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Prevalance of Anti-HLA antibodies in parous female blood donors: A pilot study from tertiary care hospital of North India

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Abstract

BACKGROUND: Various studies have implicated that plasma causing transfusion-related acute lung injury is from alloimmunized females. The frequency of sensitization to human leukocyte antigen (HLA) was found to correlate with their parity score. No literature on the prevalence of anti-HLA antibodies in Indian blood donors is available to date. Hence, this pilot study was done to know the frequency of HLA alloimmunization in Indian blood donors.

MATERIALS AND METHODS: A total of 192 consenting voluntary blood donors from blood donation camps were enrolled in the study. Test group: Parous female donors (n = 96) and control group: Nulliparous female donors (n = 48) and male donors (n = 48). HLA alloimmunization was tested on the Luminex platform by screening assay to detect IgG antibodies to HLA Class I and II molecules of human origin. A mean fluoresence index of more than 2000 was considered as a positive reaction, considering the high sensitivity of Luminex assay.

RESULTS: Sixty-three out of 192 donors (32.8%) tested positive for anti-HLA antibodies, out of which 23 donors were in the control group (23.9%), and 40 donors were in the test group (41.7%); P = 0.002. On gender-based comparison, 9 out of 48 male donors (18.7%), as compared to 54 out of 144 female donors (37.5%), tested positive for HLA antibodies (P = 0.02). Based on an increase in parity score, the frequency of HLA alloimmunization was found to be significantly correlated (P = 0.002). A decrease in the trend of HLA alloimmunization was observed as the duration from the last pregnancy increased. A higher frequency of HLA alloimmunization was observed in female donors with a history of transfusion and bad obstetric history.

CONCLUSION: The present study substantiates that plasma from parous female donors has a higher chance of containing anti-HLA antibodies as compared to nulliparous female and male donors.

Keywords:

Anti-HLA antibodies, mean fluoresence index, parous female blood donors, transfusion-related acute lung injury

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Introduction

The use of fresh frozen plasma is indispensable in the management of massive transfusion and in acute conditions such as burns or shock. The major side effects of plasma transfusion range from allergic reactions, anaphylaxis, transfusion-related acute lung injury (TRALI), and transfusion-associated circulatory overload (TACO). TRALI is under-diagnosed[1] and under-reported since its first description in 1957 by Brittingham,[2] but recent reports have shown that it is the most important and common cause of morbidity and mortality related to

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transfusion.[3,4] According to the US Food and Drug Administration, TRALI and TRALI like cases were the most common cause of death after transfusion (34%).[5]

It is considered that the source of plasma, which leads to TRALI, are alloimmunized female blood donors, and their frequency of sensitization to human leukocyte antigen (HLA) correlate with their parity score.[6] After adopting the "Male-only plasma" policy, there has been a significant drop in the incidence of TRALI in the UK,[7,8] USA,[9] and Netherlands.[10]

From India, there are very few case reports on TRALI. A study from our center reported one case of TRALI out of 56503 blood component transfusions.[11] There are no studies on the prevalence of anti-HLA antibodies in Indian blood donors. Hence, we planned this study to know the frequency of HLA alloimmunization in blood donors from our population. This was aimed to have evidence to decide if we should adopt the "Male only plasma" strategy for clinical transfusion and selectively divert female donor plasma for manufacturing plasma derivatives as done in the West.

Materials and Methods

Study design

This is a pilot cross-sectional study conducted among whole blood donors who donated blood in Chandigarh and the neighboring areas in northern India, where blood donation camps were being conducted by the tertiary care institute for 1 year (2016–2017) [Figure 1].

Study process flow

Institute ethical committee clearance was obtained for the study. The blood donors were screened according to departmental standard operating procedure in accordance with national guidelines.[12] Target enrollment of parous female donors and nontarget enrollment of males and nulliparous female donors was done. A detailed obstetric history and history of transfusion was taken which could be additional source of exposure for HLA alloimmunization. Red cell contaminated and lipemic samples were excluded from the study.

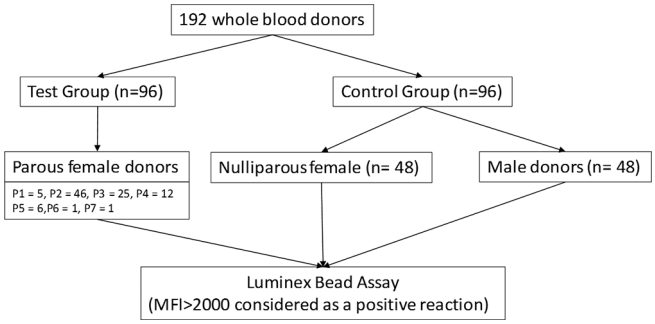

Figure 1: Study design

Blood samples from donors were taken in plain vials, and the serum was separated (within 6 h of collection) after centrifugation (3000 rpm for 3 min) and stored in aliquots at −80°C till testing was done. Tests were done in a batch, according to the manufacturer's specifications. Alloimmunization was tested by Luminex bead assay (LIFECODES Life Screen Deluxe assay, Immucor, Georgia, USA), LifeScreen Deluxe is composed of Luminex Beads to which affinity purified Class I HLA and Class II HLA glycoproteins are conjugated to detect IgG antibodies to HLA Class I and Class II molecules of human origin.[13] An mean fluorescence index (MFI) of >2000 was considered as cut off for defining a sample as positive.

Statistical analysis

Discrete categorical data were represented in the form of either a number or a percentage (%). Proportions were compared using Chi-square or Fisher's exact test, depending on their applicability. All the statistical tests were two-sided and were performed at a significance level of $P = 0.05$. The analysis was conducted using IBM SPSS STATISTICS version 22.0 Statistical product and service solution (SPSS, Chicago, IL, USA).

Results

During the study period of 1 year, 192 donors were enrolled in the study. The test group consisted of 96 parous females and the control group consisted of 48 nulliparous females and 48 males. The mean age of males in the control group was 22.83 years (range: 18–37 years) and of nulliparous females in the control group was 24.16 years (range: 18–41 years) and of parous females in the test group was 39.61 years (range: 26–58 years).

Overall, 63 out of 192 donors (32.8%) tested positive for anti-HLA antibodies. Thirty-Seven donors (19.3%) had anti-HLA Class I antibodies and 51 donors (26.6%) had anti-HLA Class II antibodies. Twenty-six donors (13.5%) had both Class I and II antibodies [Table 1]. Overall, 23.9% of donors in the control group and 41.7% of donors in the test group tested positive for anti-HLA antibodies ($P = 0.002$).

Analysis of human leukocyte antigen alloimmunization by gender

Nine out of 48 males (18.7%) and 54 out of 144 females (37.5%) tested positive for anti-HLA antibodies ($P = 0.02$). Of the 54 females who tested positive, 14 were nulliparous and 40 were parous ($P = 0.004$).

Correlation of human leukocyte antigen alloimmunization based on bad obstetric history

Out of 96 parous female donors, 17 females (17.7%) had bad obstetric history (BOH) history in the form of 1st or 2nd trimester abortions, stillbirths, neonatal deaths, preterm

labor, and fetal anomalies. Out of these 17 females, nine tested positive (52.9%), which was high as compared to parous females without a BOH (39.2%) but was not statistically significant, as shown in Table 2.

Analysis based on parity score

The parity score in our study population ranged from 1 to 7, and the rate of alloimmunization in increasing order of the parity score was 40%, 30.4%, 52%, 66.6%, 16.6%, 100%, 100% implying that increase in parity increased the likelihood of HLA alloimmunization.

Table 1: Comparison of human leukocyte antigen alloimmunization among male, nulliparous and parous female donors

HLA antibodies	Overall (*n*=192), *n* (%)	Male (*n*=48)	Nulliparous female (*n*=48)	Parous female (*n*=96)
Overall frequency	63 (32.8)	9 (18.7)	14 (29.2)	40 (41.7)
Class I	37 (19.3)	3 (6.2)	8 (16.7)	26 (27.1)
Class II	51 (26.6)	7 (14.6)	13 (27.1)	31 (32.3)
Both Class I and II	26 (13.5)	1 (2.1)	7 (14.6)	17 (17.7)

HLA: Human leukocyte antigen

Table 2: Effect of parity, history of transfusion, and bad obstetric history on frequency of human leukocyte antigen alloimmunization

Parity	Nulliparous females (*n*=48)	Parous females (*n*=96)	*P*
Overall frequency	14 (29.2%)	40 (41.7%)	0.04
Class I	8 (16.7%)	26 (27%)	0.03
Class II	13 (27.1%)	31 (32.3%)	0.21
Both Class I and II	7 (14.6%)	17 (17.7%)	0.31
History of transfusion in parous females (96)	**Transfused (*n*=3)**	**Nontransfused (*n*=93)**	***P***
Overall frequency	2 (66.6%)	38 (40.8%)	0.14
Class I	2 (66.6%)	24 (25.8%)	0.083
Class II	1 (33.3%)	30 (32.2%)	0.788
Both Class I and II	1 (33.3%)	16 (17.2%)	0.54
Bad obstetric history	**Present (*n*=17)**	**Absent (*n*=79)**	***P***
Overall frequency	9 (52.9%)	31 (39.2%)	0.433
Class I	7 (41.2%)	19 (24%)	0.448
Class II	6 (35.3%)	25 (31.6%)	0.168
Both Class I and II	4 (23.5%)	13 (16.4%)	0.296

Table 3: Mean fluorescence index for Class I and Class II human leukocyte antigen antibodies in the study population with positive test result (mean fluorescence index >2000)

Study group	Total (*n*)	Positive (*n*=63)	Mean MFI	
			Class I	Class II
Males	48	9	3074	3032
Nulliparous females	48	14	3244	3539
Parous females	96	40	5571	6439
P1	5	2	4855	2900
P2	46	14	4182	5809
P3	25	13	4900	6928
P4	12	8	8789	9688
P5	6	1	-	2211
P6	1	1	2378	2645
P7	1	1	3660	4935

MFI: Mean fluorescence index

Correlation of human leukocyte antigen alloimmunization based on the duration from the last pregnancy

The parous females in the study population were divided into three groups based on the duration from their last delivery (within the past 10 years, 10 to 20 years, more than 20 years). On comparative analysis, a decrease in the trend of HLA alloimmunization was observed as the duration from the last pregnancy increased in female donors: 52%, 41%, and 0%, respectively, but was not statistically significant.

Comparison of mean fluoresence index range

On comparing the mean MFI among donors who tested positive, males had the lowest mean MFI (Class I-3074 and Class II-3032.3) and parous females the highest (Class I– 5571.3 and Class II– 6438.5). An increasing trend of the mean MFI can be observed with increase in parity score (at least till the lower parity scores), but the same did not continue up to P7 as shown in Table 3. The sample size was very low in higher parity score to comment on this observation.

In male donors, the majority of them, i.e., 77% had MFI <1000 for Class I antibody and 47.9% had MFI of 1000–2000 for Class II antibody. Very few of the male donors had an MFI >4000 (0 for Class I and 4.16% for Class II antibodies).

Discussion

The overall HLA alloimmunization was significantly higher in the test group (41.7%) than in the control group (23.9%); $P = 0.002$. A decrease in frequency of HLA alloimmunization was observed as the duration from the last pregnancy increased (similar findings observed in a previous study by Truilzi *et al*.[14]), suggesting decrease in antibody titers. Further, the HLA alloimmunization is higher in females (37.5%) than males (18.7%); $P = 0.02$. The presence of alloimmunization in unexposed males could be explained by antibodies produced due to an epitope sharing by various microbial organisms, allergens, and food proteins as observed by Morales-Buenrostro *et al*,[15] in which 424 healthy male donors without any history of transfusion or transplantation were studied out of which 63% had anti-HLA antibodies (positive cut off >1000 MFI). Similar results were observed in another study where HLA alloimmunization rate of 34% was found in unexposed donors.[16] Author in this study observed that unexposed donors with an history of infection in the past 3 years had twice the rate of positive results concluding the need

for separate guidelines on MFI interpretation in healthy donors.[16] As India is a tropical and socioeconomically developing country, the presence of such cross-reacting antibodies could be one possibility. In addition, the HLA E antibodies present in healthy male population could cross-react with antibodies against HLA– Ia. The allele specificity of HLA antigen is based on various exposures to pathogens, meat proteins in the diet. Hence, such vast diversities in the alleles of HLA give chances for antigen mimicry and cross reactivity.[17]

Although the manufacturer considers an MFI >1000 as a positive reaction for transplant recipients which is standard in most of the centers in the country, the cut off is not defined for healthy blood donors. Studies from different parts of the World have used their population specific cut-off. For example, Truilzi *et al*.[14] have used positive cut off as mean + 3SD of the MFI of 1% nonalloexposed healthy population. Powers *et al*.[18] used an MFI of 2.4-fold above a normalized background as the positive cut off. De Clippel *et al*.[19] and Xia *et al*.[20] have used a 2000 and 2500 MFI as the positive cut off, respectively, in their studies [Table 4]. As this is first of its kind study from this part of the world and we have no reference of cut off for blood donors in our region, we have taken cut off as MFI >2000, which is two times the cut off for patient population considering high sensitivity of Luminex assay and the possibility of overestimate of the HLA antibodies in an otherwise healthy population. It was also observed that the MFI value did not have a uniform incresing trend with the rise in parity score of female donors [Table 3], may be due to lower sample size in higher parity scores. This finding re-establishes the fact that MFI does not necessarily indicate the strength or titer of antibody.[21,22]

This study demonstrate that around one-third plasma units from parous female donors contain anti-HLA antibodies. It is already known that these HLA Class 1 and Class 2 antibodies are implicated in the pathogenesis of TRALI. A study from our center showed only 8.3% of our donor pool is constituted by female donors.[23] Hence, selective diversion of female plasma to fractionation as primary prevention strategy of TRALI will not impact our plasma supplies as female donor percentage are very low in our country. This will further augment availability of plasma for fractionation in our country.

Conclusion

The present study substantiates that plasma from parous female donors has a higher chance of containing anti HLA antibodies as compared to male and nulliparous female donors. An increase in trend in the frequency of HLA alloimmunization was seen as the parity score increased. The frequency of HLA alloimmunization was higher in donors with a history of transfusion and a BOH. Hence, as a primary prevention strategy, it is better to avoid clinical use of plasma from parous female donors and divert them for fractionation.

Table 4: Comparative analysis of studies on the frequency of human leukocyte antigen alloimmunization

Author, year, country	Study population	Test method	Cut off for a positive test	The frequency of HLA alloimmunization
Truilzi *et al*.[14] 2009, USA	8171 whole blood donors, 6011 females, 2160 males	Luminex bead assay	Mean + 3SD MFI of 1% nonallo-exposed normal population	Nontransfused males - 1% Transfused males - 1.7% Females - 17.3% Nulliparous females - 1.7% Parous females - 24.4%
Powers *et al*.[18] 2008, USA	2429 whole blood donors, 1020 females, 1409 males	Flow cytometry	2.4 fold above a normalized background	Females - 25.4% Nulliparous females - 5.9% Parous females - 52.6% Transfused males - 12%
De Clippel *et al*.[19] 2014, Germany	1018 platelet pheresis donors, 947 females, 93 males	Luminex bead assay	Positive - MFI above 2000	Transfused males - 1.3% Nulliparous females - 4.2% Parous females - 31%
Xia *et al*.[20] 2015, China	1014 whole blood donors, 560 females, 454 males	Luminex bead assay	MFI above 2500	Males - 4.6% Females - 19.6% Nulliparous females - 4.9% Parous females - 24.7%
The present study, 2017 India	192 whole blood donors, 144 females, 48 males	Luminex bead assay	MFI above 2000	Males - 18.7% Females - 37.5% Nulliparous females - 29.2% Parous females - 41.7%

MFI: Mean fluorescence index, SD: Standard deviation, HLA: Human leukocyte antigen

Limitation

The limitation of this study is the low sample size because of cost bound factors. An overall large sample size (especially with higher parity scores) will be required to comment on the findings like increase in alloimmunization with parity. Further analysis of positive reactions for HLA antibodies must be done to know whether they were true positives due to HLA alloimmunization or mere cross-reactivity of other acquired antibodies.

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One kit (96 Tests) for HLA antibody screening was provided by Immucor India.

Conflicts of interest

There are no conflicts of interest.

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