Objective: The present study aimed to determine the prevalence and risk factors for HLA alloimmunization in multiple transfused patients with hematologic malignancies at Siriraj Hospital.

Materials and Methods: The present research was a cross-sectional study. The samples comprised 100 cases of hematologic malignancy patients who had a history of at least three episodes of transfusion with cellular blood components. All patients were tested for anti-HLA class I and class II antibodies by Luminex assay.

Results: The overall prevalence of HLA alloimmunization in multiple transfused patients with hematologic malignancies was 40%. Anti-HLA class I, class II, and both class I and class II antibodies were detected in 35%, 30%, and 25% of the patients, respectively. Factors related to the development of anti-HLA antibodies were previous pregnancy (OR 4.82, 95% CI 1.62 to 14.35, p=0.005), five or more transfusions (OR 7.82, 95% CI 2.22 to 27.55, p=0.001), and diagnoses with myelodysplastic syndromes (MDS) antibodies (OR 5.88, 95% CI 1.98 to 17.47, p=0.001).

Conclusion: Patients with hematologic malignancies who required long-term blood transfusion support could be at high risk for the development of anti-HLA antibodies. Important risk factors are pregnancy, multiple transfusions, and MDS.

Keywords: HLA alloimmunization, Anti-HLA antibodies, Blood transfusions, Hematologic malignancy

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Red blood cell (RBC) and platelet transfusions are essential therapy for patients with hematologic malignancies[1]. These patients who often receive intensive chemotherapy become anemic with thrombocytopenia. Most patients need transfusion support during the course of their disease. Although blood transfusion is life-saving, it can sometimes result in significant adverse events. In multiple transfused patients, the main concern with alloimmunization should be the development of alloantibodies with complications and risks of erythrocyte and leukocyte alloantibodies. RBC alloimmunization is a severe problem due to limited availability of compatible blood and higher risk of transfusion reactions. Additionally, alloimmunization to human leukocyte antigen (HLA) is a significant complication for adverse reactions, such as febrile non hemolytic transfusion reactions, transfusion-related acute lung injury (TRALI), and platelet refractoriness[2]. In the meantime, platelet transfusion refractoriness is an important clinical problem in those requiring long-term platelet supportive care similar to hematological patients with HLA-selected platelet transfusions[3]. Moreover, donor-specific anti-HLA antibodies can cause graft failure in allogeneic hematopoietic stem cell transplantation[4].

HLA alloimmunization can be induced by exposure to allogeneic HLA. Studies demonstrated that HLA sensitization occurs after transfusion, pregnancy, and transplantation[2]. In blood donors, the number of prior pregnancies could be associated with the prevalence of HLA antibodies[5,6]. In renal transplant cases, the risk factors of HLA sensitization are pregnancy, blood transfusion, and retransplantation[7-9]. In stem cell transplant recipients,

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HLA antibodies are related to prior pregnancy and multiple transfusions[10-12]. Previous studies yielded a higher prevalence of HLA alloimmunization in patients with aplastic anemia, thalassemia, and other chronic hemolytic anemias[13-17].

Nonetheless, little information has been known on the frequency and risk factors of HLA antibodies in hematologic malignancy patients receiving multiple transfusions. Therefore, the main purpose of the present study was to determine the prevalence and risk factors of antibodies against HLA in multiple transfused patients with hematologic malignancies.

Materials and Methods

Study design and study populations

The present study was a cross-sectional study. One hundred patients with hematologic malignancies who received transfusion from 2009 to 2022 at the Hematology Clinic, Department of Medicine Siriraj Hospital were included in the study. Plasma samples were collected between January and May 2022. Sample size was calculated by using the Cochran formula. Data on potential risk factors inducing the HLA alloimmunization, such as history of pregnancy, transfusion, and transplantation, were collected for analysis. All patients received more than two blood transfusions with cellular blood components.

The present study was approved by the Ethics Committee of Siriraj Hospital, Mahidol University (Thailand), the registration number was COA 1029/2021 (SIRB Protocol No.995/2564).

Anti-HLA antibody testing

All samples were tested for the presence of anti-HLA class I and class II antibodies by the Luminex xMAP technology. Antibody screening was performed by the LABScreen Mixed kits (One Lamda Inc., Canoga Park, CA, USA). The value of panel reactive antibody (PRA) for HLA specificities was identified by using the LABScreen PRA: LS1PRA and LS2PRA commercial (One Lamda Inc.) in accordance with the manufacturer's instructions. The cut-off value of mean fluorescence intensity (MFI) considered as PRA positive was 1,000.

Statistical analysis

Baseline demographic characteristics were analyzed by descriptive statistics of the IBM SPSS Statistics, version 21.0 (IBM Corp., Armonk, NY, USA). A comparison between patient groups was performed using the Mann-Whitney test and chi-square test, or Fisher's exact test, for categorical variables. Binary logistic regression tests with multivariable analyses were applied for the association of risk factors and the prevalence of anti-HLA antibodies. The prevalence of various anti-HLA antibodies was compared by using the McNemar test. A p-value of less than 0.05 was considered statistically significant.

Results

One hundred patients with hematologic malignancies, including 56 males and 44 females, were recruited. The median age was 69 (range of 25 to 87) years. Thirty-six females had previous pregnancies. The median number of prior transfusions was 9 (range of 3 to 107). The range number of transfused units of RBC was one to 136. The range number of transfused units of PLT was zero to 82. The majority of patients received non-leukoreduced blood components. Eleven patients had RBC alloantibody and seven patients had a history of stem cell transplantation. Thirty patients had myelodysplastic syndromes (MDS) and 15 patients had acute myeloid leukemia (AML). Five patients were noted with acute lymphoblastic leukemia (ALL), eight with chronic myeloid leukemia (CML), two with chronic lymphocytic leukemia (CLL), 16 with lymphomas, 13 with multiple myeloma (MM), and 11 with myeloproliferative neoplasms (MPN).

The comparison between patients with PRA positive and PRA negative is shown in Table 1. No statistically significant difference in age was observed between PRA-positive and PRA-negative groups. The number of males were significantly higher in the PRA-negative group at 70% than the PRA-positive group at 35% ($p<0.001$). Whereas, the number of females was significantly higher in the PRA-positive group at 65% than the PRA-negative group at 30.0% ($p<0.001$). Most of them had prior pregnancies, with significantly higher in the PRA-positive group at 52.5% than the PRA-negative group at 25.0% ($p=0.005$). Additionally, the frequency of patients with two or more prior pregnancies was significantly higher in the PRA-positive group at 47.5% than the PRA-negative group at 16.7% ($p<0.001$).

The frequency of those with five or more total transfusions was significantly higher in the PRA-positive group at 87.5% than the PRA-negative group at 55.0% ($p<0.001$). The frequency of patients with less than five total transfusions was higher in the PRA-negative group at 45% than the PRA positive group at 12.5% ($p<0.001$).

Moreover, the frequency of patients with MDS

Table 1. Comparison between positive PRA and negative PRA groups

Patients	Positive PRA (n=40)	Negative PRA (n=60)	p-value
Age (years); median (range)	69 (25 to 86)	69 (28 to 87)	0.969
Sex; n (%)			
Male	14 (35.0)	42(70.0)	<0.001
Female	26 (65.0)	18 (30.0)	<0.001
Prior pregnancies; n (%)	21 (52.5)	15 (25.0)	0.005
Number of prior pregnancies; n (%)			
1	2 (5.0)	5 (8.3)	0.699a
≥2	19 (47.5)	10 (16.7)	<0.001
Number of prior total transfusions; n (%)			
<5	5 (12.5)	27 (45.0)	<0.001
≥5	35 (87.5)	33 (55.0)	<0.001
Exclusively RBC leukoreduction; n (%)	2 (5.0)	12 (20.0)	0.034
Diagnosis; n (%)			
MDS	21 (52.5)	9 (15.0)	<0.001
Leukemia	12 (30.0)	18 (30.0)	1.000
Lymphomas	3 (7.5)	13 (21.7)	0.058
Others	4 (10.0)	20 (33.3)	0.007
Presence of RBC antibody; n (%)	8 (20.0)	3 (5.0)	0.025

PRA=panel reactive antibody; RBC=red blood cell; MDS=myelodysplastic syndromes

[a] Fisher's exact test

was significantly higher in the PRA-positive group at 52.5% than the PRA-negative group at 15.0% (p<0.001). The frequency of patients with AML was also higher in the PRA-positive group than the PRA-negative group, but with no statistical significance. However, the frequency of patients with ALL, CML, CLL, NHL, MM, and MPN was higher in the PRA-negative than the PRA-positive group. The frequency of patients with only leukoreduced blood products was higher in the PRA-negative group at 20% than the PRA-positive group at 5% (p=0.03).

The prevalence of anti-HLA antibodies is shown in Table 2. The overall prevalence of anti-HLA class I and/or class II antibodies was 40%. There was 35% positive for class I and 30% positive for class II. The median MFI of PRA for class I and class II was 8,383, with a range of 1,035 to 23,544 and 8,885 with a range of 1,142 to 23,769, respectively. There was no significant difference in the prevalence and the MFI between class I and class II. Of these, 35 patients were positive for class I when compared with 30 patients positive for class II. Twenty (57.1%) and 19 (63.3%) patients had PRA greater than 50%, respectively. The frequency of antibodies against different antigens showed that 31% of the patients had anti-HLA-A, followed by anti-HLA-B at 31%, anti-HLA-C at 7%, anti-HLA-DR at 27%, anti-HLA-DQ at 21%, and anti-HLA-DP at 1%.

Table 2. Prevalence and mean fluorescence intensity value of anti-HLA class I and II antibodies

Type	MFI; median (range)	Frequency (%)
HLA class I	8,383 (1,035 to 23,544)	35
HLA-A	5,702 (1,057 to 23,384)	31
HLA-B	7,355 (1,035 to 23,544)	31
HLA-C	5,165 (3,268 to 6,812)	7
HLA class II	8,885 (1,142 to 23,769)	30
HLA-DR	8,193 (1,158 to 23,769)	27
HLA-DQ	9,884 (1,142 to 23,749)	21
HLA-DP	2,272	1
Only HLA class I	4,443 (1,057 to 17,650)	10
Only HLA class II	2,544 (1,142 to 3,490)	5
Both HLA class I and II	10,002 (1,035 to 23,769)	25

MFI=mean fluorescence intensity; HLA=human leukocyte antigen

A multivariable logistic regression model revealed that prior pregnancies, five or more prior transfusions, and diagnosis of MDS were independent factors for HLA alloimmunization (Table 3). Those data indicated that patients with prior pregnancies were associated with HLA alloimmunization (OR 4.82, 95% CI 1.62 to 14.35, p=0.005). Meanwhile, those with five or more transfusions were more likely to develop anti-HLA antibodies than patients with less than five prior transfusions (OR 7.82, 95% CI 2.22 to 27.55, p=0.001). Moreover, it was found

Table 3. Multivariable logistic regression model of HLA alloimmunization

Parameters	Adjusted OR	95% CI	p-value
Prior pregnancies (vs. no pregnancy)	4.82	1.62 to 14.35	0.005
Number of transfusions: >5 vs. <5	7.82	2.22 to 27.55	0.001
Diagnostic: MDS vs. non-MDS*	5.88	1.98 to 17.47	0.001
RBC antibody positive	4.82	0.90 to 25.82	0.067

OR=odds ratio; CI=confidence interval; MDS=myelodysplastic syndromes; RBC=red blood cell

* Leukemia, lymphoma, and others

that the diagnosis of MDS was associated with the development of anti-HLA antibodies (OR 5.88, 95% CI 1.98 to 17.47, p=0.001). Interestingly, patients with RBC alloimmunization were more likely to develop anti-HLA antibodies when compared to those with RBC antibody negative (OR 4.82, 95% CI 0.90 to 25.82, p=0.067).

Discussion

The overall prevalence of HLA alloimmunization in the present study was 40% with similar frequency and strength of class I and class II antibodies. This was higher than that reported in transplant candidates who had hematological disease with anti-HLA antibodies ranging from 20.2% to 28.9%[10-12]. However, one study in hemato-oncological patients with platelet refractoriness reported that HLA antibodies were found in 42%, similar to the present study due to multiple blood transfusions[18].

The variance of prevalence of HLA immunization between the present study and the others depended on study population, patient characteristics, sensitization events, testing performed, and different cut-off MFI. Sensitization events such as blood transfusion, pregnancy, and transplantation caused HLA alloimmunization[19]. In the present study, Luminex method, a sensitive technique for HLA antibody, was used with the same cut-off routine testing for transplantation.

The present study found the frequency of HLA alloimmunization in transfused patients with a history of pregnancy higher than those without pregnancies. Furthermore, the prevalence of anti-HLA antibodies increased with the number of pregnancies, similar to the other studies[20,21]. In addition, the female gender was associated with the presence of anti-HLA antibodies[10,11]. There were similar results for females at greater risk of sensitization than males. However, in multivariate analysis, the female gender was not associated with HLA alloimmunization, while pregnancy was significantly related to HLA alloimmunization.

Studies reported that transfusion had less effects than pregnancies and transplantation[7,20,22]. This might be due to a small number of transfusions in these patients, which seemed different from the present study patients. Despite reports on the relevance between blood transfusions and HLA antibodies in many studies, the number of transfusions remained unclear. The present study data yielded the association between the number of five or more transfusions and the development of HLA antibodies. This was compatible to the results reported by other studies in populations with six or more transfusions of associated HLA alloimmunization[10]. Moreover, a previous study showed that platelet transfusion, not RBC transfusions, was an important risk factor[23]. However, the non-leukoreduced RBC transfusions in the present study were also crucial for the production of HLA antibodies.

In the present study, the diagnosis with MDS was an independent risk factor for HLA alloimmunization. The higher frequency of HLA alloimmunization in MDS patients was similar to a previous study in China[10,12]. The possible cause may be based on higher number of transfusions in those with MDS than other diseases. RBC transfusions were necessary for most MDS patients with treatment for regular blood transfusion[24]. In particular, AML patients in the present study appeared to be more likely associated with HLA immunization. Whereas, in other disease subgroups including ALL, CLL, CML, lymphoma, MM, and MPN, the number of patients with PRA negative were higher than those with PRA positive, but too small sample size for analysis in each subgroup. In other hematologic diseases, patients with thalassemia and aplastic anemia showed a higher prevalence of HLA antibodies despite the leukoreduced blood[14-16]. The production of antibodies might be from the immune mechanism of those diseases.

The prevalence of HLA antibodies in Thai patients was also reported as high in chronic hemolytic anemia[17]. However, only a small number of Thai patients received leukoreduced RBCs. In the present study, most patients received non-leukoreduced blood components, which was a cause of HLA alloimmunization. Among 14 patients with leukoreduced RBC, only two patients had HLA antibodies. Studies reported a decreased incidence of HLA alloantibody formation in patients receiving transfusions with leukoreduced blood components[2].

The present study also revealed a higher proportion of patients with RBC alloantibodies of HLA-sensitized than the non-HLA-sensitized patients, which was compatible to the previous studies[25]. However, only a few patients with multiple transfusions in the present study had RBC antibodies.

The data on HLA antibodies could be useful for the management of platelet refractoriness and HSCT transplantation in those patients. The limitation of the present study was the small sample size, which could limit comparison and the inference of statistical significance, especially in disease subgroups. Thus, a larger sample size in the multicenter study would be recommended for future study.

Conclusion

The results of the present study yielded a high prevalence of HLA class I and II antibodies in multiple transfused patients with hematologic malignancies. Previous pregnancy, number of transfusions, and diagnosis with MDS were found to be important risk factors for antibodies in HLA in multiple transfused hematologic malignancies. Hence, the prevention of HLA alloimmunization should be performed by leukocyte-reduced transfusions.

What is already known on this topic?

The prevalence of HLA antibodies and risk factors were reported in hematological patients in other populations. The studies were limited to Caucasian and Chinese patients who received leukoreduced blood.

What does this study add?

The prevalence of HLA antibodies was detected by Luminex and risk factors were reported in Thai patients with hematological malignancies by the patients who still received transfusions with non-leukoreduced blood.

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Conflicts of interest

The authors declare no conflict of interest.

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