Red cell alloimmunization in multi-transfused patients with sickle cell anemia in Benin City, Nigeria

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Abstract

Background: Sickle cell anemia (SCA) is an inherited hemoglobin disorder characterized by chronic anemia and occasional crises. Clinical features are variable. While some individuals are relatively stable and rarely require blood transfusion, others often require blood transfusion. Multiple blood transfusion is associated with complications including alloimmunization, infections, and iron overload.

Aims and Objectives: The study aimed at determining the prevalence of red cell alloimmunization among multi-transfused patients with SCA.

Materials and Methods: A cross-sectional study of adult SCA patients who have received multiple blood transfusion and those who have never received blood was done. Antibody screening and identification were carried out using gel technology with commercially made panel of cells.

Results: A total of 145 SCA subjects were studied. They were made up of 86 test group (those that had received two or more units of blood) and 59 control group (those that had never received blood transfusion). Prevalence of red cell alloantibody among multi-transfused patients with SCA was found to be 9.3%. Alloantibodies identified were mainly against Rhesus antigens contributing 87.5% (anti-E 37.5%, anti-C 25%, anti-D 12.5%, anti-e 12.5%). A combination of Kell and Lutheran blood group antigens contributed 12.5%. No antibody was detected among the control group.

Conclusion: Blood transfusion is associated with the development of alloantibodies. Routine blood grouping for multi-transfused patients with SCA should be extended to include other blood group antigens in addition to Rhesus D and ABO antigens.

Key words: Alloimmunization, blood transfusion, sickle cell anemia

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Introduction

Sickle cell disorders (SCD) are genetic disorders resulting from the presence of a mutated form of hemoglobin (Hb), hemoglobin S (HbS). Homozygous inheritance of HbS, known as sickle cell anemia (SCA), is characterized by chronic anemia and occasional crises. The highest frequency of SCD is found in tropical regions, particularly sub-Saharan Africa, India, and Middle East.[1] Migration of people from these high prevalence areas has raised the frequency of the genes in America and Europe.[2] Three-quarters of sickle cell cases occur in Africa.[3] Nigeria has the highest prevalence of SCA in the world.[4] Currently, stem cell transplantation is the only treatment option that has the potential to cure SCD in carefully selected patients, although it is associated with high risk of morbidity and mortality.[5] Treatment is, therefore, largely supportive. Blood transfusion may also be indicated depending on the individual’s clinical condition. Multiple blood transfusion is associated with complications including alloimmunization, iron overload and infections such as HIV and hepatitis.[6] Different studies have reported varying prevalence of red cell alloimmunization.[7,11]
The aim of this study is to determine the prevalence and pattern of red cell alloimmunization among SCA patients who have received two or more units of blood transfusion.

### Materials and Methods

This was a cross-sectional study conducted in Benin City, Edo State Nigeria between September 2011 and December 2011. Approval for the study was gotten from the Ethics and Research Committee of University of Benin Teaching Hospital. The study population was made up of adult SCA patients aged 18 years and above. They were divided into two groups: Test group (SCA patients who have received two or more units of blood) and control group (SCA patients who have never received blood transfusion). Subjects for the study were recruited consecutively from the hematology out-patient clinic of the University of Benin Teaching Hospital and also from sickle cell center, Benin City, who gave their consent to participate in the study. A questionnaire of personal characteristics including history of blood transfusion, the total number of units of blood received, indications for blood transfusion, history of blood transfusion reaction and demographic variables were completed for each participant. In addition, 5 mL of blood were collected from each participant – 3 mL of blood were dispensed into plain bottles for antibody screening and identification while the remaining 2 mL were dispensed into ethylene diamine tetraacetic acid bottle to get patient’s cell for auto control. The sera were used for antibody screening and identification by gel technology using commercially made panel of cells – “ID-DiaCell” (lot number 45404.50.1) for antibody screening and “ID-DiaPanel” (lot number 45161.62.1) for antibody identification manufactured by DiaMed GmbH, 1785 Cressier FR, Switzerland.

Principle of the procedure involves testing unknown serum/plasma against a set of group O reagent red cells that together contain most of the antigens necessary to detect clinically significant antibodies. Reagent red cells in a hypotonic buffered saline solution were combined with patient’s serum to allow for antigen/antibody interaction in the upper chamber of the microtube containing gel. Incubation of the microtube content at 37°C for 30 min was carried out to promote antibody uptake. This is followed by controlled centrifugation of the red blood cells through dextrane-acrylamide gel. The micro typing system’s anti-IgG card restricts the unbound IgG from moving through dextrane-acrylamide gel. The micro typing system’s anti-IgG card restricts the unbound IgG from moving through the gel during centrifugation. Thus, unbound IgG does not neutralize the anti-IgG incorporated in the gel. Agglutinated red cells become trapped in or above the gel (positive reaction). Unagglutinated red cells travel through the gel particles and form a pellet at the bottom of the microtube (negative reaction).

Antibody identification was performed to determine the specificities of irregular antibodies detected in the antibody screen. This involves testing the serum against a panel of group O cells of known composition. Positive reactions were compared to known antigens on an identigram. The reaction patterns were evaluated to identify the antibodies present.

To ensure quality control, manufacturer’s instructions were strictly adhered to. Each batch of test was run with both positive and negative control. Patient’s own red cells and sera were set up (autocontrol) in order to rule out the presence of autoantibody.

Data collected from this study were analyzed using Statistical Package for Social Sciences (SPSS) software, version 16. Descriptive statistics was used to compute percentages and averages. Fisher’s test was used to test for associations between variables in test group and control. Level of significance was set at 0.05.

### Results

A total of 145 participants satisfied the inclusion criteria

#### Table 1: Age and sex distribution of the participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of subjects in test group</th>
<th>Number of subjects in control group</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30</td>
<td>69</td>
<td>51</td>
<td>120</td>
<td>82.8</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>6</td>
<td>17</td>
<td>11.7</td>
</tr>
<tr>
<td>41-50</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>5.5</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>59</td>
<td>145</td>
<td>100</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>28</td>
<td>73</td>
<td>50.3</td>
</tr>
<tr>
<td>Female</td>
<td>41</td>
<td>31</td>
<td>72</td>
<td>49.7</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>59</td>
<td>145</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Table 2: Alloantibody and autoantibody screening results of the participants

<table>
<thead>
<tr>
<th>Antibody detected</th>
<th>Test group (%)</th>
<th>Control group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloantibody</td>
<td>8 (9.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Autoantibody</td>
<td>1 (1.2)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>No antibody</td>
<td>77 (89.5)</td>
<td>57 (96.6)</td>
</tr>
<tr>
<td>Total</td>
<td>86 (100)</td>
<td>59 (100)</td>
</tr>
</tbody>
</table>

Fisher’s test \( P = 0.027 \)

#### Table 3: Antibodies identified in participants with alloimmunization

<table>
<thead>
<tr>
<th>Type of antibody</th>
<th>Number</th>
<th>Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-E</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>Anti-C</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Anti-D</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Anti-e</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Anti-k, kp, Js, Lu+</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>100</td>
</tr>
</tbody>
</table>
and were recruited for the study. They comprised of 86 SCA patients with a history of multiple blood transfusion and 59 control subjects who were SCA patients without a history of blood transfusion. The study group was made up of 73 males (50.3%) and 72 females (49.7%). While the test group consisted of 45 (52.3%) males and 41 (47.7%) females, the control subjects were made up of 28 (47.5%) males and 31 (52.5%) females [Table 1].

The test subjects were aged between 18 and 48 years with a mean of 26 ± 7.4 years whereas the control subjects were aged between 18 and 50 years with a mean of 23 ± 7 years. The most frequent age group in this study was 18–30 years. This age group accounted for 82.8% (n = 120) of the subjects, while the age group with the least frequency was 41–50 years, accounting for 5.5% (n = 8) [Table 2].

Of the 145 samples analyzed, 11 showed positive results during antibody screening. Eight of these were alloantibodies while three were autoantibodies. The prevalence of red cell alloantibody among SCA patients with multiple blood transfusion was 9.3% (8/86). None of the patients in the control group had alloantibodies. Blood transfusion was found to be significantly associated with alloimmunization (P = 0.027) [Table 3]. Out of the three autoantibodies detected, one was from the test group while two were from the control group.

Antibody identification tests carried out on the eight samples with alloantibodies showed that seven (87.5%) of the samples reacted positively to Rhesus blood group antigens, specifically three (37.5%) of the subjects reacted to E antigen, 2 (25%) to C antigen, 1 (12.5%) of the subjects reacted to D and e antigens each. One of the subjects reacted to a combination of K, k, Kp, and Js antigens of the Kell blood group system as well as Lu^b^ antigens of the Lutheran blood group system.

Of the eight subjects that have alloantibodies, 3 (37.5%) received <5 units of blood, 2 (25%) received between 5 units and 10 units of blood while 3 (37.5%) received >10 units. Development of alloantibody in relation to the number of units of blood received was not statistically significant (P = 0.116).

Five (62.5%) out of the eight subjects that have alloantibodies were males while 3 (37.5%) were females. Development of alloantibodies in relation to sex was not statistically significant (P = 0.470).

Four of the eight subjects who developed alloantibodies fall within the age group of 18–30 years. Two subjects fall within 31–40 years and 41–50 years age group each. Development of alloantibodies in relation to age was not statistically significant (P = 0.517).

Five (5.8%) of the 86 test subjects had transfusion reactions. Three (60%) had febrile transfusion reaction while 2 (40%) had urticarial rash.

**Discussion**

Red blood cell transfusion is a key component of therapy in the successful management of sickle cell disease. Transfusion therapy facilitates improved blood and tissue oxygenation reduces the propensity for sickling by diluting the host cells and temporarily suppresses the production of red cells containing HbS.\(^{[12]}\) Despite the beneficial effects of transfusion therapy in sickle cell disease, there are still adverse effects associated with transfusion that can lead to serious short- and long-term complications including transmission of infections, transfusion hemosiderosis, alloimmunization among others.\(^{[13]}\)

This study showed that the prevalence of red cell alloimmunization among multi-transfused patients with SCA is 9.3%. This finding further reaffirms that blood transfusion is associated with the development of alloantibodies. None of the patients in the control group had alloantibodies; as was also observed in previous studies.\(^{[7,8]}\) The prevalence of 9.3% found in this study is similar to that of an earlier study done in Nigeria.\(^{[7]}\)

Alloimmunization to red blood cell antigens has been widely reported in patients with sickle cell disease living in industrialized countries, with a varying incidence from 8% to 50%.\(^{[14]}\)

The specificities of red cell alloantibodies detected in this study were against Rhesus, Kell and Lutheran blood group antigens. Rhesus antibodies were found to be the most prevalent accounting for 87.5%. Findings from this study is in keeping with previous studies\(^{[7,8]}\) where 66.7% of patients reacted to Rhesus blood group antigens. The high contribution of Rhesus alloantibodies is probably because of the high immunogenicity of Rhesus antigens.\(^{[15]}\) Unlike ABO antibodies that are naturally occurring, Rhesus antibodies are immune antibodies and only occurs when an individual who lacks the antigen is exposed to it through blood transfusion or pregnancy. Rhesus antibodies are mainly IgG type and reacts better at 37°C and so are clinically important antibodies known to cause hemolytic transfusion reactions and hemolytic disease of the new born (HDN). Rhesus antibodies rarely, if ever, bind complement and therefore red cell destruction is mediated almost exclusively via macrophages in the reticuloendothelial system (extravascular hemolysis). Anti-D, anti-C, anti-E and anti-e have all been involved in hemolytic transfusion reactions, particularly delayed reactions.\(^{[15]}\) Routine blood typing for Rhesus D status in both blood donors and transfusion recipients has reduced the incidence of transfusion reaction caused by anti-D but sensitization to other Rhesus antigens can
be a problem in transfusion medicine, particularly in multi-transfused patients.

Apart from Rhesus, Kell and Lutheran alloantibodies together contributed 12.5% of the antibodies found. Kell alloantibodies found in this study, as commonly reported in previous studies, is attributed to the high immunogenicity of the antigen. Kell antigens have been reported to be the third most potent, after ABO and Rhesus antigens at triggering an immune reaction. Antibodies produced against Kell antigens are usually IgG type, does not bind complement and hemolysis is usually extravascular in nature. Anti-Kell antibodies can cause hemolytic transfusion reactions and HDN. In contrast to ABO and Rhesus sensitization, HDN attributable to Kell sensitization is caused by maternal anti-Kell suppressing fetal production of red blood cells. Anti-Kell promotes the immune destruction of K-positive erythroid early progenitor cells by macrophages in the fetal liver. Because the red blood cell precursors do not contain Hb, less bilirubin is released during the hemolysis and jaundice in the newborn period is less common. However, the underlying anemia may be severe. Alloantibodies to Lutheran blood group antigens are rare antibodies that have been reported to cause delayed hemolytic transfusion reaction (DHTTR). Anti-Lu antibodies are found in Lu (a-b-) individuals following blood transfusion or pregnancy.

Low frequency of acute transfusion reactions (5.8%) found in this study is probably because the alloantibodies identified are known to cause mainly DHTTRs. Delayed transfusion reactions may occur long after the patient has been discharged from the hospital and can be mistaken for crisis. Autoantibodies were detected in 2.1% of the subjects. Finding of autoantibodies both in test group and control group suggests that there is no association between the presence of autoantibodies and blood transfusion as reported in an earlier study.

Development of alloimmunization is affected by many factors such as age of the patient, number of units of blood received and antigenic differences between the donor and recipient population. Development of alloimmunization is higher in women than men. Contrary to previous studies, males were found to have a higher frequency of alloimmunization (62.5%) than females (37.5%). This is probably because the majority of the females were unmarried, thus pregnancy which is thought to be a contributory factor in other studies were eliminated.

Blood transfusion remains a mainstay of management for patients with sickle cell disease. Transfusion in patients with sickle cell disease can result in the development of alloimmunization to red blood cell antigens. Lack of extended red cell typing among multi-transfused patients with sickle cell disease further predisposes them to development of alloantibodies. Development of alloimmunization predisposes patients to the risk of developing DHTTRs. Symptoms of DHTTR may mimic serious complications of sickle cell disease and in some cases even precipitate crisis. In addition, DHTTRs can be a serious and potentially life-threatening complication. Extended red blood cell grouping in sickle cell disease patients is recommended to prevent or reduce red blood cell alloimmunization in sickle cell disease population.

References


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