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Original Article

Transfusion Medicine Section

Type and Screen Method versus Antihuman Globulin Crossmatch in Pretransfusion Testing: A Cross-sectional Study

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ABSTRACT

Introduction: Blood transfusion remains the primary modality of treatment for many serious and common diseases. According to the International Society of Blood Transfusion (ISBT), there are about 349 blood group antigens, out of which only about 25-28 antigens are known to cause acute or delayed type of haemolytic transfusion reactions which could be prevented by Pretransfusion Testing (PTT). Regulated pretransfusion tests include ABO blood grouping, Rh typing, antibody detection, antibody identification and compatibility testing. The purpose of compatibility tests is to demonstrate in-vitro red cell antigen-antibody reaction. The Antihuman Globulin (AHG) crossmatch testing can assure ABO compatibility between donor and patient blood as well as detect most clinically significant antibodies. Type and Screen (T&S) is a procedure carried out as part of PTT in which the recipient's blood sample is tested for ABO group, RhD T&S for unexpected antibodies.

Aim: To compare T&S method of PTT with AHG crossmatch.

Materials and Methods: This cross-sectional study was conducted in the Department of Transfusion Medicine at Government Royapettah Hospital, Chennai and The Tamil Nadu Dr. MGR Medical University, Chennai, Tamil Nadu, India from June 2012 to December 2013. T&S was performed on 1,040 recipients' (510 males and 530 females) samples. All these samples were subjected to AHG crossmatch with ABO group and RhD type matched donor samples to assess the compatibility between donor and recipient by using column agglutination technology. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 11.0.

Results: The prevalence of unexpected antibodies in the recipient population was 1.06%. Among the 1,040 recipients' blood samples, 11 samples were found to have unexpected antibodies. Out of these 11 samples, 10 showed exact antibodies and the remaining one sample with negative antibody screening was found to be incompatible with AHG crossmatch. The sensitivity and specificity of T&S method in comparison to AHG crossmatch was 87.50% and 99.71%, respectively.

Conclusion: The sensitivity and specificity of T&S is as acceptable as AHG crossmatch. However, in view of one sample with false negative antibody screening in the study population, it is imperative to know the phenotyping of Red Blood Cells (RBC) antigens of the native population before getting away with AHG crossmatch.

Keywords: Antibody screening, Clinically significant antibodies, International society of blood transfusion

INTRODUCTION

The concept of PTT of blood had evolved following the discovery of blood groups A, B and O by Karl Landsteiner in 1901 and group AB by Decastello and Sturli in 1902. In 1908, Ottenberg took the first step of crossmatching of blood and demonstrated the importance of compatibility testing for the prevention of transfusion related accidents [1].

Regulated PTT include ABO blood grouping, RhD typing, antibody detection, antibody identification and compatibility testing [2]. PTT involves the detection of clinically significant unexpected antibodies, which results in both acute and delayed haemolytic transfusion reactions.

The purpose of compatibility tests is to demonstrate in-vitro red cell antigen-antibody reaction. The major and minor crossmatch was initially carried out to demonstrate the compatibility between the donor and the recipient [3].

The major crossmatch is conducted in two phases, immediate spin phase and the AHG phase. The immediate spin phase is designed to detect ABO incompatibility between the donor RBC and the recipient serum. The AHG phase helps to detect unexpected antibodies that were not detected during the immediate spin phase [2].

The minor crossmatch detects compatibility between the donor serum and the recipient RBCs. In 1970's, American Association of Blood Banks (AABB) had made the minor crossmatch optional [4].

In the 1980's, T&S policy (antibody detection and antibody identification) gained importance and the AABB set up the standards that if the antibody screening was negative, it is permissible to omit the AHG phase and issue blood after immediate spin phase of crossmatch. Further, if the recipient is found to have clinically significant antibodies, RBC units lacking relevant antigens should be issued after AHG crossmatch [4].

In the mid 1990's, with the safe complement of the T&S policy, the computer/electronic crossmatch was started and was validated with set standards. The computer crossmatch had evolved over the years and in Western countries if there are no clinically significant antibodies in antibody screening, the group compatible blood units are issued without immediate spin crossmatch/AHG crossmatch [5].

According to ISBT (https://www.isbtweb.org/isbt-working-parties/rcibgt.html), there are about 349 blood antigens, which have been divided into 43 blood group systems [6]. However, not all of the antigens will lead to formation of clinically significant antibodies. Only about 25-28 blood group antigens are known to cause haemolytic transfusion reactions. Haemolytic transfusion reaction (1.1-9 per 1,00,000 transfusions) is an irreversible unfavourable event that can occur at the time of transfusion (immediate) or 3-7 days after transfusion (delayed) which can be prevented by PTT [3].

Thus, PTT should eliminate the possibility of a haemolytic transfusion reaction and increase the duration of survival of RBCs in the recipient. Only 0.3-2% of the general population have unexpected antibodies

and the incidence is higher in multipara and patients with history of multiple transfusions [7-10]. The AHG crossmatch testing can assure ABO compatibility between donor and patient blood as well as detect most clinically significant antibodies. T&S is a procedure conducted as part of PTT in which the recipient's blood sample is tested for ABO group, RhD T&S for unexpected antibodies and then the sample is stored in the blood bank serology laboratory for future crossmatching, if a unit is needed for transfusion [2].

T&S has been considered as a preferred method over AHG crossmatch because of several advantages mainly better inventory management, reduced turnaround time and reduced exposure of technical personnel to blood samples [2].

Further, the time from a request to issue of ABO group and RhD specific blood is reduced to five minutes without immediate spin crossmatch and 15 minutes with immediate spin crossmatch in electronic crossmatch when compared to one hour for an antiglobulin crossmatch. This helps to reduce turnaround time [11].

The purpose of present study was to demonstrate whether the T&S procedure is a safe and sensitive method of PTT in our population, when compared to the AHG crossmatch currently in use.

MATERIALS AND METHODS

This cross-sectional study was conducted in the department of Transfusion medicine at Government Royapettah Hospital, Chennai Tamil Nadu, India and The Tamil Nadu Dr. MGR Medical University, Chennai from June 2012 to December 2013 after getting approval from Institutional Ethical Committee (IEC) (ECMGR0309016).

Inclusion criteria: All patients requiring blood transfusion and blood donors who were willing to participate in the study were included in the study.

Exclusion criteria: Patients who required emergency blood transfusion and those not willing to participate in the study were excluded from the study.

Procedure

- T&S was performed independently on 1,040 recipients' (510 males and 530 females) samples. T&S procedure included ABO grouping, RhD typing, antibody screening with Asia 3-cell screening panel and identification with 11-cell panel along with autocontrol on microcolumn agglutination gel cards. Turnaround time for T&S and antibody identification if three cell panel positive was 45 minutes and one hour respectively. Turnaround time for Issue of blood on request after T&S is five minutes without immediate spin crossmatch and 15 minutes with immediate spin crossmatch. All the above 1,040 recipients' samples were subjected to AHG crossmatch with ABO group and RhD type matched donor samples to assess the compatibility between {donor cells and recipient serum (major crossmatch)} and {recipient red cells and donor serum (minor crossmatch)} on microcolumn agglutination gel cards. Incompatible samples were then subjected for antibody screening and identification along with autocontrol on microcolumn agglutination gel technique. (When specific antibodies were identified, antigen-negative units were selected for the AHG cross-match). Turnaround time for AHG crossmatch was 45 min-1 hour and turnaround time for Issue of blood on request was one hour.

Interpretation of results: After centrifugation, positive reactions were indicated by RBC agglutinates trapped anywhere in the column of the gel. Positive reactions can be graded from 0 to 4+ [3].

1. A 4+ reaction is indicated by a solid band of RBCs on top of the gel.
2. A 3+ reaction displays agglutinated RBCs in the upper half of the gel column.
3. A 2+ reaction is characterised by RBC agglutinates dispersed throughout the length of the column.
4. A 1+ reaction is indicated by RBC agglutinates mainly in the lower half of the gel column with some unagglutinated RBCs pelleted at the bottom.
5. Negative reactions display a pellet of RBCs at the bottom of the microtube and no agglutinates within the matrix of the gel column [3].

STATISTICAL ANALYSIS

Analysis was carried out using SPSS version 11.0. Data were expressed using descriptive statistics such as frequencies and percentage. Sensitivity, specificity, positive predictive value, negative predictive value was done to compare the two methods of PTT. Chi-square test was used to compare the number of positives in T&S to AHG crossmatch and level of significance was evaluated. Measure of agreement (KAPPA) between the two tests was done. All statistical analysis was carried out at 5% level of significance and p-value <0.05 was considered as significant.

RESULTS

During the study period, T&S was done on 1,040 recipients' (510 males and 530 females) samples. Out of 1040 recipients, O positive {355 patients (34.14%)} followed by B positive {349 patients (33.56%)} were the most prevalent blood groups among the recipient population [Table/Fig-1] and antibody screen was positive in 10 out of 1040 recipients [Table/Fig-2]. Autocontrol was negative in all recipients.

S. No.	ABO blood group and RhD type	Frequency	Percentage
1	A Positive	191	18.37
2	B Positive	349	33.56
3	AB Positive	90	8.65
4	O Positive	355	34.14
5	A Negative	10	0.96
6	B Negative	23	2.21
7	AB Negative	1	0.09
8	O Negative	21	2.02
Total		**1040**	**100**

[Table/Fig-1]: ABO and RhD type of the recipients (n=1040).

Test result	Antibody screening (n)	Percentage
Positive	10	0.96
Negative	1030	99.04
Total	**1040**	**100**

[Table/Fig-2]: Antibody screening of the recipients.

Antibody identification revealed anti-D followed by anti-c and anti-e as the most common antibodies [Table/Fig-3]. Since, the above antibodies were identified in the AHG phase; they were considered as significant antibodies. The prevalence of red cell antibodies among the recipient population was 0.96% (10 out of 1040 recipients).

S. No.	Antibody identified	Frequency
1	Anti-D	3
2	Anti-Le^b	1
3	Anti-c	2
4	Anti-C	1
5	Anti-e	1
6	Anti-e and Anti-M	1
7	Anti-Jk^a and Anti-Fy^a	1
Total		**10**

[Table/Fig-3]: Antibody identification of the recipients (n=10).
(Anti-Le- lewis system, Anti Jk- Kidd (JK) system, Anti-Fy- duffy system)

All the 1040 recipient samples were subsequently subjected to AHG crossmatch with ABO group and RhD Type matched donor samples

to assess the compatibility between donor and recipient by using column agglutination technology. Among the donor population, O positive {355 blood donors (34.14%)} followed by B positive {349 blood donors (33.56%)} were the most prevalent blood groups [Table/Fig-1]. Out of 1040 AHG crossmatches, eight were incompatible and 1032 were compatible [Table/Fig-4,5]. The reason for incompatibility was confirmed by antibody screening in 7 of the 8 incompatible crossmatches. Seven of these incompatible crossmatches were due to the presence of alloantibodies that were identified by antibody detection. For the remaining one of the incompatible crossmatch, exact antibody could not be detected. Hence, to find out whether this incompatible crossmatch was due to the antibodies against low incidence/unknown antigens, other rare possibilities of incompatible AHG crossmatches like Direct Antiglobulin Test (DAT) on donor cells were ruled out.

Test result	Number of compatible/incompatible crossmatches	Percentage
Incompatible	8	0.77
Compatible	1032	99.23
Total	1040	100

[Table/Fig-4]: Major crossmatch results.

Test result	Number of compatible/incompatible crossmatches	Percentage
Incompatible	0	0
Compatible	1040	100
Total	1040	100

[Table/Fig-5]: Minor crossmatch results.

AHG crossmatch detected eight incompatible crossmatches, while the T&S method detected ten recipient samples with unexpected antibodies. The T&S had detected anti-D in three recipients but the three recipient samples were compatible in AHG crossmatch as the crossmatch blood selected was RhD negative donor. The AHG crossmatch detected one incompatible crossmatch but the recipient had a negative antibody screen. The prevalence of unexpected antibody detection was 0.77% by AHG Crossmatch and 0.96% by T&S method.

The sensitivity, specificity, positive predictive value and negative predictive value of the T&S method were 87.50%, 99.71%, 70% and 99.90%, respectively with respect to the AHG crossmatch [Table/Fig-6].

AHG crossmatch T&S	Incompatible	Compatible	Total
Positive	7	3	10
Negative	1	1029	1030
Total	8	1032	1040

[Table/Fig-6]: Comparison between T&S and AHG crossmatch results.

Using Chi-square Test, when comparing the number of positives in AHG crossmatch to T&S, "p-value" was found to be statistically significant.

Measurement of agreement: The agreement between the "T&S" method and "AHG Crossmatch" method was 77.6 % (kappa=0.776), which is a good agreement. On statistical evaluation for causes of positive pretransfusion test, there was a significant correlation between history of blood transfusion (p-value of 0.021, r=0.398), history of abortion/pregnancy (p-value of 0.037, r=0.442), female gender of the recipients (p-value of 0.019, r=0.163) and positive pretransfusion test, respectively [Table/Fig-7-9].

H/O previous transfusion	Positive antibody screening by T&S	Incompatible AHG crossmatch
Yes	7	7
No	3	1

[Table/Fig-7]: History of previous transfusion.
Significant p-value <0.05

H/O abortion/pregnancy	Positive antibody screening by T&S	Incompatible AHG crossmatch
Yes	8	6
No	2	2

[Table/Fig-8]: History of abortion/pregnancy.
Significant p-value <0.05

Gender	Positive pretransfusion tests (n=11)				Percentage
	T&S	AHG XM	Both T&S and AHG XM	Total	
Male	-	-	1	1	9%
Female	3	1	6	10	91%

[Table/Fig-9]: Gender of the recipients with positive pretransfusion tests.
Significant p-value <0.05

DISCUSSION

The present study was undertaken to compare the "T&S" method with the conventional "AHG crossmatch" method in PTT of blood. AHG crossmatch was considered as the only safe method to identify perfectly compatible blood for transfusion until 1960s [12]. However, after the advent of antibody screening technique using Group O reagent cells expressing clinically significant known antigens representing the native population, blood transfusion service has slowly switched over to this method over AHG crossmatch as an accepted method after many studies on larger number of samples [9,12,13]. However, accepting 'T&S' as the method of choice for PTT over 'AHG crossmatch' needs careful evaluation as the former method cannot be introduced without studying the representative antigens of the indigenous population.

In the present study, the prevalence of unexpected antibodies in the recipient population was 1.06%. The study by Chaudhary R and Agarwal N on 2026 samples from northern India showed almost the same prevalence rate of 1.28% [14]. Boral LI and Henry JB; and Chow E had also reported prevalence rates of 2.20% and 1.85%, respectively in New York and Hong Kong, which was comparatively higher than the present study [9,15]. These studies were done to ensure expected survival of transfused RBCs in recipients.

Present study population comes under the 'to be evaluated group' with respect to collective data on phenotyping of RBC antigens, in spite of a few studies for and against considering T&S as a preferred method of PTT [14,16-19]. In order to compare the two methods of PTT, 1,040 recipient blood samples were subjected for 'T&S' and 'AHG crossmatch' independently. The study identified clinically significant unexpected antibodies in 10 samples by T&S method and eight samples by AHG method of crossmatching. The details of the recipients with the presence of unexpected antibodies by both the methods were collected and compared. On comparison, 7 of the 10 samples with positive antibody screening belonged to RhD positive recipients and the remaining were from RhD negative recipients. The same details were obtained for AHG crossmatch in addition to one RhD positive sample with negative antibody screening which was found incompatible by AHG crossmatch method. Hence, there were in total eight RhD positive samples and three RhD negative samples with presence of unexpected antibodies detected by either of the two methods.

Seven of these eight RhD positive samples showed presence of unexpected antibodies by both methods, the remaining one sample with negative antibody screening was found to be incompatible with ABO RhD matched donor red cells by AHG crossmatch. Three of the RhD negative samples which were found to be compatible with ABO RhD compatible donor RBCs showed the presence of anti-D antibodies by T&S method. Since none of the antibodies were other than anti-D in these three RhD negative samples, which were expected to be compatible with RhD negative ABO group matched blood by AHG method, it has been considered unrelated to include these three samples for comparison. Hence, the remaining eight

samples out of 11 with unexpected antibodies were compared to assess the efficiency between the said two methods.

In comparison to AHG crossmatch the sensitivity of T&S method was 87.5%, the specificity was 99.71% in present study. In a study by Chaudhary R and Agarwal N on 2026 samples, the sensitivity and specificity of T&S in comparison to AHG crossmatch was found to be 91.6% and 99.25%, respectively, which was almost similar to present study [14]. In another study by Boral LI and Henry JB, the sensitivity of T&S was found to be 96.11%, which opined that T&S as a safe method of detecting unexpected antibodies [9].

Present study with 1,040 samples had identified 11 samples with unexpected antibodies were identified. In concordance to present study, 'Boral LI and Henry JB' (283 out of 12,848 samples) Chaudhary R and Agarwal N (26 out of 2026 samples), Pathak S et al., (68 out of 45373 samples), and Heisto H (178 out of 23,857 samples) had reported high frequency of antibody detection against antigens of Rh, Lewis and MNS blood group system [9,14,19,20].

One out of 11 (9.09%) recipients with unexpected antibodies showed incompatible AHG crossmatch with negative antibody screening in present study. Similar pattern of one out of 12 recipients (8.33%), nine out of 84 recipients (10.71%) and 16 out of 101 recipients (15.84%) of non reactive antibody detection test on AHG incompatible crossmatched samples had been reported by Chaudhary R and Agarwal N Oberman HA et al., and Mintz PD et al., respectively [14,16,18].

Such kind of samples need further study to find out the reason for incompatible AHG crossmatch with negative antibody screening. The possible reasons are due to antibody against unidentified/low incidence antigen, donor red cells having positive DAT and antibodies which react with red cells having stronger expression of a particular antigen (dosage) or variation in antigen strength [2]. However, the donor DAT in this particular crossmatch was found to be negative. Since there is a lack of accepted database for antigen phenotype of the study population, the antibody missed by the panel cells used in this study could be against a low incidence/unidentified antigen.

Limitation(s)

Present study was carried out on limited number of samples, further studies with larger number of samples is necessary to arrive at a definitive decision.

CONCLUSION(S)

The safety of T&S is almost comparable to AHG crossmatch. However, in view of one sample with false negative result in the study population, it is imperative to know the exact phenotyping of RBC antigens of the native population before replacing AHG crossmatch by T&S. Hence, in order to issue antigen negative RBCs immediately for these patients in emergencies, phenotyping of donor RBCs for antigens prevalent in the native population is necessary. In a country like India where demand is always more than supply, if T&S is introduced with indigenous cell panel representing red cell antigens pertained to the population, it would pave way for better inventory management and better care by decreasing the turnaround time.

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