



Risk Factors for Red Blood Cell Alloimmunization in Multi-Transfused Oncology Patients at Moi Teaching and Referral Hospital, Kenya

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Abstract

BACKGROUND

Multiple blood transfusions may result in the production of alloantibodies against one or more red blood cell antigens which might make it more challenging to execute subsequent transfusions. Despite age and gender being risk factors for transfusion and being associated with alloimmunization frequency, they are not routinely taken into account before transfusion. This study assessed red blood cell alloimmunization and its association with risk factors among multi-transfused oncology patients at Moi Teaching and Referral Hospital Cancer Centre, Kenya.

METHODOLOGY

The study employed a cross-sectional study design and focused on multi-transfused oncology patients at the Moi Teaching and Referral Hospital Cancer Centre. A sample size of 162 was used in the study based on Fisher's exact test formulae and a consecutive sampling technique was applied. The gel-based antibody screening and identification were performed with "ID-Diacell I-III®" panel cells. The frequency, mean, median, and dispersion of descriptive statistics were shown and the association between alloimmunization with the number of transfusions, age and sex were determined by Spearman's correlation analysis. Statistical significance was established at $P < 0.05$ and statistical tests were run at a 95% level of significance.

RESULTS

This study established no association between alloimmunization and the number of transfusions ($P = 0.753$). There was also no association between alloimmunization and age ($P = 0.159$). However, there was a significant positive association between alloimmunization with gender ($P = 0.01$). The study had a 6.2% prevalent rate of red blood cell alloimmunization, females had a greater prevalent rate than male patients. Anti-E and anti-K were the most prevalent alloantibodies.

CONCLUSION AND RECOMMENDATION

There is a need to improve current blood grouping and cross-match practices in most Kenyan hospitals by performing antibody screening and antibody identification tests. This study suggests routinely assessing alloimmunization in patients getting several transfusions while taking gender into account.

Keywords: Alloimmunization, Transfusion, Oncology, Risk Factors, Kenya.

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Introduction

Red blood cell (RBC) antigen alloimmunization is a risk linked with blood

transfusions, even though they can save lives [1]. One of the dangers associated with blood transfusions is alloimmunization to red blood cell



antigens brought on by genetic differences between the donor and recipient [2]. Alloantibodies against one or more red blood cell antigens may be produced as a result of repeated blood transfusions, which can complicate subsequent transfusions [3]. Alloantibodies can hinder cross-match testing, which can delay the process of getting compatible blood and occasionally be linked to a delayed type of hemolytic transfusion reaction.

There is a theoretical chance of developing an alloimmunization to red blood cell antigens is about 1% per transfused unit, and it is higher in patients who have received multiple transfusions [4]. The frequency and volume of transfusions, the immunogenicity of the antigen, and the immunological response of the recipient all contribute to the probability of alloimmunization. Ethnic and antigenic pattern differences between donors and receivers can affect the outcome [5]. Sickle cell disorders, aplastic anaemia, myelodysplastic syndrome, chronic myeloproliferative disease, and various cancers are a few of the illnesses that call for periodic red cell transfusions [6].

According to a Malaysian study, the frequency of antibody testing (single versus serial), the number of transfusions, the time between transfusion events, the number of alloimmunized patients, and the specificity of the antibodies were all factors in the rise in alloimmunized patients [7]. In Indonesia, 72.1% (72 instances) of participants with a history of prior transfusions experienced transfusion responses [8].

Due to exposure to HLA (Human Leucocyte Antigen) or HPA (Human Platelet Antigen) through prior transfusions, also known as alloimmunization, individuals who have a history of repeated transfusions will experience sensitization, which results in the formation of alloantibodies in the recipient's body. Systemic inflammation is brought on by the production of the cytokine when donor antigens interact with

recipient antibodies that have already been sensitized [9]. Patients with thalassemia are alloimmunized 4-50% of the time, those with haemato-oncology conditions are alloimmunized 1.9-13% of the time, and those with renal disease are alloimmunized 1.27-13.1% of the time [10]. As erythrocyte exposure increases, so does the risk of alloimmunization. Up to 30% of patients who required repeated transfusions developed erythrocyte alloantibodies.

Obtaining appropriate blood is extremely challenging and can potentially result in a delayed haemolytic transfusion reaction (DHTR), which can be fatal in some cases [1]. Thus, phenotypic matching between donor and recipient erythrocytes is strongly advised to prevent sensitization in chronically transfusion-dependent patients. Although there are few studies of alloimmunization prevalence in multi-transfused oncology patients in Kenya, the relationship between alloimmunization and transfusion frequency among oncology patients is yet to be studied.

The patient's age has frequently come to light as the primary risk factor for RBC alloimmunization in patients with transfusion-dependent diseases, among other risk factors [13]. Age at the beginning of transfusion therapy is a significant risk factor, with older patients being more vulnerable [14]. For instance, patients who started receiving transfusions before they were between two and three years old had a reduced rate of alloimmunization [15]. A lower risk of alloimmunization may be caused by a form of immunotolerance that is produced by the still undeveloped immune system of young patients [16]. While the early implementation of transfusion therapy following diagnosis may be an intriguing strategy to reduce the likelihood of alloimmunization, it could also put cancer patients at an increased risk of various transfusion-related problems [17]. Ageing causes significant changes to the immunological and endocrine systems, making people more



susceptible to infectious diseases and reducing the effectiveness of immunization. Both the innate and the adaptive immune system are affected by immunosenescence [18]. Naive T cell production is reduced as a result of thymus involution, and the T-cell repertoire is constrained by the accumulation of antigen-experienced T cells. More pro-inflammatory cytokines are produced by highly differentiated effector T cells, which coupled with activated innate immunity cells help create a systemic pro-inflammatory environment as people age [19]. The incidence of alloimmunization among sickle cell disease and cancer patients has been reported in earlier research conducted in Kenya [24], however, there is a paucity of data to date on how age relates to RBC alloantibodies in cancer patients.

The genetic disparity between donors and recipients of RBCs caused by racial disparities is one of the most significant factors contributing to the development of alloimmunization [25]. The reported range may be explained by genetic heterogeneity between the donor and recipient populations, variations in transfusion practices, and variations in the specificity and sensitivity of the test methodologies [27]. It is recommended that preemptive antigen matching for Rh (C, c, E, e) and K be performed on oncology patients and women of reproductive age to prevent alloimmunization and increase transfusion safety by lowering alloantibody formation [23].

Finding solutions to lessen negative reactions in females and enhance immune responses in males will be made easier with knowledge of the mechanisms underlying sex discrepancy in immune responses. With the long-term goal of tailoring therapy for men and women, this is required to appropriately protect both sexes against immune-mediated illnesses including alloimmunization and infectious diseases [30]. Despite mounting evidence that indicates sex-based differences in immune

responses, susceptibility to infectious illnesses, and incidence of autoimmune diseases, most immunological studies either do not disaggregate and analyze data by sex or do not mention the sex of their subjects. In addition to the underlying variations in sex hormones, the X chromosome gene contributions and the influence of environmental factors are also linked to the fundamental disparities in immune systems between males and females [31, 32] Prevalence among oncology and sickle cell disease patients with a history of many transfusions were the subject of earlier Kenyan studies (24), however, no research has been done to determine how gender relates to RBC alloantibodies in cancer patients. This study therefore assessed alloimmunization and its association with transfusion frequency, age and gender among oncology patients at Moi Teaching and Referral Hospital, Kenya.

Methodology

Study design and area

The study used a cross-sectional study design. It was conducted between July 2021 and September 2022 at Moi Teaching and Referral Hospital, which is situated in the Kenyan Rift Valley in the county of Uasin Gishu. The hospital is host to the Chandaria Oncology Center, which sees 11500 transfusions annually and averages between 1800 and 2400 cancer patients. Also, the hospital has a certified laboratory that is well-stocked with the haematology and blood transfusion tools needed for the investigation.

Study population

Fisher's exact test formulas were used to establish a sample size of 162 people for the study [36]. A successive sampling strategy was employed, selecting each individual who met the inclusion criteria until the desired sample size was reached. Patients who had a history of malignancy and had received more than one blood transfusion were included, while those who had received only one transfusion and those with autoimmune conditions including idiopathic

thrombocytopenia, lupus nephritis and systemic lupus erythematosus were excluded from the study.

Sample collection and analysis

Before collecting venous blood samples from the participants, informed consent was administered and records of their age, gender, and history of transfusions were made. Gel-based antibody screening and identification were carried out using "ID-Diacell I-III®" panel cells made by Diamed GmbH, Pra Rond 23, 1785 Cressier FR and Switzerland, as was done previously [37]. To facilitate antigen/antibody interaction, the recipient's serum was combined with group O reagent red cells in the upper chamber of the micro-tube containing anti-globulin and low ionic strength saline (LISS) reagent. They were kept warm, at 37 C. The controlled centrifugation concept and the distinct passage of free and agglutinated red blood cells via a micro-tube column made of dextran-acrylamide gel make up the fundamental principle of the gel card method. Red blood cells that have been agglutinated stick to the gel or its surface. Rh (D, C, E,c,e, Cw), Kell (K,k, Kpa,kpb, Jsa, Jsb), Duffy (Fya, Fyb), Kidd (Jka, Jkb), Lewis (Lea,-Leb), P (P1), MNS (M, N, S,s), and Lutheran (Lua, Lu) were the three panels of reagent red cells with the highest antigenic specificity used (Xga). Red cell antibodies were recognized using the manufacturer's antigram. Three-panel reagent red cell panel was used, as before, for the antibody screening with the aforementioned antigenic specificity [39].

Quality assurance of the data

Only blood samples that met the laboratory sample acceptance requirements were used and blood samples were taken by a trained phlebotomist. The blood samples were handled with care during storage and transportation. Before testing, the separated and kept samples were brought to a temperature in line with the recommendations of the manufacturer. Throughout the investigation, internal and

external quality controls were confirmed and guaranteed. Recorded results were counter-checked for accuracy. The lab used was a participant in Human Quality Assessment Services' (HUQAS) External Quality Assurance System for Blood Transfusion Science (BTS), Chemistry, which includes the antibody screening test. The Kenya Accreditation Services (KENAS) has also granted the Blood Transfusion Science (BTS) scope accreditation, ensuring the integrity of the data gathered.

Data management and analysis

The data analysis and development of the visuals were done using the Statistical Program for Social Science (SPSS V.24). The descriptive statistics' frequency, mean, median, and dispersion were displayed. By using Spearman's correlation analysis, it was possible to determine the relationships between alloimmunization and age, sex, and the quantity of transfusions. The threshold for statistical significance was set at $P \leq 0.05$.

Data analysis by Spearman's ranking: The study used Spearman's Rank Correlation (R) which is suitable for ranks that cannot be quantified that is qualitative variables. The classification can be classified as either Positive or Negative. The distribution between the 2 depends on the direction of change of values of variables under investigation. When the variables increase, the correlation is termed positive and if the variables decrease they are termed Negative

Ethical considerations

The Moi Teaching and Referral Hospital's Institutional Research and Ethics Committee (IREC) approved the study (# IREC/2016/252), and the National Commission for Science, Technology, and Innovation (#885337) granted permission for it to be conducted. Informed consent was sought and obtained from each participant in writing.

Results

As shown in Table 2, there was no statistically significant association between

alloimmunization and age among oncology patients ($p= 0.415$). There was no statistically significant association between red cell alloimmunization and the number of (frequency) of transfusion ($P= 0.753$) as shown in Table 2.

There was a statistically significant association between alloimmunization and gender among multi-transfused oncology patients ($p= 0.01$) with females being more alloimmunized than males. Table 3.

Discussion

This sought to assess alloimmunization and its association with transfusion frequency, age and gender among oncology patients at Moi Teaching and Referral Hospital, Kenya. Interestingly, alloimmunization was not

statistically associated with the number of transfusions ($P= 0.753$).

The current study findings are similar to the findings of a study done in Tanzania which demonstrated that there was no significant statistical association between the number of transfusions and the risk of alloimmunization [12].]. This finding is similar to those of Wafa *et al* and Rofinda *et al* who reported the incidence of alloimmunization in patients with transfusion-dependent thalassemia patients did not significantly correlate with the frequency of transfusions and that erythrocyte antibodies are not substantially correlated with alloimmunization on repeated transfusion [40, 41].

Table 1:

Association between alloimmunization and age among multi-transfused oncology patients.

			Alloimmunization	Age
Spearman's rho	Alloimmunization	Correlation Coefficient	1.000	0.415
		Value. (2-tailed)		0.159
		N	13	13
Age		Correlation Coefficient	0.415	1.000
		Value. (2-tailed)	0.159	
		N	13	13

Table 2:

Association between alloimmunization and transfusion frequencies among multi-transfused oncology patients

			Alloimmunization	Transfusions
Spearman's rho	Alloimmunization	Correlation Coefficient	1.000	-0.147
		Value. (2-tailed)	.	0.753
		N	7	7
Transfusions		Correlation Coefficient	-0.147	1.000
		Value. (2-tailed)	0.753	.
		N	7	7

Table 3:

Association between alloimmunization and gender among multi-transfused oncology patients

			Alloimmunization	Gender
Spearman's rho	Alloimmunization	Correlation Coefficient	1.000	1.000
		Value. (2-tailed)	0.01	0.01
		N	2	2
Gender		Correlation Coefficient	1.000**	1.000
		Value. (2-tailed)	.	.
		N	2	2



Immunogenicity, dosage, and clinical factors including pro-inflammatory conditions all play a role in alloimmunization. Furthermore, some patients fail to respond even after being exposed to large concentrations of RBC antigens (39). These study findings, however, contradict a study done in Brazil which demonstrated that the number of transfusion episodes and blood units received are related to the development of alloimmunization [42]. The present findings also contradict the findings of a study conducted in India which demonstrated that first transfusion before 2 years of age and red blood cell units received/duration of blood transfusion/transfusion frequency were related to red cell alloimmunization(24). The results of the current study also conflict with the findings of a study done in the Netherlands which indicated that Alloimmunization rates rise with the quantity of transfusions, although the transfusion course in patients might vary from receiving numerous units at once to receiving them over a longer period [43]. The present study findings further contradicted the findings of a study done in Columbia which suggested that reticulocytes in donated red blood cell units affect the quality of transfused blood, are directed to a specific compartment, and could represent an unappreciated risk factor for red blood cell alloimmunization [44]. Alloimmunization and transfusion frequency were not related in the current study, this could be attributed to the fact that immunogenicity, dose, and clinical factors like pro-inflammatory circumstances all play a role in alloimmunization. Furthermore, some patients fail to respond even after being exposed to large concentrations of red blood cell antigens (42). This may also be attributed to the likelihood that low titer antibodies may have gone undetected as specified time intervals following blood transfusion were not taken into account before antibody screening and detection in identification in the current investigation. It is important to undertake a large-scale study where

antibody detection is done following blood transfusion at predetermined intervals.

This study established that there was no statistically significant association between alloimmunization and age ($P= 0.159$). This could be attributed to the study population which comprised a majority of adult participants. The immune systems gradually deteriorate with age, and these changes include diminished T cells and modifications to the antigenic repertoire that underlies both humoral and cell-mediated immune responses. The key abnormalities have been demonstrated in the T cells which experience functional changes, such as a decrease in precursor frequencies of helper and cytotoxic T-cells, and an increase of T cells which are unresponsive activators [45, 46]. These findings agree with a study carried out in the Netherlands which also established no significant association between alloimmunization and age [47]. These findings also concurred with the findings of a study conducted in Brazil (42). However, these findings contradicted studies conducted in Brazil [48] and Palestine (27) respectively which revealed a significant association between age and alloimmunization. In the current study, alloimmunization rates were higher among participants under 40 than among those in other age groups. Ages in the current study ranged from 1 to 97. These findings concur with a study carried out in the United States of America [49]. The cause of this is unclear; however, it could be because individuals in this age group have stronger immune systems than those in older age groups. Also, they might not have comorbid conditions or other aging-related variables that could lower immune-competence. To this strength, this study proposes the assessment of individuals' immune-competence status when studying alloimmunization.

Finally, this study established that there was a statistically significant positive association between alloimmunization and gender ($P= 0.01$), with many incidences being recorded in female



than male patients. Since women are exposed to alloantigens throughout pregnancy and childbirth, this is biologically plausible [50]. According to research, more allogeneic exposure leads to a higher rate of alloimmunization, which is correlated positively with the number of prior pregnancies (42). This is because if the mother's HLA alleles and those of her foetus are different, alloantibody production is more likely to occur [51]. According to some studies, the recipient's and donor's genders should be matched [52]. Studies have also shown that receiving red blood cells from female donors who have a history of pregnancy is linked to higher mortality rates in male recipients [53]. To ascertain the importance of these findings and define the underlying process, more research is required. In some situations, some 'naturally occurring' red blood cell antibodies can be produced without prior exposure to foreign red blood cells (42). It is unclear what causes the formation of the majority of these antibodies, however, it is frequently hypothesized that environmental or microbial chemicals that share antigenic properties with blood group antigens may act as a trigger [57]. These study findings however contradict the findings of a study done by Rofinda *et al.* which demonstrated no significant association of red blood cell alloimmunization with gender (41). The present study findings also contradicted the findings of a study by Aldakheel *et al* which found that there is no association between gender and the production of antibodies [58]. The current study findings did not agree with a study conducted in Tehran which recorded that Age, gender, history of pregnancy, and splenectomy are not contributing factors to the antibody presence in multi-transfused patients [59]. These study findings also contradicted a study done in Asia which demonstrated that alloimmunization is not significantly associated with gender and splenectomy status of a multi-transfused patient [60]. The discrepancies in the findings could be attributed to different study populations. To

strengthen this, the current study proposes routine consideration of gender when assessing alloimmunization among multi-transfused oncology patients.

Conclusion

Alloimmunization did not correlate with age or the number of transfusions. Yet, among patients receiving many transfusions for cancer, there was a positive correlation between gender and alloimmunization that was statistically significant.

Recommendations

The authors recommend routinely taking gender into account when evaluating alloimmunization in patients receiving several transfusions.

Competing interests: The authors declare no competing interests.

Authors contributions: The study's concept, planning, data collection, data analysis, writing, and editing were carried out by ZBW, TWW, PMM and WIE. The article was reviewed and approved by all authors.

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