ORIGINAL ARTICLE

Frequency and Specificity of Red Blood Cell (RBC) Allo-immunization in Multiple Transfused Breast Cancer Patients

Dr. Ambreen Sheikh[1], Neha Baqai[2], Dr. Saima Minhas[3] and Dr. Rafat Amin[4*]

[1]Department of Pathology, Dow Medical College, Dow University of Health Sciences, Baba-e-Urdu Road, Karachi
[2]Dow Research Institute of Biotechnology and Biomedical Sciences, Dow University of Health Sciences, Ojha Campus, Karachi
[3]Department of Pathology, Dow International Medical College; Dr. Ishratul Ebad Khan Institute of Blood Disease and Oncology, Bone Marrow Transplantation and Blood Banking, Karachi
[4]Dow College of Biotechnology, Dow Research Institute of Biotechnology and Biomedical Sciences, Dow University of Health Sciences, Ojha Campus, Karachi

ABSTRACT

Background & Objective: RBC transfusion is an immediate rescuing strategy for patients of critical health conditions including cancer patients. Suppression of immune system is associated with various cancer types. This study is designed to compare the frequency and specificity of RBC alloantibodies between multiple transfused breast cancer patients and individuals with no cancer history receiving repeated blood transfusions.

Methods: This case-control study was conducted from July 2016-August 2017. 34 confirmed breast cancer patients and 18 surgery, accident and emergency patients were enrolled from the allied sectors of Dow University of Health Sciences. Followed by blood group testing, alloantibody screening and identification tests were performed.

Results: Altogether 12 out of 52 studies subjects were found positive for alloantibodies indicating a prevalence of 23% in this study. 11 (91.6%) among those were breast cancer patients whereas only one subject belonged to the control group. The distribution of different alloantibodies among breast cancer patients is, Anti-D (05; 45.4%), Anti-E and Fy-a (each in 2 patients, 18%), Anti-Fy-b and Anti-jka were positive for 1 breast cancer patient. The only RBC alloantibody identified in the control group is Anti-K. A significant association was observed between mean number of repeated transfusion and the formation of alloantibodies (p=0.005).

Conclusion: Considering a high prevalence of alloimmunization observed in this study, we recommend revising our laboratory guidelines by adding a pre-transfusion antibody screening and identification test for transfusion safety across Pakistan.

Keywords: Blood transfusion, RBC antigens, RBC alloimmunization, Breast cancer, Alloantibodies screening.

INTRODUCTION

Blood transfusion is widely practiced in numerous medical conditions to replenish specific blood constituents whose level drop due to a particular disease. Besides saving thousands of lives, transfusion of blood or blood components are reported to be associated with multiple complications including alloimmunization, immunomodulation and blood borne infections [1]. Approximately 0.5-1.0% of all blood transfusions are accompanied by some sort of adversative effects [1, 2].

Every individual carries a distinctive set of inherited RBC antigens responsible for his characteristic blood group. More than 230 different RBC surface antigens have been identified [3]. Suitability of blood products depends on compatibility (matching) of these proteins between donor and recipient. Due to an additional cost factor, most of the clinical setups across the globe do not run a pre- transfusion screening test to match all the antigens present on the donor's red blood cells with the

recipient or *vice versa*. Blood transfusion with foreign RBC antigens elicits the formation of alloantibodies and according to the published data transfused patients are 6-36% more prone of developing alloantibodies if they are given transfusion therapies for longer periods [4]. Alloimmunization of red blood cells causes severe immunological complications which include acute and delayed hemolytic reactions [5, 6]. Alloantibodies do not only reduce the life span of the transfused RBCs thus increase the need of more blood transfusions, but also upsurge the development of autoantibodies and cause a high morbidity and mortality in chronic disease patients such as cancer patients [7, 8].

Health status of an individual is an important determinant for evaluating its alloimmune response targeting RBC antigens [9-11]. Modulation of this competent defense mechanism is well reported in various pathological conditions including Louis-Bar syndrome, complement impairment, certain chronic infections and many types of malignancies [12-14]. Cancer patients face immune-suppressive challenges due to the combine effects of their disease and immune suppressive medications [15]. Despite the fact that immune system impairment in cancer patients may lead to lower alloantibody

development as compared to the healthy population, a number of studies have reported contradictory results with varied frequencies of RBC alloantibodies [16-20]. This discrepancy in the rate of alloimmunization is because of the genetic differences in studied populations, regarding RBC antigens and the type of cancer [4, 21].

Breast cancer is the most common cancer affecting women world-wide. It is the leading cause of cancer-related deaths throughout the world. In Pakistan, the incidence of breast cancer is 2.5 times higher than of its neighboring countries such as India and Iran [22]. In Pakistan one out of every nine women is at the risk of developing this cancer. Chemotherapy, radiotherapy and/or surgical procedures to treat such patients depress blood cell count, causing the need of repetitive blood transfusions. This study is conducted to evaluate the prevalence of alloantibodies among breast cancer patients and compare it to the non-cancerous, multi-transfused patients.

MATERIAL/SUBJECTS/PATIENTS AND METHODS

This study was carried out at Dow University of Health sciences (DUHS) during a period of July 2016-August 2017 after obtaining an ethical approval from the Institutional review board, DUHS (IRB-714/DUHS/Approval/2016/266). Participants of the study had a history of receiving more than five transfusions either with ABO/D compatible packed RBCs or whole blood with a case control ratio of 2:1.

A total of 52 females that include 32 cases and 18 controls who gave their consent were enrolled into the study.

34 breast cancer patients, diagnosed on the basis of fine needle aspiration biopsy reports during January 2015 to June 2016, were selected from the histopathology section of Dow Diagnostic Research and Reference Laboratory (DDRRL), DUHS, Karachi. These chosen patients were called for blood sample collection *via* telephone. At the time of sample collection, information regarding age, sex, treatment record and transfusion history (date of transfusion, type of blood component, volume transfused, total number of transfusions and time period from last transfusion) was noted.

Eighteen patients with no cancer history were taken as controls include surgery, accident and trauma patients from the emergency department, Dow University Hospital. All enrolled patients in this group were having previous accident or surgery histories and received blood transfusions multiple times. Pregnant females and multigravida with known alloantibodies and malignant patients with no transfusion history, thalassemia or surgical patients with other malignances were excluded from the study.

From those patients who met the inclusion criteria and gave their consent, 4.5 ml blood was drawn aseptically ensuring minimal risk to the study subjects in a vacutainer without additives. All the collected specimens were properly labeled and transported promptly in cold packs to the laboratory for warranting sample integrity. Serum was obtained by centrifuging the samples at 2000g for 10 minutes at room temperature (20-22 ˚C), separated in aliquots and stored at -80˚C until further processing.

Samples were tested for blood group and presence of alloantibodies. Commercially available three cells panel was used for antibody screening (Bio Rad kit) following the manufacturer's procedure. Identification of alloantibodies was performed with eleven cells panel antibody identification kit (Bio Rad). Interpretation was done using suggested "three cell ruling out" procedure.

Statistical Package for the Social Sciences (SPSS 16.0) was used for data administration and analysis. Categorical variables were summarized using frequencies and percentage. Pearson chi-square test was applied to find association of study variables with formation of alloantibodies and p value ≤ 0.05 was considered significant.

RESULTS

A total of 52 females who gave their consent were enrolled in the study. Randomly selected confirmed breast cancer patients comprised 65% of the total participants whereas control group be presented at 35%. Their age range was between 25-54 years with a mean age of 39.5 years. Age of the surgery, accident and emergency patients ranged from 33-53 years with a mean age of 41 years.

ABO blood groups and their associated rhesus factor of the study subjects were recorded. Both cases and control patients had almost similar distribution pattern where breast cancer patients had (B > O = A > AB) and controls had (B > O > A > AB) blood group distribution arrangement. The highest percentage of blood groups among study participants was related to blood group B (34.6%). Among cases B+ was found to be the most frequent one with 29% (n=10) whereas B- was the most dominant blood group in control group (n=7; 22%). Frequencies of other ABO and Rh blood groups in study population are presented in Table **1**.

The number of blood bags transfused to the study participants ranged between 5-13 units. The majority of the participants (30; 57.6%) had received 6-7 blood transfusions. Mean number of transfusions is 7.3 and 6.5 blood bags per participant in case and control group respectively.

The antibody screen was positive in 12 patients among 52 study subjects, showing a prevalence of about 23% in this study. Among the patients showed positive results for the presence of alloantibodies, 11 (91.6%) were belonging to the breast cancer patients whereas only one subject of the controls participant displayed positive result. The prevalence of alloantibodies among

cases and control groups is 32% (11/34) and 5.5% (1/18) respectively. Table **2** describes the specificity of alloantibodies detected in the study subjects. The distribution of different alloantibodies among 11 positive patients in the case group is as follows, Anti-D is the most frequent alloantibody identified in 5 breast cancer patients (5/11; 45.4%) followed by Anti-E and Fy-a found in 2 cases (2/11; 18%) each. Both Anti-Fy-b and Anti-jka were positive for 1 breast cancer patient (**Fig. 1**). The only single RBC alloantibody identified in the patient of control group is Anti-K.

To find out the association of age, blood group and number of transfusions with the production of alloantibodies, the Pearson Chi-square test was used. Significant association (p=0.005) was observed between mean number of repeated transfusion and formation of alloantibodies (Table **3**). However, association was found insignificant in case of age of the patient with alloantibodies (p=0.137) and blood groups of the patients to alloantibodies (p=0.247).

Table 1: ABO and Rhesus (Rh) blood group distribution among cases and control groups of study population.

| Alloantibody		Cases		Controls	
		n =34	%	n =18	%
A	A -ve	3	8.8	3	16.6
	A +ve	7	20.5	2	11.1
AB	AB -ve	0	0	0	0
	AB +ve	3	8.8	0	0
B	B -ve	1	2.9	4	22 .2
	B +ve	10	29.4	3	16.6
O	O -ve	1	2.9	3	16.6
	O +ve	9	26.4	3	16.6

Table 2: Frequency and specificity of RBC alloantibodies among the Patients.

Alloantibodies	No. of Study Participants	Percentage
Anti-D	5	41.6
Anti-E	2	16.6
Anti-K	1	8.3
Fy-a	2	16.6
Fy-b	1	8.3
Anti-jka	1	8.3

Table 3: Association of alloantibody formation with the number blood transfusions.

No. of transfusions	Alloantibodies			
	Positive Frequency (%)	Negative Frequency (%)	Total	P value
1-5	1(14.28)	7 (87.5)	8	
5-10	8(24.24)	33(80.48)	41	*0.005
10-15	3 (100)	0 (0)	3	

*P-value <0.05 was taken as significant.
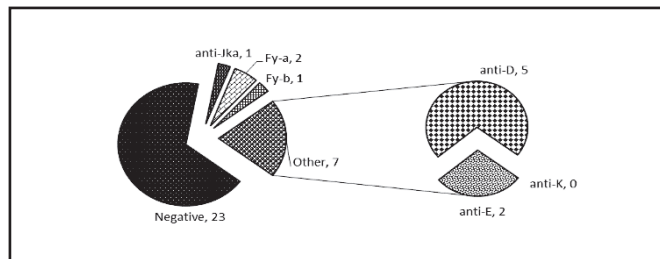
Fig. (1): Distribution of various anti-RBC alloantibodies in multi-transfused breast cancer patients.

DISCUSSION

RBC alloimmunization is of prime concern in cancer patients receiving multiple transfusions. Such patients develop transfusion related complications which directly affects their life expectancy. Preferably cancer patients should receive red blood cells from donors, phenotype or match for as many RBC antigens as possible. Antibody screening is not routinely done as a part of pre-transfusion testing in most of the hospitals across Pakistan. The pre-transfusion testing routinely performed in our clinical settings currently includes ABO and Rh-D typing only whereas recently published 31st edition of the Standards of practice in blood banking and transfusion medicine from American Association of Blood Banks states that antibody detection, whole blood and granulocyte components must be part of pre-transfusion compatibility testing for allogeneic transfusions along with routine ABO grouping and Rh typing.

This case-control study was conducted to determine the prevalence and specificities of RBCs alloantibodies among multiply transfused breast cancer patients. The control group included multi-transfused patients from accident and emergency department. The mean ages of cases and control subjects were 39.5 and 41 years respectively (**Fig. 1**). The mean number of transfusions in cases was 7.3 blood bags compare to the control group (6.5 blood bags).

Blood group B was found to be the most prevalent type amongst this studied population followed by O group (Table **1**). Our results are similar to the findings of Rahman *et al.* 1975, Anees and Mirza, 2005, Pathan *et al.* 2008, Khattak *et al.* 2008, Rehman *et al.* 2015 and Mehmood *et al.* 2018, who reported the predominance of blood group B among people belonging to the different regions of Pakistan [23-28].

In this study, antibody screening test was performed using three cell panel and our results showed that a significant proportion of the studied population developed RBC alloantibodies. Out of 52 patients, 12 (23%) were found positive for RBC alloantibodies. Overall rate of RBC alloimmunization in our study is high; however it is comparable with the frequencies reported in multi-transfused thalassemia patients [29, 30].

The prevalence of alloantibodies among cases and control subjects is 32% and 5.5% respectively (**Fig. 1**).

Our results show high RBC alloimmunization in breast cancer patients irrespective of receiving chemotherapy which is reported to have a shielding effect against alloimmunization. This frequency is on the higher side if compared to that reported in Australia; 11% in myelodysplastic syndrome [20], Brazil; 2.79% in breast cancer [31], Uganda; 8.3% amongst cancer patients [18], Pakistan; 6% in non-hematological malignancies [17], Kenya, 2.2% in cancer patients [8]. On the other hand our results are in accordance to the results of the studies done in India; 25% in chronic myelogenous leukemia (CML), 23% in myelodysplastic syndromes (MDS) [32], Texas; 22.6% in solid cancers, 23% in myelodysplastic syndrome and 7% in hematologic malignancies [16], Poland; 33.4% in myelodysplastic syndrome [19].

Several risk factors are predicted to be associated with these discrepancies in the incidences of alloimmunization including status of their immune system and their disease. Overall rate of the alloimmunization in solid tumors is reported to be higher than those in hematological malignancies [16, 19, 33]. This may be due to the chronic inflammatory state in solid malignancies with an increased immune activation [33]. Hendrickson *et al.* 2006 suggested that the inflammatory status of human transfusion recipients may regulate the immunogenicity of transfused RBCs [34]. Studies based on animal model of transfusion demonstrated that alloantibody formation after blood transfusion against a RBC antigen depends on Tregs cells and spleen antigen presenting cells (APC) that present foreign RBC antigens to the T cells [35]. An increased number of Tregs cells are reported to be observed in cancer patients with a higher rate of consumption of transfused RBCs by splenic dendritic cells that explain the consequently higher rates of alloimmunization in solid tumors [36]. Gender of the patient is another risk factor of alloimmunization and women are reported to have comparatively higher prevalence of alloantibodies due to their exposure to alloantigens during pregnancy and childbirth [37].

Earlier studies have established an association between the types of blood group and rate of alloimmunization. A higher prevalence of alloimmunization was reported in Rh negative patients [38, 39]. Our results however showed an insignificant relationship between ABO-Rh blood groups and alloimmunization. These results are similar to the findings of Usman *et al.* 2011 and Al-Joudi *et al.* 2011 [40, 41]. We were also unable to find out a significant association between age of the patients and the frequency of alloimmunization that is contradictory to the previous reports published by Xu *et al.* 2014 and Jan *et al.* 2009 [42, 43].

When investigated the connotation between the number of transfusion events and the rate of alloimmunization, an association was observed (Table **3**). This observation is in agreement with the previous studies [11, 17, 32, 44]. The distribution of alloantibodies amongst the positive participants of our study is anti-D (5), anti-E (2), Fy-a (2),

Fy-b (1), anti-K (1) and anti-jka (1). Anti-D was found to be the most prevalent type that was observed in 41.6% of our study patients. These results are in accordance to the anti-D frequency (33.3%) reported in multiple transfused cancer patients from Pakistan [17]. Another study from the same region reported 26.6% prevalence of anti-D alloantibody in transfusion dependent thalassemia patients [41]. The D antigen belonging to the Rh group is the most important RBC antigen that is known to easily immunize D-negative individuals.

A smaller group of breast cancer patients was conscripted in our study. Further research include larger patient cluster with similar and other malignancies is required in order to evaluate the RBC alloimmunization in patients receiving multiple blood transfusions.

CONCLUSION

Considering a high prevalence of alloimmunization observed in this study, we need to revise our laboratory guidelines for transfusion safety across Pakistan not only for the cancer patients but also for the benefits of all other patients who need regular blood transfusion supply to carry-on their lives. We recommend that pre-transfusion antibody screening and identification test should be included for the patient's safety particularly for oncological patients receiving multiple transfusions.

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