COMPARISON BETWEEN CONVENTIONAL TUBE TECHNIQUE AND COLUMN AGGLUTINATION TECHNIQUE FOR ANTIBODY SCREENING AND IDENTIFICATION AT MGM BLOOD BANK, NAVI MUMBAI

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ABSTRACT

BACKGROUND

Pretransfusion testing is one of the most important section of the entire transfusion process. Conventional tube technique (CTT) is the most commonly employed technique in blood grouping, cross-matching and for the detection of antibodies. It is still considered as a gold standard in the pretransfusion testing but it has some inherent limitations. Newer techniques have been introduced to overcome the shortcomings of the CTT. One of them is Column agglutination technique (CAT). The present study was aimed to evaluate the efficacy in terms of sensitivity and specificity of CAT over the CTT in our setup for antibody screening and identification.

MATERIALS AND METHODS

The prospective comparative study was conducted for a period of 6 months. A total of 2258 patients' samples were screened for antibody detection during the pretransfusion testing process. Each sample was tested with CTT parallelly with CAT for antibody screening and identification. The results of CTT and CAT were compared using Student 't' test, p value of <0.05 was considered significant. The sensitivity and specificity were calculated by the standard manual method.

RESULTS

Out of 2258 samples screened, a total of 11 cases with antibodies were identified. Of these, all 11 could be identified by CAT and only 7 were determined by CTT. The overall incidence of alloimmunisation was 0.5%. Out of 11 antibodies, 10 were clinically significant and one was clinically insignificant. The most commonly found antibody was Anti-D followed by Anti-c, Anti-M, Anti-Fy(a) and Anti-Le(a). The results obtained by CTT and CAT showed statistical significant difference, p value=0.04. The sensitivity of CTT was 63.63% while the specificity was 100%. The sensitivity and specificity of CAT were 100%.

CONCLUSION

The gel technique is now considered better and has been introduced as a replacement to conventional tube technique on an automation platform. Though CTT is still considered gold standard in pretransfusion testing, it still has various disadvantages and depends on accurate hand to eye work of the laboratory personnel. The CAT, although being costly affair, still has several advantages over the tube technique. Therefore, it is highly recommended to be used routinely in the pretransfusion testing.

KEYWORDS

Pretransfusion Testing, Conventional Tube Technique, Column Agglutination Technique.

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BACKGROUND

Pretransfusion testing is one of the most important section of the entire transfusion process.[1] ABO and Rh blood grouping, cross‑matching between donor and recipient and antibody screening and identification to detect clinically significant antibodies are the basic steps of the pretransfusion testing in blood banking.[2] Antibody screening and identification tests are performed to detect "irregular" or "unexpected" antibodies, as opposed to the "expected" antibodies of the ABO system in recipients' serum.[3,4]

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These are the principle tools to select compatible blood for recipients to prevent haemolytic transfusion reactions and alloimmunisation.

Conventional tube technique (CTT) is the most commonly employed technique in blood grouping, cross-matching and for the detection of antibodies. It is still considered as a gold standard in the pretransfusion testing but it has some inherent limitations like multiple washing steps, the instability of the reactions and subjective nature of grading by the technologist, etc. However, in the recent years, newer techniques have been introduced which have not only tried to overcome the shortcomings of the conventional tube technique but have also showed substantial improvement in the quality of results.[5,6] One of the newer technique is Column agglutination technique (CAT) which is also known as Micro typing gel method. It is based on the principle of controlled centrifugation of RBCs through dextran acrylamide gel that contained predispensed reagents. This gel column agglutination test was developed by Dr. Lapierre in 1985 who

used Sephadex gel within the microtube.[7] This gel technique is advantageous in being simple, less labour intensive, offers consistent and objective stable results. It requires small amount of sample volume and it includes no washing steps or controls for antiglobulin tests.[5,6]

Therefore, the present study was carried out to evaluate the efficacy in terms of sensitivity and specificity of Micro typing gel method over the conventional tube method in our setup for antibody screening and identification

MATERIAL AND METHODS

The prospective comparative study was conducted in the Department of Immunohaematology and Blood Transfusion, MGM Medical College and Hospital, Kamothe, Navi Mumbai for a period of 6 months from January 2017 to June 2017. A total of 2258 patients' samples were screened for antibody detection during the pretransfusion testing process. Anticoagulated EDTA (ethylenediaminetetraacetic acid) and clotted (plain) samples were used for testing. Each sample was tested with Conventional Tube technique (CTT) and Column agglutination technique (CAT) for antibody screening and identification. The study was approved by Institutional ethical committee.

Conventional Tube Technique

It was performed by taking 25 µL of red cell suspensions [ID-DiaCell I-II-III, Biorad, Diamed, Switzerland] and 50 µL of patient's serum sample. They were mixed and kept for incubation at 37°C for 60 min. Then they were centrifuged and the results were noted. If negative, the tubes were washed three times with normal saline and polyspecific Anti-human globulin reagent, AHG (Diaclon Coombs, Diamed, Switzerland) was added. After centrifugation, the results were noted. The procedure was in accordance with the standard method described by AABB.[8] The samples coming positive on screening cells were then subjected to identification panel cells [ID-DiaPanel (11 cell), Biorad, Diamed, Switzerland]. The negative tests after AHG phase were validated by presence of agglutination on addition of Coomb's control cells (in-house). All reactions were graded and recorded.

Column Agglutination Technique

It was performed by adding 50 µL of red cell suspension [ID-DiaCell I-II-III, Biorad, Diamed, Switzerland] in low ionic strength solution (LISS) to appropriately labelled microtube of the ID cards (polyspecific AHG, LISS Coombs card, Diamed, Switzerland). Then, 25 µL of patient's sample was added in each microtube of Id-Cards. After incubation at 37°C for 15 min., they were centrifuged in a dedicated centrifuge device (Diamed, Switzerland). Samples reactive with screen cells were then similarly tested for identification panel cell [ID-DiaPanel (11 cell), Biorad, Diamed, Switzerland]. The findings were graded and documented. The gel technique was performed following manufacturer's instruction.

Statistical Analysis

The statistical analysis was done using Microsoft excel. The results of Conventional tube technique and column agglutination technique were compared using Student 't' test, p value was calculated and a value of <0.05 was considered significant. The sensitivity and specificity, positive predictive value and negative predictive value were calculated by the standard manual method. On theoretical basis, conventional tube technique in AHG phase was assumed as standard reference for sensitivity and specificity.[1,4,5]

RESULTS

During the 6-month study period, 2258 samples were screened with conventional tube technique in parallel with column agglutination technique. Out of 2258, 1027 (45.48%) were males and 1231 (54.51%) were females. The mean age of the patients was 27.90 years. The age distribution of the patients is summarised in Table 1.

Age Group (In Years)	No. of Patients (N)	N%
0-10	241	10.67
11-20	524	23.20
21-30	683	30.24
31-40	387	17.13
41-50	158	6.99
51-60	96	4.25
61-70	93	4.11
71-80	76	3.36
Total	2258	100

Table 1. Age Distribution of Patients

A total of 11 cases with antibodies were identified. Of these, all 11 were identified by Column agglutination method (CAT) and only 7 were determined by conventional tube technique (CTT). The overall incidence of alloimmunisation was 0.5%. Antibody specificity was not associated with age and sex of the patient. Table 2 shows comparison of the results by conventional tube technique and column agglutination technique.

Result	CTT	CAT
Positive	7 (63.63%)	11 (100%)
Negative	4 (36.36%)	0 (0%)

Table 2. Comparison of the Results Obtained by CTT and CAT

Out of 11 cases, 10 were clinically significant and 1 clinically insignificant. Table 3 and Figure 1 summarises the results obtained by both techniques which determines the number of antibodies.

Antibody Identified	Column Agglutination Technique (CAT)	Conventional Tube Technique (CTT)
Clinically Significant Antibodies		
Anti-D	7	5
Anti-c	1	1
Anti-M	1	1
Anti-Fy (a)	1	0
Clinically Insignificant Antibodies		
Anti-Le (a)	1	0
Total	**11**	**7**

Table 3. Number of Antibodies Identified by CAT and CTT

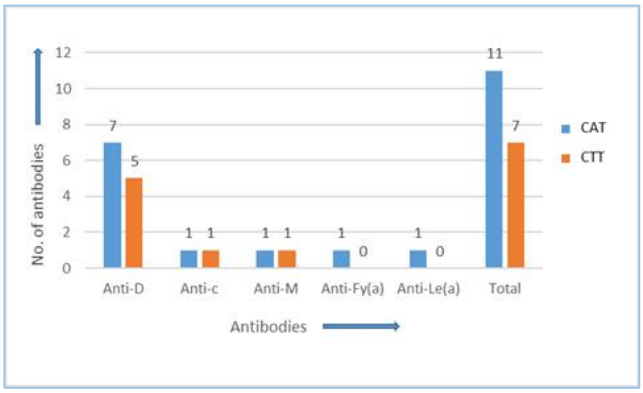

Figure 1. Number of Antibodies Identified by CAT and CTT

Column agglutination technique detected all 10 clinically significant antibodies and one clinically insignificant antibodies. Conventional tube technique detected 7 clinically significant antibodies when proceeded to anti-human globulin phase but failed to detect other clinically significant and insignificant antibodies. Out of 11 cases of alloantibodies, 7 cases of anti-D were detected in multigravida females with Rh D negative blood groups, 1 case of anti-c and anti-M each were identified in thalassaemia patients, 1 case of anti-Fy(a) was a patient who received multiple transfusions for cardiac surgery and 1 case of Le(a) was a patient who received multiple transfusions for refractory anaemia. [Table 4 and Figure 2].

Antibody Identified	Indication for Transfusion	No. of Antibodies (N)	No. of Antibodies (N %)
Anti-D	Multigravida females	7	63.63%
Anti-c	Thalassaemia	1	9.08%
Anti-M	Thalassaemia	1	9.08%
Anti-Fy(a)	Multiple transfusions for cardiac surgery	1	9.08%
Anti-Le(a)	Multiple transfusions for refractory anaemia	1	9.08%
Total		**11**	**100%**

Table 4. Antibodies Identified with Indication of Transfusion

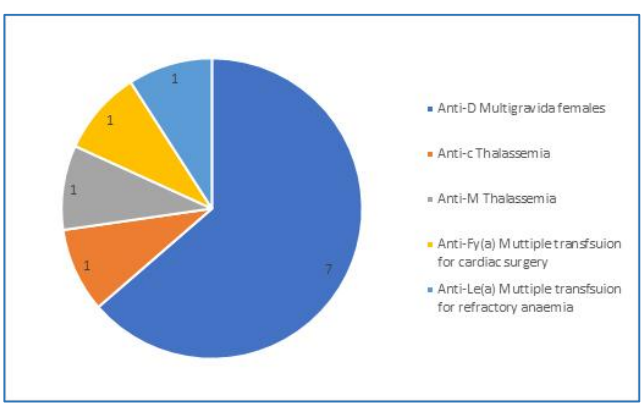

Figure 2. Graphical Representation of No. of Antibodies Identified with Indication of Transfusion

On statistical analysis, the results obtained by conventional tube technique and column agglutination method showed statistical difference, p value=0.04, which was significant ($p<0.05$). The sensitivity of determining antibodies by Conventional tube technique was 63.63% while the specificity was 100%. The sensitivity and specificity of determining antibodies by column agglutination method were 100% each. The positive predictive value and negative predictive value for conventional tube technique were 100% and 99.8% respectively, while for column agglutination technique, the positive and negative predictive values were found to be 100% each.

DISCUSSION

Cross-matching as a part of pretransfusion testing is performed in all blood centres before transfusion to prevent alloimmunisation. The main aim of cross-matching is to detect clinically significant antibodies to the maximum. Since the introduction of concept of cross-matching, rationale has been changing from time to time. One of them is type and screen policy, which is widely practised in western countries but only few centres in developing countries in India have been working on it to make a part of their protocols. Red cell antibodies screening is important as these antibodies when clinically significant mediate haemolytic transfusion reactions or haemolytic disease of new-born and foetus.[9,10]

At present, there are various methods of antibody screening available in various blood centres. They are Conventional Tube Technique (CTT), LISS-IAT, Column agglutination Technique (CAT), polyethylene glycol (PEG) tube test, solid phase red cell adherence assay (SPRCA) etc.[11] We compared the commonly used techniques in our setup, the conventional tube technique and column agglutination technique also called as Micro typing gel technique. During the 6-month study period, 2258 samples were screened with most of the patient group lying between the age group 21-30 years (30.24%).

A total of 11 alloantibodies were identified in 2258 patients during the study period making overall alloimmunisation rate to 0.5%. This was comparable to the study by Pathak S et al[10] which reported an overall rate of alloimmunisation of 1.5% in 45,373 patients. These frequencies were low as compared to 2.71% in 4569 patients and 5.5% in 200 patients reported by Agarwal A et al[12] and Philip J et al.[13] Therefore, the reported prevalence of alloimmunisation in multi-transfused patients in India varies from approximately 3% to 10%.[14,15,16] The rate of alloimmunisation globally was variable with 1.35% in Denmark,[17] 0.78% in Germany,[18] and 0.3-2% in the USA[19,20] to 21.1% in Greece,[21] 30% in Kuwait[22] and 37% in Taiwan.[23]

The most commonly found clinically significant antibody in our study was Anti-D (7 out of 11 cases) followed by one case each of Anti-c, Anti-M, Anti-Fy(a). One case of clinically insignificant antibody was also identified, Anti-Le(a). Similar set of antibodies frequencies were reported in studies by Pathak S et al[10] (Rh> MNS> Kell) and Philip J et al.[13] (Rh>MNS>Lewis). We found multigravida females followed by thalassaemia as the most common alloimmunised cases which was comparable to the study by Patel et al.[24]

Out of the 11 cases of alloantibodies identified, Column agglutination technique could identify all 11 cases while conventional tube technique identified 7 cases. These 7 cases

identified by conventional tube technique were 5 cases of Anti-D, one case each of Anti-c and Anti-M while the other antibody cases were not detected by it. The number of antibodies detected by both the methods showed significant statistical difference ($p<0.05$). This was also demonstrated by specificity and sensitivity calculated for both techniques for determining potentially significant antibodies. The sensitivity of conventional tube technique was 63.63% and specificity was 100% while the sensitivity and specificity were 100% for column agglutination technique. Our findings were in accordance with the other studies of Swarup D. et al,[25] Reis et al[26] and Pinkerton et al[27] where these techniques were compared.

Our study demonstrated that the results by conventional tube technique were comparable to column agglutination technique when subjected to IAT phase like the study by Swarup D. et al.[25] The gel technique in our study could also identify cold reacting antibody, Anti-Le(a) apart from warm reacting antibodies. The detection of cold reacting antibodies was demonstrated in the past studies also by Lapierre et al,[7] Bromilow et al[28] and Kretschmer et al.[29] Therefore, gel technique is considered better than column agglutination technique. There are lower chances of false positive or false negative results by gel technique because there is increased serum to cell ratio with no washing step, thereby reducing possibility of elution of weakly bound antibodies from red blood cells.[28] The time taken by gel technique procedure is 15-20 min. compared to tube technique procedure which is about 45-60 min. The sensitivity, specificity, positive predictive value and negative predictive value were found better for column agglutination technique on comparing with conventional tube technique which was comparable to other studies. [1,2,9,25,30,31] Column agglutination technique is, therefore, simple, rapid, more sensitive and helps in standardisation of laboratory results with objective haemagglutination findings.[32] The only limitation is the cost of gel cards and commercial screening panel. In all blood centres, due to high costs per test it is very difficult to screen each and every patient for antibodies by gel technique and commercial screening panels. Though being the most sensitive way to detect alloantibodies, many blood centres are still reliant upon screening done by in-house prepared pooled cells which may not cover all antigens in tube technique.

In addition to antibody identification, various studies have shown that column agglutination technique gives better and accurate results in ABO and Rh grouping, dilutional titration and in evaluation of DAT (Direct antiglobulin test) when compared to conventional tube technique.

CONCLUSION

In the recent years, with advancement in blood banking, column agglutination technique has been introduced as a replacement to conventional tube technique on an automation platform.[3] Though conventional tube technique is still considered gold standard in pretransfusion testing, it still has various disadvantages and depends on accurate hand to eye work of the laboratory personnel. The column agglutination technique on other hand, although being costly affair, still has several advantages over the tube technique. Therefore, it is highly recommended to be used routinely in the pretransfusion testing.

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