Original Research Article

ERYTHROCYTE TRANSFUSION AND ALLOIMMUNISATION PATTERNS AMONG SICKLE CELL DISEASE PATIENTS, BENIN CITY, NIGERIA

ABSTRACT

INTRODUCTION: Blood transfusion remains a mainstay therapy in sickle cell disease (SCD).Transfusional therapy may be complicated by allo-immunisation due to exposure to foreign red cell antigens. However, the prevalence and patterns of atypical antibodies in Nigerian SCD has been sparsely reported majorly due to underdeveloped blood banking systems. A prospective study was therefore undertaken to assess patterns of blood transfusion and allo-immunisation among SCD patients in Benin City, Nigeria.

METHODOLOGY: The study was conducted among adult and paediatric SCD subjects seen at a sickle cell centre in Benin City, Nigeria. All subjects (parents in case of children) who gave consent/assent to the study were interviewed using a structured questionnaire to obtain details on bio-data, SCD history and blood transfusion history. Blood specimen obtained from each participant was subjected to antibody screening/identification test using tube agglutination technique. Association of categorical variables was tested using chi-square or fisher exact test as appropriate.

RESULTS: Fifty five SCD patients were studied with a mean(SEM) age of 22.95 (1.66) years. More of the subjects (67.3%) were aged 15 years and above. 74.5% of the subjects have a past history of blood transfusion. Four (7.3%) of the subjects had unexpected erythrocyte allo-

antibodies. Antibodies belonging to the Rh and Kell blood group systems were implicated. The risk of alloimmunisation increased with total lifetime transfusions (p = 0.002)

CONCLUSION: Erythrocyte alloimmunisation is a significant therapy related complication in Nigerian SCD. There is need to upgrade local/regional transfusion services to include routine allo-antibody screening/identification as part of precompatibility testing particularly SCD patients who have had more than 10 units of red cell transfusion.

Keywords: Erythrocyte transfusion, blood transfusion, alloimmunisation, sickle cell diseasee, Benin City, Nigeria.

INTRODUCTION

Sickle cell disease (SCD) remains a significant public health problem in sub-Saharan Africa particularly Nigeria [1-3]. Its management and control is bewildered with limited resources for early diagnosis, optimal treatment, prevention and treatment of complications including erythrocyte allo-immunisations [4, 5]. However, transfusional therapy in form of simple (top-up), exchange or chronic (prophylactic) blood transfusions remains a mainstay [6].

Previous local studies have reported prevalence of blood transfusion to be as much as 44% among Nigerian patients [7-9]. This implies that a high proportion of Nigerian SCD patients are exposed to foreign antigens through blood transfusions, which is a major risk factor for erythrocyte allo-immunisation [10, 11]. However, there is insufficient local data on the frequency and specificities of these implicating allo-antibodies. This is partly due to gross deficiencies in local blood banking systems including absence of routine antibody screening (and identification) on both donor and recipient samples (pre-compatibility testing). Hence, there is a

need for prospective studies to evaluate the patterns of transfusion and allo-immunisations in Nigerian SCD.

Erythrocyte alloimmunisation is a clinico-laboratory phenomenon characterized by presence of immune (atypical) allo-antibodies in an individual following exposure to foreign blood group antigens through transfusions, pregnancy and other sensitizing events [10, 12, 13]. The resultant clinical sequalae include haemolytic transfusion reactions and difficulty in finding compatible blood units for transfusion of affected individuals [12, 14, 15]. There is therefore a continual need to evaluate the prevalence and patterns of blood transfusion and allo-immunisation among Nigerian SCD patients. Information obtained will help to engage, advocate and deliver proper measures/protocols for transfusional therapy in SCD, as well as prevention/treatment of this untoward immune-haematological complication.

This study therefore aimed to characterize the pattern of blood transfusion among SCD patients in Benin City. It also aimed to determine the prevalence and specificities of the implicating allo-antibodies, as well as the possible associated factors.

METHODOLOGY

A prospective, cross-sectional, hospital based study was conducted over a period of two months (June to July 2015) among 55 sickle cell disease subjects in Benin City, Edo State. Subjects included adult and paediatric SCD patients seen at Sickle cell centre, GRA, Benin City, who gave consent/assent (parental consent in case of children) to the study after detailed explanation of the purpose of the study and its protocol. Ethical approval was sought from Edo State Ministry of Health, Benin City.

Relevant data including bio-, clinical data and details of transfusion history were gotten consecutively from each patient during clinic consultations, using a structured, interviewer administered questionnaire. Five milliliters of venous blood specimen was taken from each study participant, 3 mls was dispersed into plain tubes to obtain sera, remaining 2 mls into EDTA anticoagulated tube. Sera were separated after standing for at least 15 minutes and stored at -30^{o}C prior to analysis. All laboratory analysis were performed within 1 month of sample collection. Tube agglutination technique was used for antibody detection and identification. Sample detected positive for allo-antibodies were subjected to identification test using crossing out (exclusion) method. All samples which were screening positive were also subjected to direct antiglobulin test and auto-control. Positive and negative controls were run with each batch of test to ensure quality. Screening cells and panel of cells (albacyte reagent red cells) for detection and identification of unexpected allo-antibodies were procured from Lorne Laboratories UK, with Lot Numbers V159123 and V159601/V159602 respectively. Screening cells were $R_1^wR_1$, R_2R_2 and rr cells. The identification panel have known antigram containing Rh-hr (D, C, E, c, e, f, V, C^w), Kell (K, k, Kp^a, Kp^b, Js^a, Js^b), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), Lewis (Le^a, Le^b), MNS (M, N, S, s), P1, Lutheran (Lu^a, Lu^b), Additional antigens (Xg^a, Wr^a).

Descriptive and inferential statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 16, USA. Association of allo-immunisation status with variables such as transfusion history, age, total lifetime transfusions were tested using Chi square analysis or Fisher's exact test as appropriate. Statistical significance was set at the level of $p = 0.05$. Results are presented in tables and frequencies.

RESULTS

A total of 55 subjects with ages ranging from 2 to 51 years were recruited into the study. Mean(SEM) age of the subjects was 22.95 (1.66) years (Table 1). More of the subjects (67.3%) were aged 15 years and above. Mean (SEM) age at diagnosis of SCD was 5.98 (0.93) years. Male:Female Ratio was 1.39:1, while the predominant SCD haemoglobin phenotype (90.9% of the subjects) is HbSS (Table 1).

About three-quarter (74.5%) of the subjects have had at least one episode of blood transfusion prior to the study (Table 2). The remaining 14 subjects were blood transfusion naïve. The mean(SEM) age at first episode of blood transfusion was 10.83 (1.49) years. The most frequent indication (97.6%) for blood transfusion was simple (top-up) transfusion (Table 2). A few of the patients (7.3% each) have had exchange and chronic blood transfusions respectively. Mean(SEM) for total lifetime transfusions was 4.47 (1.19) Blood Units. Eleven of the transfusion experienced subjects (20% of all subjects) had at least one episode of blood transfusion in the preceeding three months. The most frequent transfusion reaction (43.7%) as reported by the subjects was febrile non-haemolytic transfusion reaction (FNHTR), followed by allergic reactions (37.5%).

Four (7.3%) of the subjects were observed to be positive for unexpected allo-antibodies (Table 2). All samples were DAT and Autologous control Negative. The distribution of the identified allo-antibodies includes Anti-C (33.3%), Anti-E (33.3%), Anti-k (16.7%), and Anti-Le^a (16.7%) (Table 4). All allo-immunised subjects have had a prior history of transfusion of at least two units of red cells (multiple transfusion), however, no statistically significant relationship was established ($p = 0.297$) (Table 3). Similarly, no statistically significant association was found

between allo-immunisation status and other variables including age groups, time of last episode of transfusion, and haemoglobin phenotypes (Table 3). However, allo-immunisation rate is found to be significantly and statistically related to total lifetime transfusions (p = 0.002).

DISCUSSION

A high prevalence of blood transfusion, about 75% was observed in this study group.This is not surprising as transfusion has remains a major therapeutic modality in SCD worldwide. Earlier studies in Nigeria have reported prevalence as high as 44% in paediatric age group [8, 9]. The frequency of blood transfusion was also observed to increase with age. This possibly explains the significantly higher prevalence of blood transfusion observed in this study since more of the subjects were adults. Erythrocyte allo-immunisation was observed in 4(7.3%) of the subjects. Five (83.3%) of the six identified allo-antibodies were clinically significant. These clinically significant erythrocyte allo antibodies were observed to be associated with transfusion of more than 10 red cell units. In a similar Nigerian study, Ugwu et al reported a slightly higher prevalence of 9.9% among adult SCD patients. This difference can be account for by the age distribution of the subjects. The index study encompassed both children and adults. Moreover, the prevalence of allo-immunisation appears to be age-related (more prevalent in subjects aged 15 years and above).

Prevalence of allo-immunisation among SCD subjects vary widely from about 5 to over 50% in different parts of the world [11,16,17]. In Sudanese, Tanzanian and Ugandan SCD patients, alloimmunisation prevalences of 4%, 4.1% and 6.1% were reported respectively [18-20]. In Saudi Arabia, allo-antibodies were identified in 13.7% of a cross section of SCD patients retrospectively [21]. A recent report from Detroit, US revealed allo-immunisation prevalence of

56.2% among chronically transfused SCD patients [`22]. Similarly, in Brazil, Zanette et al reported a prevalence of 51.8% among transfused SCD patients [23]. This is a contrast to a prevalence of 7.3% (9.8% of transfusion-experienced subjects) in index study. The lower prevalence among indigenous Nigerians is possibly due to less racial disparity in the donor-recipient population and less frequent transfusions due to insufficient blood supply system in developing nations (in contrast with liberal blood supply and use in developed nations) [24,29].

In the index study, the risk of alloimmunisation is significantly related to total lifetime transfusions. This observation has also been demonstrated in other climes of the world [25, 26]. Furthermore, 83% of the identified allo-antibodies were clinically significant and belonged to the the Rh and Kell blood group systems. The clinical significance of an allo-antibody is a function of its ability to cause in vivo destruction of erythrocytes [27, 28]. Anti-C and Anti-E of the Rh system were the most frequent alloantibodies in the study group. Anti-k (antibody to the KEL2) was observed in one of the subjects. Additionally, Anti-Le^{a}, a less clinically significant allo-antibody was detected in one of the multitransfused patients. In consonance with findings from other local and foreign studies, antibodies belonging to the Rh system (in particular Anti-E, Anti-C) appear to be the most frequently implicating allo-antibody [20, 21, 25, 30]. However, occurrence of Anti-K (KEL1) appears to be rare in Nigeria in compared to Anti-k (KEL 2), which was observed in index study. Anti-K alloimmunisation was not observed in the previous local study and index study [30]. Moreover, there is sparse report on frequency distribution of Rh and Kell antigen phenotypes in the Nigerian population. There is therefore a need to characterize the frequencies of the corresponding antigens (Rh and Kell) in Benin City and other parts of Nigeria through further research.

CONCLUSION

Erythrocyte alloimmunisation is a significant problem in Nigerian Sickle cell disease. The rate of alloimmunisation is significantly related to total life time transfusions. Current evidence suggests that extended red cell phenotyping for at least Rh and Kell antigens reduces alloimmunisation rates [31]. Author recommends upgrade of local/regional transfusion services to include routine extended red cell phenotyping and matching for SCD patients who are billed for transfusional therapy. Multi-transfused SCD subjects (with ≥2 blood units) should be routine screened for red cell allo-antibodies as part of precompatibility testing. Particular emphasis on allo-immunisation screening should be placed for adult Nigerian SCD who had received more than 10 units of red cells, patients on exchange or chronic (prophylactic) red cell transfusions.

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TABLE 1: PATIENT CHARACTERISTICS

VARIABLES		Frequency (n)	Percentage (%)
AGE (years)			
	< 15	18	32.7
	≥ 15	37	67.3
Mean(SEM) = 22.95 (1.66), Median = 23, Min = 2, Max = 51			
GENDER			
	Male	32	58.2
	Female	23	41.8
Male:Female Ratio = 1.39:1			
AGE AT DIAGNOSIS (years)			
	Infancy	20	36.4
	1 – 5	14	25.5
	6 – 14	16	29.1
	≥ 15	5	9.1
Mean (SEM) = 5.98 (0.93), Median = 4, Min = 1, Max = 31			
HAEMOGLOBIN PHENOTYPE			
	SS	50	90.9
	SC	5	9.1

N = 55 (100%)

255 TABLE 2: DETAILS OF PATIENTS' TRANSFUSION HISTORY

VARIABLES		Frequency (n)	Percentage (%)
TRANSFUSION HISTORY			
	Transfusion naïve	14	25.5
	Transfused	41	74.5
DISTRIBUTION BY AGE			
	<15 years	11	20.0
	15 or more	30	54.5
	Not transfused	14	25.5
AGE AT FIRST TRANSFUSION (years)			
	Infancy	7	12.7
	1 – 5	8	14.5
	6 – 14	15	27.3
	≥15	11	20.0
	Not transfused	14	25.5
Mean (SEM) = 10.83 (1.49), Median = 8 , Min = 1, Max = 37			
TOTAL LIFETIME TRANSFUSION			
	<2	9	41.8
	2 – 10	27	49.1
	>10	5	9.1
	Not Transfused	14	25.5
Mean (SEM) = 4.47 (1.19), Median = 2, Min = 1, Max = 55			
TIME OF LAST TRANSFUSION			
	Non transfused	14	25.5
	Less than 3 months	11	20.0
	More than 3 months	30	54.5
INDICATIONS FOR TRANSFUSION*			
	Top-Up	40	97.6
	Exchange	3	7.3
	Chronic/Prophylactic	3	7.3
	Non Transfused	14	25.5
PATTERNS OF TRANSFUSION REACTIONS**			
	FNHTR	7	43.7
	ALLERGIC	6	37.5
	DHTR/DSTR	2	12.5
	IRON OVERLOAD	1	6.3

256 N = 55 (100%), *multiple responses, **Frequency/Percentages of the reported reactions

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TABLE 3: ASSOCIATION BETWEEN ALLO-IMMUNISATION AND OTHER VARIABLES

VARIABLES		ALLO-IMMUNISATION STATUS		AOR; p-value
		POSITIVE	NEGATIVE	
AGE GROUPS (years)				4.97; 0.194
	≥ 15	4	33	
	< 15	0	18	
Haemoglobin Phenotypes				1.06; 0.675
	SS	4	46	
	SC	0	5	
Previous transfusion				3.48; 0.297
	Transfused	4	37	
	Not Transfused	0	14	
Total LifeTime Transfusion				0.002*
	<2 or none	0	23	
	2 – 10	1	26	
	>10	3	2	
Time of Last Transfusion				10.87; 0.052
	Less than 3 Months	3	8	
	More than 3 Months	1	29	

N = 55 (100%), AOR = Adjusted Odds Ratio (adjusted by adding 0.5 in each cell), *significant p-value, AOR not estimated

TABLE 4: SPECIFICITIES OF THE IMPLICATING ALLO-ANTIBODIES

PATIENTS	Allo-antibod(ies)	DETAILS
Patient 1:	Single: Anti-Le[a]	28 year old SS male with TLT of 8 units
Patient 2:	Multiple: Anti-C, Anti-E	34 year old SS male with TLT of over 60 units
Patient 3:	Single: Anti-k	46 year old SS male with TLT

		of 18 units
Patient 4:	Multiple: Anti-C, Anti-E	51 year old SS female with TLT of 20 units
6 allo-antibodies identified in 4 subjects, 5 (83.3%) clinically significant		