Original Paper

Prevalence of Anti-Red Cell Antibodies in Repeatedly Transfused Patients

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Abstract

Introduction: The development of anti-RBC antibodies (alloantibodies and/or autoantibodies) can significantly complicate transfusion therapy, particularly in patients needed repeated transfusion.

Objectives: To find out the prevalence of alloantibodies and autoantibodies in repeatedly transfused patients so that serious hazards due to immune reaction may be avoided.

Materials and Methods: This descriptive cross-sectional study was carried out in Department of Immunology, BIRDEM and Armed Forces Institute of Pathology (AFIP) during the period of July 2015 to June 2016. Total 370 patients who had received at least five units of transfusions were enrolled in this study but known patients of auto immune haemolytic anaemia, patients in whom antibody was previously detected and pregnant women were excluded from the study. Blood grouping and Direct Anti-globulin Test (DAT) were performed with cell suspension using a poly- specific Coombs reagent. In cases of a positive DAT, further investigation using specific monoclonal reagents to detect IgG or a complement (C3d) was carried out. Serum was used to detect red cell alloantibodies using standard blood bank methods. Antibody identification was performed in antibody screening positive samples using red cell Identicells.

Results: Maximum 132 (35.7%) patients were in the age group 1-10 years. The male-female ratio was 1.2:1. Among 370 total patients 290 were Hereditary Haemolytic Anaemia (HHA) and 80 were non-Hereditary Haemolytic Anaemia (HHA). Antibody was detected in only 17(4.59%) patients. Among the Hereditary Haemolytic Anaemia (HHA) patients it was 11 (3.79%) but among the non-HHA patients it was 6(7.5%). Out of 8 auto-antibodies, 5 were anti IgG followed by 3 were anti C3d. Out of 14 alloantibodies, 4(28.6%) were anti E, 3(21.4%) were anti K and in 3(21.4%) cases specificity of alloantibody was not detected.

Conclusion: Prevalence of anti-RBC antibodies was not so uncommon in multiple transfused patients.

Key-words: Red cell alloantibody, Auto-antibody, Repeatedly transfused patients.

Introduction

Red blood cell transfusions are a valuable health care resource, especially for patients with thalassaemia, myeloproliferative disorders, haematological disorders, end-stage renal failure, leukaemia and organ transplant patients[1]. Chronic red cell transfusions can cause unwanted complications called transfusion reactions in a patient[2]. Development of alloantibodies to red cell antigens is an important immune-mediated delayed hemolytic transfusion reaction. It is a matter of great concern in multi-transfused patients and in patients who have had multiple pregnancies[1]. Alloimmunization results from the disparity between the donor and patient antigens. Prior exposure to donor antigens can lead to an anamnestic or secondary response where even very small amounts of donor antigenic RBCs can elicit an alloimmune response resulting in increased antibody production leading to red cell destruction since the patient is already immunized[3]. Alloimmunization occurs when incompatible antigens introduced in an immune-competent host evoke an immune response leading to irregular antibody formation. Alloimmunization against red blood cell (RBC) can result in delayed hemolytic transfusion reactions which can range from destruction of RBCs within hours or even minutes, leading to reduced survival by a few days or can cause hemolytic disease in newborns. Development of alloantibodies thus complicates and limits transfusion therapy, contributing not only to technical complications but also morbidity and mortality[4].

Observational studies in random patients, who most often receive incidental transfusions, and pregnant women, estimated the antibody prevalence between less than 1 to 3%; however prospective systematic studies done by Walker et al[5] in multi-transfused patients reported on an up to over 70% alloimmunization incidence. Three large retrospective studies on alloimmunization in random hospitals transfusion recipients showed that antibodies to Rh and K blood group antigens comprise almost 80% of clinically significant non-D antibodies[6]. Transfusion dependent diseases are characterized

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by a high alloimmunization frequency and are highest in sickle cell patients. Combined data from 18 studies on 4005 sickle cell patients and 11 studies regarding 3394 thalassaemia patients showed an overall alloimmunization risk of 22% and a total of 1606 RBC antibodies were reported in 675 sickle cell patients and 834 antibodies in 446 thalassaemia patients[7]. Multiple antibody specificities were present in 35% of patients[8].

The immunization rate[1], expressed as the number of antibodies per 100 transfusions, varied between 1.7 and 4.0. A dysfunctioning immune system, either hyper- or hypo sensitive can result in an enhanced immune response/antibody production or reduced antibody production. Patients with myelodysplastic syndrome are fully immunocompetent, associated with a high incidence of autoimmune phenomena, and also in a high immune response against allogeneic RBC antigens[9]. On the other hand, patients with lymphoid leukaemia, AIDS and hematopoietic stem cell transfusion show a highly reduced RBC alloimmune response probably related to the disease's pathophysiology or the intensive immunosuppressive therapy[10]. However, the immunosuppressive therapy in myeloid leukaemia, organ transplant and the impaired immune response in end-stage renal disease do not prevent alloimmunization against allogeneic RBC antigens[11].

RBC alloimmunization mainly occurs after the first few transfusions. RBC antibodies, which can become undetectable over time, can cause delayed transfusion reactions after incompatible blood transfusions. Several authors advocated that transfusions given to patients who are likely to become transfusion-dependent over a longer period of time should be matched for antigens other than ABO and Rh (D antigen only) in an attempt to prevent alloimmunization[12]. However, cost-effective considerations make this policy a matter of debate. Patients undergoing chemotherapy, especially those with leukaemia, exhibit a lesser antibody response than do other patients. Impairment of the immune status as a result of the malignant process or transient immunosuppression due to intensive chemotherapy could enable patients to develop tolerance or unresponsiveness to incompatible transfusion[13,14].

Compatibility testing is routinely carried out with respect to major blood group antigens i.e. ABO and Rh antigens. There is always a high probability that the donor might have minor blood groups antigens which may not be present in the recipient blood which will lead to RBCs alloimmunization[1]. Reaction to these alloantigens may also vary from person to person. Clinically significant alloimmunization develops with the Rhesus, Kell, Duffy and Kidd system[1]. Allo antibody against red cell antigen develops this will increase the need for transfusion and can complicate transfusion therapy further. Therefore it has been advocated that transfusion-dependent patient should be advised blood matched for antigens other than ABO and D to prevent alloimmunization. Red cell allo and autoantibodies should not be overlooked in multiply transfused patients. It should always be considered if the patient repeatedly suffers from haemolytic transfusion reactions or not being able to maintain haemoglobin at a desired level in spite of regular transfusions. Transfusion of packed red cells after matching for all major antigens associated with clinically significant antibody production can to a great extent ameliorate the problem. After the antibody screening and identification corresponding antigen negative blood should be given. As this exercise is laborious and expensive, it will be beneficial for only a few patients who could develop these problems. In Bangladesh, data regarding red cell antibodies (allo as well as autoantibodies) in multiply transfused patients is scarce. A study regarding alloantibodies in repeatedly transfused patients done by Khatun A[13] found that frequency of alloimmunization is 6% in thalassaemia patients and Rh and Kell antibodies are alloantibodies commonly identified. As transfusion is vital to the management of patients with hematologic disorders and malignancies, clinically significant RBC alloantibodies and autoantibodies develop in these patients who receive multiple transfusions and can pose major problems in case of long-term transfusion therapy. Allo and autoantibodies can lead to difficulty in finding compatible RBC units because of the presence of clinically significant RBC antibodies and transfusion reactions.

Materials and Methods

This descriptive cross-sectional study conducted from July 2015 to June 2016 in the Department of Immunology, BIRDEM and Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment. A total Number of 370 patients were enrolled in this study after fulfilling the inclusion and exclusion criteria. Diagnosed patients with Hereditary Haemolytic Anaemia (HHA) and non–HHA, haematological diseases and malignancy, chronic renal failure on dialysis, non-haematological malignancy irrespective of age and sex who received at least five units of blood transfusion admitted in Department Haematology and Bone Marrow Transplant in Dhaka Medical College Hospital, Dhaka and Bangladesh Thalassaemia Foundation Hospital, Dhaka were taken as study population. Known patients of Auto Immune Haemolytic Anaemia (AIHA), patients in whom antibody was previously detected and pregnant women were excluded from the study. The sample was taken at least 04 weeks from the last transfusion date who had received previous transfusions. About 5ml of venous blood was collected from each patient. Blood was centrifuged at 100-120 rcf/min for 10 minutes to separate into cell and serum then 5% cell suspension was made. Blood grouping and direct anti globulin (DAT) test were performed with cell suspension of all samples. A poly-specific Coombs reagent (blend of IgG and C3d) was used. In cases of a positive DAT test, further investigation

using specific monoclonal reagents to detect IgG or a complement (C3d) was carried out. Serum was used to detect red cell alloantibodies using standard blood bank methods (albumin, enzyme and indirect Coombs test method). Serum was stored in -20°C freezer. Antibody identification (anti-D, anti-C, anti-c, anti-E, anti-e, anti-K, anti-Jka, anti-JKb, anti-Lea, anti-Leb, anti-Fya, anti-Fyb, anti-M, anti-N, anti-S) was performed in antibody screening positive samples using red cell identicells.

Results

Among 370 patients, maximum 132(35.7%) patients were in age group 1-10 years followed by 117(31.6%) in age group 11-20 years. Among total patients, 204(55.1%) were male and 166(44.9%) were female and male-female ratio was 1.2:1. About 361(97.6%) patients were Rh positive and only 9(2.4%) Rh were negative (Table-I). In non-HHA patients, the age of the first transfusion was more than HHA patients. The number of blood unit transfused was more in case of HHA (75.70±89.03) than non-HHA (8.11±6.25). But the time interval between subsequent transfusions was almost equal (Table-II). Maximum 290(78.4%) were of HHA and remaining 80(21.6%) patients were non-HHA. Out of 80 non-HHA patients, 50(13.5%) were with haematological diseases and malignancy like aplastic anaemia, acute myeloblastic leukaemia, acute lymphoblastic leukaemia, myelodysplastic syndrome, hodgkin and non hodgkin lymphoma and 30(8.1%) patients had non-haematological malignancy and diseases like carcinoma colon, carcinoma stomach, carcinoma breast, chronic renal failure on dialysis (Table-III). The antibody was detected in 11(3.79%) patients with HHA and 6(7.5%) patients of non-HHA patients. Among HHA patients, only auto-antibody, only alloantibody or both were detected in 2, 6 and 3 patients respectively and among Non-HHA patients it was 1, 3 and 2 patients respectively (Table-IV). Out of 8 auto-antibodies, 5(62.5%) were anti IgG followed by 3(37.5%) were anti C3d. Out of 14 alloantibodies, 4(28.6%) were anti E, 3(21.4%) were anti K and in 3(21.4%) cases specificity of alloantibody was not detected (Table-V).

Table-I: Distribution of patients according to age, sex and blood group (n=370)

Characteristics			Frequency	Percentage
Age (years)	1 - 10		132	35.7
	11 - 20		117	31.6
	21 - 30		70	18.9
	31 - 40		24	6.5
	>40		27	7.3
Sex	Male		204	55.1
	Female		166	44.9
Blood Group	Positive	O	113	30.5
		B	133	35.9
		A	91	24.6
		AB	24	6.5
		Total	361	97.6
	Negative	O	2	0.5
		B	3	0.8
		A	4	1.1
		Total	9	2.4

Table-II: Features of patients according to transfusion history (n=370)

Transfusion History	HHA	Non-HHA
Age of 1st transfusion (Mean ±SD years)	3.09 ± 4.89	21.90 ± 12.88
Total number of transfusion (Mean ±SD units)	75.70 ± 89.03	8.11 ± 6.25
Time interval between transfusion (Mean ±SD weeks)	4.07 ± 1.04	4.22 ± 1.06

Table-III: Distribution of patients according to diagnosis (n=370)

Diagnosis		Frequency	Percentage
Hereditary hemolytic anaemia (HHA)		290	78.4
Non-HHA	Non-haematological	30	8.1
	Haematological	50	13.5
	Total	80	21.6

Table-IV: Distribution of patients according to diagnosis and types of antibody

Diagnosis	Types of Antibody	Frequency	Percentage
HHA	Only Autoantibody	2	0.68
	Only Alloantibody	6	2.07
	Both Antibodies	3	1.03
	Total	11	3.79
Non-HHA	Only Autoantibody	1	1.25
	Only Alloantibody	3	3.75
	Both Antibodies	2	2.50
	Total	6	7.50

Table-V: Different anti-RBCs antibodies in multiple transfused patients

Types of Anti-RBCs Antibodies		Frequency	Percentage
Autoantibodies	Anti IgG	5	62.5
	Anti C3d	3	37.5
	Total	8	100.0
Alloantibodies	Anti E	4	28.7
	Anti K	3	21.4
	Anti c	2	14.3
	Anti JKa	1	7.1
	Anti Fya	1	7.1
	Non-specific	3	21.4
	Total	14	100.0

Discussion

In this study, mean total no of transfusion were75.70±89.03 and 8.11±6.25 for HHA and non-HHA patients. Non-HHA patients developed antibody though they received much less unit of transfusion than HHA patient. The reason behind this could be higher age of starting transfusion among non-HHA patients. Mean age of starting transfusion for HHA and non-HHA patients were 3.09±4.89 and 21.90±12.88 years.

Singer et al[25] showed that starting of transfusion at an early age may offer some immune tolerance and protect against alloimmunization.

This study was conducted to find out the prevalance of red cell autoantibodies and alloantibodies in repeatedly transfused patients and identification of allied alloantibody so that pretransfusion screening in these patients can be evaluated. Identification of the type of autoantibody was done for treatment and prognosis purpose. The rate of immunization has been variably reported across the world. In this study, the frequency of development of red cell auto and alloantibody was 3.79% among patients with HHA and 7.5% among patients with other non-HHA who had undergone repeated transfusions. Sood et al[1] showed the incidence of RBC alloimmunization ranges between 1-6% in occasionally transfused and up to 30% in poly-transfused patients. A study done by Dhawan et al[15] reported the frequency of alloimmunization in multi-transfused patients in India is comparatively low varying from approximately 3% to 10%. In other studies, alloimmunization to RBC was positive in 7.4% patients in Iran done by Natukunda et al[16] 9.2% in Pakistan done by Mohsin et al[17], 22% in Saudi Arabia done by Shamsian et al[18], 6.1% in Uganda done by Bilwani et al[19] in patients who received multiple transfusions with different diseases.

The factors for alloimmunization are complex and involve at least 3 main contributing elements e.g. the RBC antigenic difference between the donor and the recipient, the immune status of the recipient and the immunomodulatory effect of the allogeneic blood transfusions on the recipient's immune system. Karimi et al showed a low rate of alloimmunization may be expected when there is the homogeneity of RBC antigens between the blood donors and recipients[20] when transfusion of extended phenotype blood group matched blood units[21] and leucodepleted blood[22] is being practised. The study subjects were transfused neither extended phenotype nor leucodepleted blood. Previous data on homogenous populations in Greece and Italy showed an overall low rate (5-10%) of alloimmunization in a study done by Hmida et al[22] Lower rate of alloimmunization was reported in Thalassaemia patients from Iran (5.3%) by Karimi et al[20], Pakistan (6.2%) by Bhatti et al[25] and in Malaysia (8.6%)[24]. Very few studies from India reported alloimmunization in multiply transfused patients including Thalassaemia patients. A study from North India reported alloimmunization rate 3.4% in multiply transfused patients[15].

Among 370 patients alloantibodies were found in 9(64.28%) of HHA and in 5(35.71%) of non-HHA patients. Autoantibodies were found in 5(62.5%) of HHA and in 3(37.5%) of non-HHA patients. A study reported[17] on 697 Thalassaemic patients who had received transfusions. Allo and autoantibodies were reported in 115(16.5%) and 34(4.9%) subjects respectively. In this study, 5 of 290 HHA patients and 3 of 80 non-HHA patients developed auto-antibody. Of these 8, 5 antibodies were typed warm IgG antibody and 3 were complement C3d. Autoimmunization was associated with alloimmunization. A similar result was shown in another study[19] which showed a relation of autoimmunization with splenectomy. Transfused RBCs had abnormal deformity profiles, more prominent in the patients without a spleen which possibly stimulated antibody production; however, none of the patients had undergone splenectomy in this study.

In this study, out of 370 patients, 14 developed alloantibody. Out of 14 alloantibodies, maximum 4 (28.57%) were Anti E, 3(21.43%) were Anti K and Anti c was found in 2(14.29%) cases and Anti JK^a & Anti Fy^a were found in 1(7.14%) case. Antibody specificity could not be detected in 3 cases. The antibodies found in this study were from Rh, Kell, Duffy, Kidd groups. Out of fourteen positive patients, 6(42.85%) patients were positive for alloantibodies against Rh system. Comparable results were reported in another study conducted at Rawalpindi demonstrating that red cell alloantibodies detected in multi-transfused patients belonged mainly to Rh system[26]. In India, among 306 multi-transfused patients, 22 alloantibodies were detected in 13 cases; the most frequent was Anti-E in six cases followed byAnti-c, Anti-JKa, Anti-K[27]. Another study in India showed, among 211 multi-transfused Thalassaemia patients frequency of alloimmunization was 3.79%. The alloantibodies identified were anti-E, anti-K, anti-D, anti-Kpa, anti-c and anti–Jka[28]. Another study conducted in India showed on 116 Thalassaemic patients; alloantibodies identified were anti-E and anti-K[29].

The specificity of most alloantibodies detected in this study was against Rh and Kell antigen systems due to their high immunogenicity, which is similar to previous reports of alloimmunization[30]. Hence, the transfusion of blood matched for Rh and K antigens could prevent alloimmunization resulting in a significant difference in the alloimmunization rates, but the potential to form RBC alloantibodies to unmatched antigens will exist[30].

Conclusion

In conclusion, this study permits to conclude that the frequency of alloimmunization and autoimmunization in repeatedly transfused patients is not so uncommon. In this study, antibodies were detected in 4.59%. Most of the alloantibodies were of Rh system that is anti-E, anti-c and anti-Kell. Autoantibodies were of IgG type. The practice of red cell auto and alloantibody screening and identification could be advised to patients requiring poly-transfusions for a better outcome of treatment.

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