

RESEARCH PAPER

**PREVALENCE OF RED BLOOD CELL ANTIBODIES AMONG
TRANSFUSED PATIENTS AT KOMFO ANOKYE TEACHING
(KATH) HOSPITAL, GHANA**

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ABSTRACT

Red blood cell (RBC) alloimmunisation is a common problem in transfused patients because of the possibility of haemolytic reaction and limited availability of compatible blood. In high-income countries, pre-transfusion antibody screening is performed routinely. In Ghana, patients are transfused with ABO Rh 'D' compatible blood without screening for immune antibodies. We therefore studied the prevalence and specificities of RBC antibodies in transfused patients at Komfo Anokye Teaching hospital, Ghana. The study was cross-sectional, involving previously transfused patients who required another transfusion. Participants' basic data on demography and transfusion history were recorded. Blood samples were screened and subsequently typed for RBC antibodies using a column gel agglutination test. A total of 106 transfused patients, 52 male and 54 females were enrolled. The patients had previously received a median of 4 RBC units (range 1-14). Of these, ten patients (9.4%) had 11 RBC alloantibodies, whose specificities were 2 anti-K; 2 anti-C; one each of anti-D, -E, -M, and -S; and 3 were pan-reactive. The number of transfusion episodes was significantly associated with the rate of alloimmunisation ($p=0.000$). In conclusion the overall alloimmunisation rate in the study was 9.4% and this was significantly associated with increasing number of transfusion episodes. Antibodies were mainly directed against antigens in the Rhesus system and K antigen. We recommend that antibody screening be incorporated into routine pre-transfusion testing procedures in Ghana.

Keywords: Alloimmunisation, multi transfusion, Alloantibody

INTRODUCTION

Transfusion therapy is vital in the management of patients with haematologic disorders and malignancies (Hoffbrand *et al.*, 2005). For these patients, it is a life-saving intervention as they are often chronically anaemic after chemo-

therapy and/or radiotherapy or usually require transfusion during illness. Although transfusion is life-saving, the development of antibodies against transfused foreign red blood cell (RBC) antigens (alloimmunisation) is one of the main risks associated with blood transfusion.

The incidence of alloimmunisation is reported to be up to about 60% in chronically transfused patients which generally include patients with haemoglobinopathies, haematologic malignancies, organ transplant recipients and patients with renal failure (Schonewille *et al.*, 1999; Angulo and Lima, 1999; Rosse *et al.*, 1990). In other less transfused groups, alloimmunisation is between 1-10%. The prevalence depends largely on how often antibody screening and identification were performed (Alves *et al.*, 2012 ; Cheng *et al.*, 2012 ; Schonewille *et al.*, 2006 ; Santos *et al.*, 2007; Melanie and Shulman, 2005 ; Aygun *et al.*, 2002).

Factors such as number and frequency of transfusions (a measure of foreign antigen exposure), ethnic differences between recipient and donor (Gader *et al.*, 2008; Shaz *et al.*, 2008), immunogenicity of RBC antigens, recipient's sex (Murao and Viana, 2005), age (Winters *et al.*, 2001), genetics (Noizat-Pirenne *et al.*, 2006; Higgins and Sloan, 2008) and underlying disease (Bauer *et al.*, 2007) have been found to influence alloimmunisation risk.

The risk of alloimmunisation increases with higher number of transfusions, however in multi-transfused patients most antibodies are formed after the first transfusions (Fluit *et al.*, 1990; Schonewille *et al.*, 1999; Zalpuri *et al.*, 2012). Chronically transfused patients have about five times an increased risk of developing multiple antibodies (Schonewille *et al.*, 2006) and are prone to developing autoantibodies which can lead to increased destruction of patients own red cells (Young *et al.*, 2004). The most frequently encountered RBC antibodies are formed against the Rhesus and Kell blood group antigens (Schonewille *et al.*, 1999; Heddle *et al.*, 1995; Seyfried and Walewska, 1990).

In most high-income countries, patients are routinely tested for the presence of clinically important RBC antibodies before each transfusion event. Once an antibody is detected, the transfusion recipient is given compatible blood that is lacking the corresponding antigen(s), to

prevent haemolytic transfusion reactions. In addition, a pre-emptive antigen (Rh/K) matching transfusion policy is often applied for chronically transfused patients and women in their (pre-) reproductive ages to reduce alloimmunisation.

In Ghana however, pre-transfusion screening involves only ABO and Rh "D" grouping and ABO compatibility testing, while antibody screening is not performed. To our best knowledge, no studies have been performed, addressing post-transfusion alloimmunisation among the Ghanaian population. As a result, not much is known about patients' antibody status and the effect of transfusion, despite the fact that alloimmunisation can significantly complicate transfusion therapy. The aim of this study was to determine the prevalence and specificities of RBC alloantibodies in previously transfused patients with different clinical conditions at Komfo Anokye Teaching Hospital, Kumasi, Ghana.

METHODOLOGY

Study site

The study was conducted at Komfo Anokye Teaching Hospital (KATH), in Kumasi, Ghana. Participants were recruited from the Medical, Dialysis, Oncology, Child Health, Surgery and Accident/Emergency Units of the hospital. KATH is the second largest and referral hospital in Ghana, serving five regions in the Northern sector of the country. It also provides specialized care to patients from neighbouring countries such as Burkina Faso, Togo, etc.

Study population

The study population included all patients over 2 years of age, with a transfusion request and with at least one previous blood transfusion episode. Written informed consent was obtained from participants 18 years and older and from guardians of those under 18 years.

Inclusion criteria

Patients over 2 years of age, with a transfusion request and with at least one previous blood

transfusion episode.

Exclusion criteria

Patients below two years and patients who had never been transfused in their lifetime were excluded.

Study design

Participants' basic demographic features, number of transfusions, last transfusion episodes, clinical history and blood component requested were retrieved from patients' medical records and recorded on a questionnaire. Whole blood samples (5ml) were obtained from consenting patients for ABO, Rh "D" grouping and antibody screening. ABO and Rh D blood grouping were performed using a manual tube method with monoclonal antisera A, B and D. Antibody screening and identification were performed at the Komfo Anokye Transfusion Medicine Unit (KA-TMU), using a three cell screen panel (Diacell I, II and III, DiaMed, Diamed Switzerland) in a low ionic strength indirect (LISS) anti-globulin test (Diamed Gel system, Dia-Med-ID, Switzerland). Antibody identification was performed on all screen-positive samples using an extended 11-cell panel in LISS (DiaMed, Switzerland). Antibody specificities that could not be identified with the LISS technique were subjected to further testing with enzyme (papain)-enhanced cards, also from Diamed (Diamed Gel system, Dia-Med-ID, Switzerland).

Ethical approval

Ethical approval was obtained from the Committee on Human Research, Publication and Ethics, (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana.

Statistical Analysis

Data were analyzed using SPSS version 16 for windows. Descriptive statistics were performed to summarize the patients' demographic and transfusion information. Chi square tests were performed to determine any possible association between factors (e.g. age, sex, number of

previous transfusion episodes, disease groups) that may influence the outcome variable of interest (prevalence rate of alloimmunisation).

RESULTS

Between January and September 2013, a total of 106 consecutive patients (52 males and 54 females, Mean age 43.2 ± 20.3 years, range 2-90 years) who had received at least one transfusion were recruited from 6 different clinical wards of KATH for antibody investigation. The highest proportion of samples screened was from the Medical (35.2%) and surgical units (30.2%) (Table 1). Almost 40% of patients had malignant disorders or renal disease. (Table 2). The patients had previously experienced a median of 4 RBC transfusion episodes (range 1-14; total 476) (Table 3). The current most frequent blood component requested was whole blood, while whole blood was issued in more than 80% of cases of a packed cell request (Table 3). The proportions of ABO blood groups of the patients were 52.7% O, 25.9% A, 19.1% B and 2.3% AB and 97.2% were Rh "D" positive.

In 10 (9.4%) patients (median age 42.5 years, range 13-60 years; male to female ratio 1:1), a total of 11 RBC antibodies were detected. Of these, 8 had clear specificity and 3 were pan-reactive alloantibodies (Table 4). One immunized patient (female with anti-K) had experienced a single previous transfusion episode, 8 patients had received 3-6, and one patient 10 transfusion episodes, before antibody detection. There was a strong statistical association between the number of transfusion episodes and the rate of alloimmunisation ($p=0.000$), while other demographic features, age ($p=0.983$), gender ($p=0.765$) and clinical diagnosis ($p=0.871$), did not appear to influence alloimmunisation.

DISCUSSION

This was a pilot cross-sectional study undertaken to determine the prevalence and specificities of RBC alloantibodies in Ghanaian patients who received blood transfusions at KATH. Our results showed that 9.4% of patients were allo-

Table 1: Proportion of patients from the various hospital units

Hospital Units	Number	(%)
Medicine	38	(35.8)
Surgery	32	(30.2)
Accident and Emergency	7	(6.6)
Dialysis	14	(13.2)
Oncology	9	(8.5)
Child Health	6	(5.7)
Total	106	100

Table 2: Disease types of patients and alloimmunisation characteristics

Disease types	Patients n (%)	Total units transfused (n)	Patients with RBC antibodies (n)	Immunized patients by disease (%)	Immunization risk per unit transfused (%)
Malignancy	23 (21.7)	121	2	8.7	1.7
Renal disease	18 (17.0)	82	3	16.7	3.3
Infectious diseases	16 (15.1)	71	2	12.5	2.3
Anaemia (unspecified)	8 (7.5)	31	1	12.5	2.7
Haemolytic condition*	8 (7.5)	29	0	0	0
Bleeding	8 (7.5)	29	0	0	0
Liver disease	4 (3.8)	12	0	0	0
Others**	21 (19.8)	101	2	9.5	3.0

* Sickle cell disease, hepatosplenomegaly with anaemia and Glucose-6-Phosphate dehydrogenase deficiency

** Vocal cord paralysis, neuropathic ulcers, head injury, fistulae, fractures, burst abdomen, diabetes, immune compromised state and unknown clinical diagnosis.

immunized, and the majority of antibodies were against antigens in the Rhesus blood group system. The alloimmunisation risk was associated with increasing number of transfusion episodes.

Our overall RBC alloimmunisation prevalence is in conformity with other published reports on

patients receiving transfusion support for haematological and other non haematological disorders in both developing and developed countries (Natukunda *et al.*, 2010; Shukla and Chaudhary, 1999). Most of our antibody specificities identified are similar to antibodies commonly encountered and reported in other pub-

Table 3: Demographic and transfusion characteristics of RBC alloimmunized and non allo-immunized patients

Demographic and transfusion characteristics of patients	RBC alloimmunized patients (n=10)	Non-immunized patients (n=96)
Median age in years (range)	42.5 (13-60)	43.0 (2-90)
Mean age in years \pm SD	40.2 \pm 15.0	43.46 \pm 20.8
Sex ratio (F:M)	1:1	~1:1
Median previous transfusion episodes (range; total)	4 (1-10; 41)	4 (1-14; 435)
Current transfusion request:		
Whole blood request (%)	5 (50)	50 (55.6)
Erythrocyte concentrate request (%)	5 (50)	40 (44.4)
Whole blood transfused (%)	8 (80)	84 (93.3)

The number of whole blood and erythrocyte requests in non-immunized patients do not add up to 96, because for 6 patients the current request was for fresh frozen plasma (n=2), platelet concentrate (n=3) and plasma + platelets (n=1).

Table 4: Red blood cell antibody specificities identified in 10 immunized patients

Antibody specificity by blood group system	Number	(%)
D, C, E	1, 2, 1	(36.4)
K	2	(18.1)
M, S	1, 1	(18.1)
Pan-reactive	3	(27.3)

lished studies (Santos *et al.*, 2007; Alves *et al.*, 2012), again confirming the high frequency of antibodies against Rhesus system antigens.

Alloantibodies production in our study was mainly found in patients with more than two transfusion episodes but one patient developed an antibody after a single episode. This finding suggests that even a single non antigen-matched transfusion may be enough to develop alloantibodies. One patient who had five transfusion episodes produced two antibodies. This

is consistent with a report of Schonewille *et al.* (2006), which found that multi-transfused patients are at high risk of developing multiple antibodies. The D antibody was unexpected since the patient was an Rh 'D' positive male. Similar findings have been reported by others (Chou *et al.*, 2013; Natukunda *et al.*, 2011). Although further D genotyping was not done, this patient may have a D variant, lacking certain epitopes to which the antibody was formed.

Our findings suggest alloimmunisation is not

influenced by age, gender and clinical diagnosis. This finding is in agreement with other studies including Usman *et al.*, (2011) and Higgins and Sloan (2008). However, Winters *et al.* (2001), Bauer *et al.* (2007) and Verduin *et al.* (2012), observed contrasting results. This disparity may be due to the difference in sample size and population. We confirmed a significant association between number of transfusion episodes and alloimmunisation (Natukunda *et al.*, 2010 and Zalpuri *et al.*, 2012).

Although most common transfusion practice in the western world is blood component therapy (UM, 2011), in Ghana close to 60% of its blood requests is whole blood. Even though 45% of the request was packed cell, patients were administered whole blood in about 80% of these cases. This may be due to lack of required human expertise and appropriate equipment to aid large scale preparation of blood components from whole blood. This does not offer maximum benefit to be derived from a unit of donated blood and may be a contributory factor to Ghana's inability to meet the units of blood required for transfusion daily (Ghana Health Nest, 2014). Patients receiving whole blood in the absence of available packed cells end up receiving components they do not need, whereas this could have been stored for patients who may need them in the future.

LIMITATIONS OF STUDY

- Some patient information were not fully captured, making it difficult for us to determine the total number of transfusion units patients had received.
- The exact moment of immunization was unknown as antibody screening was not performed prior to each transfusion.

CONCLUSION AND RECOMMENDATION

Overall, alloimmunisation prevalence rate among patients in this study was 9.4%. Although this may be lower than what is reported in literature, it is very significant, considering

the fact that Ghana's population is fairly homogenous. It is therefore necessary to expand the study to cover all the major regional and teaching hospitals in Ghana where chronically transfused patients are managed. We also recommend that antibody screening and identification tests are incorporated in the routine pre-transfusion testing procedures so that patients who develop antibodies can be issued antigen-negative blood.

ACKNOWLEDGEMENTS

The authors wish to thank Abednego Arthur, Joshua Asante and Kwabena Ameyaw Boateng of KATH Blood Bank, for the help in recruiting and drawing of blood samples. The Grants and Research Office of College of Health Sciences, KNUST, is also acknowledged for providing funds for the study.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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