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An evaluation of irregular antibody profile in voluntary blood donors and blood transfusion recipients in and around Gangtok, India

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RESEARCH

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ABSTRACT

Background
Unexpected antibodies can develop in multiple transfused patients as well as in healthy donors who were either transfused or pregnant previously. This unexpected alloantibodies can complicate transfusion process, cross matching of blood and can occasionally cause severe transfusion reactions if a large amount of plasma or whole blood is transfused as in massive transfusion and in paediatric patients.

Aims
The purpose of this study was to screen and identify irregular/unexpected antibodies in voluntary blood donors and blood transfusion recipients in and around Gangtok and to provide compatible blood and prevent transfusion reactions due to such antibodies.

Methods
A prospective cross-sectional study was carried out in a total of 2415 samples from voluntary blood donors and transfusion recipients and tested for the screening and identification of unexpected antibodies for the period of 2 years from 1st September, 2014 to 31st August, 2016 in Blood Bank of Tertiary care hospital, Central Referral Hospital, Sikkim.

Results
The positive screening rates for unexpected antibodies were found to be 1.48 per cent in donors (n=1999); and 3.03 per cent in blood transfusion recipients (n=416). Antibodies against the Kell system were the most frequent (Anti-Kp^a-32.1 per cent; Anti-K- 27.7 per cent), followed by antibodies against Lutherium system (Lu^a-22.73 per cent), against Rh system (Anti-C^w-22.73 per cent; Anti-E -18.18 per cent) and so on.

Conclusion
Since clinically significant antibodies are frequently detected in our donor as well as transfusion recipient samples, screening and identification of unexpected antibody is a must and the need of the hour. Knowledge of such alloantibodies is essential not only in the multitransfused patients but in all hospital patients who require or may require transfusion. This study not only helps in selecting appropriate RBC products for transfusion but also avoids unnecessary delays in provision of blood in case of emergencies or surgical complications.

Key Words
Screening, identification, unexpected antibodies

What this study adds:

1. What is known about this subject?
Although, WHO guidelines emphasize on the screening of donor blood for unexpected antibodies to prevent transfusion reactions, most of the blood banks are not doing so, resulting in adverse transfusion reactions especially in multi-transfused patients.

2. What new information is offered in this study?
Screening and identification of unexpected antibody is a

relatively simple test and is capable of preventing massive transfusion reaction. Such a study had never been conducted in Sikkim and the incidence of irregular antibodies in different subsets i.e., in ABO/Rh of blood groups had not been studied in blood donors and transfusion recipients in east Sikkim. This novel study could positively identify blood donors and transfusion recipients having irregular antibodies in East Sikkim.

3. What are the implications for research, policy, or practice?

The results of the study helped us in providing compatible blood to transfusion recipients thus preventing massive transfusion reactions due to such antibodies. Since clinically significant antibodies are frequently detected in our donor as well as transfusion recipient samples, it is recommended that screening and identification of unexpected antibodies should be made mandatory for every transfusion set-up.

Background

There are now 29 blood group systems recognized by the International Society on Blood Transfusion, comprising over 280 antigens.[1] After the well-known ABO and Rh systems, those considered most clinically relevant are the Kell, Kidd, and Duffy systems.[2] Clinically significant irregular antibodies are capable of causing haemolytic transfusion reactions secondary to accelerated destruction of a significant proportion of transfused red blood cells.[3] Therefore, screening and identification for unexpected antibodies should be part of all pre-transfusion testing.[4] The purpose of this study was to identify irregular antibodies in blood donors and transfusion recipients of East Sikkim and in order to provide compatible blood to transfusion recipients and to prevent future transfusion reactions in transfusion recipients due to such antibodies, using the acquired data.

The primary responsibility of the blood bank is to ensure safe blood for the recipient's routine transfusion. Detecting blood group-specific antibodies in patient sera is essential to the management of blood transfusions. Testing in the majority of the blood banks includes ABO and Rh typing along with major and minor cross-match of the donor and recipient. However, presence of red cell antibodies other than expected anti-A and anti-B, may cause transfusion reactions and these are called "irregular or unexpected antibodies".[5,6] Unexpected antibodies are referred to as irregular antibodies because their existence and type are unknown before conducting an antibody screening test.[4]

In India, more than 2,500 blood banks collect and transfuse a total of approximately eight million blood units annually.[7]

Little data are available regarding the frequencies of the blood group antigens other than ABO and RhD in the Indian population. Knowledge of the antigen frequencies is important to assess the risk of antibody formation and to guide the probability of finding antigen-negative donor blood, which is especially useful when blood is required for a patient who has multiple red cell alloantibodies. According to published data, rates of alloimmunization in random patients vary from 0 to 3 percent.[8] The alloantibodies, which frequently develop and are encountered during compatibility testing, are primarily against antigens related to Rh, Kell, Kidd, Duffy and MNSs blood group systems.[2,9,10-13] Saran et al. have reported that only 0.3 to 2.0 per cent of general population has unexpected antibodies and the incidence are higher in women due to pregnancy.[14]

WHO guidelines emphasize the screening of donor blood for unexpected antibodies to prevent transfusion reactions. Multiple transfused patients may be allo-immunized for these atypical antibodies which may result into transfusion reaction. There have been studies in the South Asian population groups on the presence of such antibodies.[5,6] Current testing procedures rely upon manual or automated agglutination reactions that use tested erythrocytes of relatively short self-life containing a variety of blood group antigens of differing liability, which produce results that can be difficult to measure and interpret objectively. Detecting blood group-specific antibodies in patient sera is essential to the management of blood transfusions.[15] Red blood cell (RBC) alloimmunization is an important adverse effect that follows repeated transfusions with allogeneic blood.[3] It results from an immune response due to the genetic differences between blood donors and recipients. Immune anti-RBC antibodies are generally formed early in the course of multiple transfusions, usually before the 10th transfusion.[16,17]

Method

A cross-sectional study was carried out in a total of 2415 samples from voluntary blood donors and transfusion recipients and tested for the screening and identification of unexpected antibodies for the period of two years from 1st September, 2014 to 31st August, 2016 in Blood Bank of Tertiary care hospital, Central Referral Hospital, Sikkim.

Settings and Design:

- ✓ **Design of study:** Hospital Based Prevalence (Cross Sectional) Study.
- ✓ **Study setting:** Tertiary care hospital (Central Referral Hospital, Sikkim).

Total of 2415 blood samples were collected into plain as

well as ethylene diamine tetra acetic Acid (EDTA) vials from all healthy voluntary blood donors and transfusion recipients and tested for the period of two years from 1st September, 2014 to 31st August, 2016 in Blood Bank of Tertiary care hospital, Central Referral Hospital, Sikkim. Deferred blood donors, Quantity Not Sufficient (QNS) cases and blood donors reactive for transfusion transmitted infections were excluded from the study. The details of the patient/donors were taken prior to the sample collection which included name, age, gender, blood group, transfusion history etc.

All the samples were centrifuged at 3000rpm for 2 to 3 minutes and plasma/serum was separated. The ABO/Rh group were determined using slide and tube Agglutination method for emergency purpose as in outdoor camps which were followed by gel technology test including forward and reverse grouping. All Rh D-negative samples were subjected to weak-D testing by an indirect antiglobulin test and the results were recorded. The patient/donor plasma were then screened and identified for commonly encountered incomplete antibodies in ID system (Gel Technology) by ID-Diacell I-II-III & ID Dia Panel 11 test cells for antibody identification, which have expression of clinically significant antigen system and homozygous expression of certain antigens in order to enhance reactivity with antibodies of certain blood group system notably Kell, Rh, Duffy and Kidd. We had further made blood group distribution study of Sikkim from the compiled data. A commercially available three cell panel (ID DiaCell I, II, III; AHG polyspecific cassette (coombs card)) was used for antibody screening procedure in which the subject's plasma was reacted with panel of red cells using low ionic strength saline (LISS) Coombs' gel card. The cards were incubated at 37°C for 15 minutes and then centrifuged for 10 minutes at 1000 rpm. An extended 11-cell panel was used for antibody identification (Dia Med 11 cell Dia Panel) for the plasma samples which were positive on antibody screen. The recordings were taken and the agglutination graded according to the kit manual. The final reporting was done on the antigen table.[18,19]

Results

A total of 2415 samples were tested of which 1999 were from donors and 416 were from recipients. Of these 2415 samples, the commonest blood group reported was A positive (32.21 per cent) and the least common was AB negative (0.25 per cent). A total of 22 cases had identifiable irregular antibodies. In the given period of time, the positive screening rates for unexpected antibodies were found to be 1.48 per cent in donors (n=1999); and 3.03 per cent in blood transfusion recipients (n=416). Antibodies against the Kell system were the most frequent (Anti-Kp^a-32.1 per cent; Anti-K-27.7 per cent), followed by antibodies against Lutherium system (Anti-Lu^a-22.73 per cent), against Rh system (Anti-C^w-22.73 per cent; Anti-E -18.18 per cent) and so on (Fig 1). In 5 cases, antibodies could not be identified as they were probably not included in the 11 cell panel used in this study and would require an extended panel for identification (Figure 1). Table 1 shows the detailed analyses of the results obtained in the study.

Discussion

The incidence of unexpected red cell antibodies in patients and donors at a hospital based study conducted in south India were found to be 4.91 per cent and 4.33 per cent respectively. Anti Lewis, anti P1 and anti Mia was the most common antibodies detected.[20] In another recent Indian study, a total of 7756 donors were screened for allo-antibodies. A total of four donors showed presence of alloantibodies in their serum (0.05 per cent) which somewhat correlates with our study (0.65 per cent). On antibody identification, two of them were anti-C, one was anti-$Lewis^a$ antibody and one was autoantibody.[21] Antibodies against antigens of the Rh and Kell blood group systems are the specificities most frequently found in alloimmunized patients in Western Europe and the United States[17,22,23] which were similar to our findings as well. The most common clinically significant alloantibodies identified in men and women were anti-K and anti-E, respectively in a study reported by Reyhaneh.[24]

Screening of the donor's and transfusion recipient's sera is a relatively simple test and is capable of detecting potent unexpected antibodies that could be the cause of massive transfusion reaction. It serves to simplify the work of cross-matching by eliminating the need for the minor cross match. However, this test is not being done in many of the hospital blood banks probably because it would add to the cost of testing of the blood unit and also because many of the blood bank staff are not aware of the utility of this test.
Such a study had never been conducted in Sikkim and the incidence of irregular antibodies in different subsets i.e., in ABO/Rh of blood groups had not been studied in blood donors and transfusion recipients in east Sikkim. Here we could positively identify blood donors and transfusion recipients having irregular antibodies in East Sikkim. We succeeded in providing compatible blood in transfusion recipients by preventing transfusion reactions in transfusion recipients due to such antibodies using the acquired data. Since clinically significant antibodies are frequently detected in our donors as well as transfused recipient's sample, screening and identification of unexpected antibody is a must and should be mandatory in every blood bank.

Knowledge of such alloantibodies is essential not only in the multitransfused patients but in all hospital patients who require or may require transfusion. This study not only helps in selecting appropriate RBC products for transfusion but also avoids unnecessary delays in provision of blood in case of emergencies or surgical complications.

However, the limitation of the study was that only a 11 cell panel was used for identification of irregular antibodies because of which 5 cases remain unidentified.

Conclusion

The index study could positively identify blood donors and transfusion recipients having irregular antibodies in East Sikkim. Therefore, we were able to provide compatible blood units to transfusion recipients and thereby were also successful in preventing transfusion reactions in them due to such antibodies, using the acquired data. This study concludes that since clinically significant antibodies are frequently detected in our donors as well as transfused recipients' sample, screening and identification of unexpected antibodies is a must and should be mandatory in every blood bank.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ETHICS COMMITTEE APPROVAL

SMIMS Institution Ethics Committee, Ref. No: SMIMS/IEC/2014-036

Figure 1: Bar diagram depicting frequency of occurrence of irregular antibodies

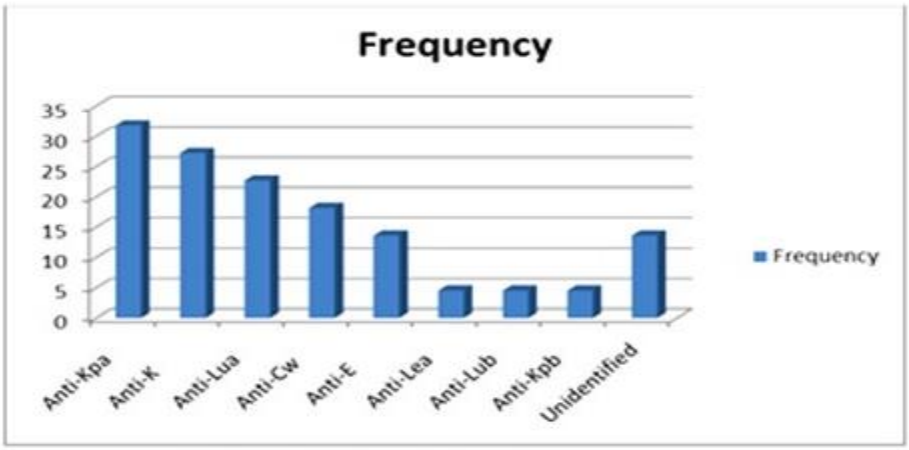

Table 1: Results Of Screening and Identification of Unexpected Antibodies

Total samples tested: 2415	
No. of donors: 1999 (82.8%) No. of recipient: 416 (17.2%)	Male: 1800 (74.5%) Female: 615 (25.5%)
Blood group distribution	
ABO/RH NEGATIVE: 56 (2.3%): A negative: 20 (0.83%) B negative: 12 (0.50%) AB negative: 06 (0.25%) O negative: 18 (0.75%)	ABO/RH POSITIVE: 2,359 (97.7%): A positive: 778 (32.21%) B positive: 621 (25.71%) AB positive: 193 (7.80%) O positive: 767 (31.76%)
Positive cases:	
Total: 22(0.91%) Donor sample (13positive): 11 positively identified unexpected antibodies (0.55%) 2case out of the 13 cases - observed to contain unidentifiable antibodies. Recipient sample (14positive): 11 positively identified unexpected antibodies (2.64%) 3 cases out of the 14 cases observed to contain unidentifiable antibodies.	
Antibody specificity: Out of all the positive results the individual frequency of occurrence of the most common unexpected antibodies are as follows(in decreasing order)	
i. Anti-Kpa: 7(31.82%) Kell ii. Anti-K: 6(27.27%) Kell iii. Anti-Lua: 5(22.73%) Lutherium iv. Anti-Cw: 5(22.73%) Rh system(Rh-hr) v. Anti-E: 4(18.18%) Rh system(Rh-hr)	vi. Anti-Lea: 3(13.64%) Lewis vii. Anti-Lub: 1(4.55%) Lutherium viii. Anti-Kpb: 1(4.55%) Kell ix. Anti-N: 1(4.55%) MNS x. Unidentified: 5(22.73%)