PREVALENCE OF ALLOIMMUNIZATION AGAINST RED BLOOD CELLS ANTIGENS IN SICKLE CELL DISEASE PATIENTS IN LATAKIA, SYRIA

Saraa Baddour*, Mohamad Ayman AWAMA and Suzanne Alshemali

Department of Biochemistry and Microbiology, Faculty of Pharmacy, Tishreen University, Latakia, Syria

Sickle cell disease (SCD) is one of the most common inherited hemoglobin disorders in most parts of the world, including Syria. Red blood cells transfusion is a supportive procedure for patients with SCD. However, repeated blood transfusion is not risk-free; one major complication is RBCs alloimmunization; which is the focus of this study. Our objective was to estimate the prevalence of alloimmunization against RBCs antigens in multi-transfused SCD patients in the coastal city of Latakia, Syria, and to identify the involved alloantibodies. The factors that might affect RBC alloimmunization in these patients will also be studied. A multicenter study was carried on 100 SCD patients who previously received blood transfusions, and medical records of these patients were consulted. Antibody screening and identification were done using indirect antiglobulin test (IAT) at 37°C in gel cards. Extended antigens phenotyping, autocontrols, and direct Coombs test (DAT) were also performed. In this first study in our region, we found that 10% of SCD patients developed clinically significant alloantibodies. None of the patients enrolled in our study had autoantibodies. Of 10 cases of alloimmunization, the alloantibodies we identified were: anti-c (3/10), anti-K alone (2/10), anti-E (1/10), anti-Fya (1/10), anti-D alone (1/10), anti-C with anti-D (1/10), anti-C with anti-K (1/10). Matching for Rh and Kell antigens will significantly decrease alloimmunization rate, since it may prevent the development around 90% of RBCs alloantibodies in a Syrian population of multi-transfused SCD patients.

Keywords: SCD, alloimmunization, antibody screening, transfusion

INTRODUCTION

Hemoglobinopathies; mainly thalassemia and sickle cell disease, are the most common inherited blood disorders in different parts of the world, including Syria, where about 7000 patients are diagnosed with thalassemia or sickle cell anemia, with a carrier rate of 5-8% (national program for Thalassemia and SCD, Syrian ministry of health website).

Sickle cell disease is an autosomal recessive disorder caused by a point mutation in the hemoglobin-Beta gene found on chromosome 11, that results in forming HbS; which leads to sickle-shaped cells in some circumstances.

In SCD patients, most patients will receive occasional RBCs transfusion at some stage of their life and 5% to 10% of those enter the chronic transfusion program, in order to prevent clinically significant vaso-occlusive events, and to improve the oxygen-carrying capacity by increasing hemoglobin concentration and reducing the level of HbS. Although long-term blood transfusion is a lifesaver in significant proportion of SCD patients -especially in the absence or limited availability of curative therapies (i.e., allogeneic haematopoietic stem cell transplantation (HSCT)); it is not risk-free. Serious complications of transfusion are well-described such as transfusion-transmitted infections, transfusion-associated lung injury (TRALI), iron overload and RBCs alloimmunization; formation of antibodies directed against foreign antigens expressed on...
the donor’s RBCs. The consequences of RBCs alloimmunization range from being asymptomatic (serological) to hemolytic transfusion reactions (HTR) up to hyperhemolysis; dropping hemoglobin level below the pretransfusion level and even death due to hemolysis of patient’s own RBCs and transfused RBCs (bystander effect). Hemolytic transfusion reaction can be rarely acute, or often delayed (DHTTR), which can be severe and life-threatening. It can occasionally mimic pain or vaso-occlusive crises in SCD patients or even trigger one. RBCs alloimmunization can also complicate transfusion therapy, shorten transfused cells in vivo survival, increase the need of blood transfusions, delay the finding of compatible units and make it more difficult to provide safe blood transfusion.

In Syria, data about alloantibodies formation is very scarce. Taken into account that transfusion-dependent patients are not usually provided with extended phenotype matched blood, and that pre-transfusion screening for irregular antibodies is not yet implemented as an integral part of laboratory testing of compatibility, highlight the need to investigate the frequency of alloantibodies in multi-transfused patients. Therefore, our objectives in this study was to estimate the prevalence of alloimmunization against RBCs antigens, determine the identities of these antibodies, and investigate the factors influencing their development in SCD patients who receive blood transfusion in our region in order to improve the quality of their life. Moreover, this will support decision-making in any future policy that aim to optimize matched-blood provision, particularly in chronic transfusion programs.

MATERIALS AND METHODS

This cross-sectional study was performed between August 2020 and May 2021. The study included 100 patients who received at least 2 blood bags. All of the enrolled patients were previously diagnosed with SCD “SS and SJ”. Patients were recruited from the three main centers in Latakia that provide blood transfusion service for SCD patients; the Center of Thalassemia and SCD (Syrian Ministry of Health), the outpatient hematology clinic based in Tishreen University Teaching Hospital (Syrian Ministry of Research and Higher Education), and the hematology ward at Latakia General Hospital. All patients at these 3 locations have been transfused with only ABO and RhD crossmatch compatible blood. All ages were included. Ethical approval for the study was attained from the Ethics and Research Committee of Tishreen University Teaching Hospital. Informed consent was obtained from all patients or their parents. The clinical and demographic data such as age, gender, history of splenectomy and transfusion history, were collected from patients and their medical records. Venous blood samples were collected in K3EDTA and plain tubes, plasma and sera were separated and kept frozen at -40°C. The plasma/serum was used for alloantibody screening and antibody identification, while red cells were used for ABO and RhD grouping, extended antigen phenotyping, and direct antiglobulin test (DAT). Antibody screening and identification were performed using indirect antiglobulin test (IAT) by column gel agglutination (CGA) technique at 37°C with LISS-Coombs gel card (INVITROGEL TEST.SYSTEM, Germany). The reagent screening cells consisted of three cell panel (ReaCell I, II, III screening cells, Hungary) which included the most clinically significant antigens of non ABO blood systems. If alloantibody screening was positive, antibody specificity detection was performed on the samples using a commercial 11 cell identification panel (ReaCell Panel, Hungary) by using the CGA. The Rh C, E, c, e and Kell phenotype was determined by using Rh Phenotype Card with Anti-K (Matrix GEL.SYSTEM, TULIP DIAGNOSTICS, INDIA).

To determine presence or absence of autoantibodies in immunized patients, autocontrol and direct antiglobulin test (DAT) were performed using polyspecific (anti-IgG + anti C3d) and monospecific (anti-IgG) Coombs gel cards (INVITROGEL TEST.SYSTEM, Germany). DAT was performed by adding 50 μl of 0.8% patient's red blood cells suspension to Coombs card well, then centrifuging the card. While autocontrol was performed by adding 25 μl of patient's plasma and 50 μl of his own red blood cells suspension (0.8%) to Coombs card well, and incubating the card for
10 min. at 37 °C. Then centrifuging the card and reading the result.

**Statistical analysis**

The Statistical Package for Social Sciences (SPSS V20) was used to analyze the data. Frequency and percentage were computed for categorical variables. Median or mean and standard deviation (SD), Standard error (SE) or range were estimated for quantitative variables. Descriptive statistics, Independent T test, Mann–Whitney test, Chi-square and Fisher’s exact test were performed and a P-value of less than 0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

**Results**

100 SCD patients (51 males and 49 females) were included in this cross-sectional study. All patients received previous blood transfusions compatible only for ABO and RhD. The median age of all SCD patients was 14 years (range: 2.5-62). The mean age at the first transfusion was 5.8 years. The rate of blood transfusion in those patients is shown in Table 1. 10% of our patients (10 out of 100 patients; 3 males and 7 females) developed at least one antibody. Their demographics and characteristics are shown in Table 2. The alloantibodies we identified were as follows: anti-c (3/10, 30%), anti-K (2/10, 20%), anti-D (1/10, 10%), anti-E (1/10, 10%), anti-Fy^a (1/10, 10%), anti-C & anti-D (1/10, 10%), anti-C & anti-K (1/10, 10%) (Figure 1).

**Table 1: Demographics and characteristics of patients included in the study.**

<table>
<thead>
<tr>
<th></th>
<th>Age (years) mean ± SE</th>
<th>Age at first blood transfusion (years) mean ± SE</th>
<th>Number of transfused blood bags mean ± SE</th>
<th>Pre-transfusion Hb mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 ± 1.3</td>
<td>5.8 ± 0.9</td>
<td>39 ± 7</td>
<td>7.1 ± 1.5</td>
</tr>
</tbody>
</table>

**Table 2: Demographics and characteristics of alloimmunized patients included in the study.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Blood Group</th>
<th>Extended phenotype</th>
<th>Number of transfused blood bags</th>
<th>Antibody Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Female</td>
<td>O^+</td>
<td>D’C’c+E’e’K'</td>
<td>12</td>
<td>Anti-E</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>Male</td>
<td>A^+</td>
<td>D’C’c+E’e’K'</td>
<td>40</td>
<td>Anti-D &amp; Anti-C</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>Female</td>
<td>AB^+</td>
<td></td>
<td>6</td>
<td>Anti-c</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>Female</td>
<td>O^+</td>
<td>D’C’c+E’e’K'</td>
<td>10</td>
<td>Anti-Fy^a</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>Female</td>
<td>B^+</td>
<td></td>
<td>2</td>
<td>Anti-c</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>Male</td>
<td>B^+</td>
<td>D’C’c+E’e’K'</td>
<td>7</td>
<td>Anti-c</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>Female</td>
<td>A^+</td>
<td>D’C’c+E’e’K'</td>
<td>13</td>
<td>Anti-D</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>Male</td>
<td>A^+</td>
<td>D’C’c+E’e’K'</td>
<td>80</td>
<td>Anti-K</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>Female</td>
<td>A^+</td>
<td>D’C’c+E’e’K'</td>
<td>250</td>
<td>Anti-K</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>Female</td>
<td>A^+</td>
<td>D’C’c+E’e’K'</td>
<td>60</td>
<td>Anti-C &amp; Anti-K</td>
</tr>
</tbody>
</table>
Fig. 1: Frequency of alloantibodies in multi-transfused SCD patient.

Table 3: Clinical and demographic profiles of alloimmunized and non-alloimmunized patients.

<table>
<thead>
<tr>
<th></th>
<th>Immunized</th>
<th>Non-immunized</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>3/7</td>
<td>48/42</td>
<td>0.196</td>
</tr>
<tr>
<td>Age (mean rank)</td>
<td>69.95</td>
<td>48.34</td>
<td>0.025</td>
</tr>
<tr>
<td>Number of blood transfusion's blood bags (mean rank)</td>
<td>53.95</td>
<td>50.12</td>
<td>0.692</td>
</tr>
<tr>
<td>Pre-transfusion Hb (mean)</td>
<td>7.7</td>
<td>6.9</td>
<td>0.128</td>
</tr>
<tr>
<td>Splenectomy(Yes/No)</td>
<td>4/6</td>
<td>39/51</td>
<td>0.558</td>
</tr>
</tbody>
</table>

Direct antiglobulin test (DAT) was negative in all the immunized patients in this study, indicating that none of our patients had either RBC autoantibodies nor experienced a haemolytic transfusion reaction (HTR) at the time of sampling. Autocontrols were also negative in all the samples, confirming -along with the negative DAT- the absence of autoantibodies. Extended RBC phenotyping was carried out, and all the immunized patients were found negative for the antigen(s) corresponding to the existing antibody/antibodies, except for one patient who formed anti-D although she was documented as RhD positive. RhD typing for this patient was repeated with the available different monoclonal blend antisera from different manufactures and the agglutination was constantly strong. Partial D was suspected. This result has been confirmed by performing extended partial-RhD phenotyping once the reagents sera become available. Furthermore, the patient DNA was extracted from peripheral blood to perform RhD prototype sequencing and confirm genetically the partial D type later.

The mean pre-transfusion hemoglobin of alloimmunized patients was 7.7 g/dl and in non-alloimmunized patients it was 6.9 g/dl. The mean rank of transfused units' number was 53.95 in alloimmunized patients and 50.12 in non-immunized patients. There was no statistical difference, too (Table. 3).

40% of immunized patient were splenectomised (mostly due to auto splenectomy) and 43.3% of the non-immunized patients were splenectomised, too. This difference was not statistically significant (\( P=0.558 \)).

As shown in Table 3, gender, number of transfused units, history of splenectomy, and pre-transfusion hemoglobin value had no effect on alloimmunization rate. While patients’ age had a statistically significant effect (\( P=0.025 \)), and the group of SCD patients whose ages were more than 18 years, was more likely to develop alloantibodies.

**Discussion**

This cross-sectional study was -to our knowledge- the first of its kind in Syria. The
main objective was to shed some light on one important sequela of repeated blood transfusion; the formation of alloantibodies. The study included 100 SCD patients. 65% of them were under the age of 18. The distribution of males and females was comparable, as this genetic disease is not sex-related.

The alloimmunization rate was 10% (10/100) in our study. This was comparable to many studies such as those done in Palestine and Brazil[21,22]. However, other studies showed significant higher rates[23,24] and this could be attributed to antigenic differences between different ethnic groups. Although it is common for alloimmunization rate to be higher in those patients because of the vast antigenic and ethnic differences between patients and donors[24], but this ethnic differences is not significantly-relevant in our study, since the donors and the patients were from the same ethnic group. So, the lower rate in our study is most likely due to the similarity in RBCs antigens between donors and recipients.

Another factor for lower alloimmunization rates in our study is that patients were not followed up over time, and most samples were taken before one transfusion episode only. Thus, some of our non-immunized patients may have developed alloantibodies at some point, which became undetected later. This phenomenon has been well-documented in the literature as the "evanescence of antibody"[21-25].

In our study, 90% of antibodies in alloimmunized SCD patients were directed against Rh and Kell antigens, which is comparable to many other studies[20,26,27]. The differences in antibody specificities could be easily attributed to the different populations.

However, some alloantibodies directed against other antigens were more popular in various studies, such as against Miltenberger antigens in Chinese patients[28].

We found anti-c to be the most common antibody, followed by anti-K. We identified anti-D in a RhD" SCD patient, suggesting that this patient had the partial D antigen, and, therefore, should be treated as D" when she receives blood transfusion. On the other hand, the formation of anti-D in one RhD" patient in our study could be mainly attributed to laboratory errors in typing or labeling blood units. Another factor to take into consideration is the absence of a strategy that detects weak D or D variants in donors in our blood banks.

Although females are more likely to be immunized according to several reports[28,29], we found that gender had no effect on alloimmunization rate in our study. This might be explained by the negligible role played by pregnancy in inducing RBCs alloantibodies in our female cohort, since more than 95% of them had neither current or previous pregnancies at the time of this study.

We observed increased alloimmunization rate with age which is comparable to the findings in various reports[21,27,30]. This is probably due to higher transfusion period, entailing higher blood transfusion rate and increased exposure to blood[30,31]. The lack of correlation between the number of blood units and alloimmunization in our study could be explained by the fact that we do not know exactly when our patient developed the antibody for the first time because antibodies screening strategy is not applied as a routine procedure in our country. However, this result contrasted many studies that reported higher alloimmunization rate in patients who were transfused with more blood units[11,21,32].

Conclusion

Significant numbers of our SCD patients had developed alloantibodies in our cohort. We suspect that many others have gone undetected. This emphasizes the need to ensure that pre-transfusion screening of clinically significant alloantibodies is implemented, especially in chronic transfusion programs, and to perform extended phenotyping at the time of diagnosis. Routine pre-transfusion matching of blood, other than for ABO and Rh "D" antigens is recommended. The specificities of the alloantibodies we identified will help in guiding any future plan to provide extended matched blood to mitigate alloimmunization. Since around 94% of RBC alloantibodies in this Syrian population of multi-transfused SCD patients were directed against Rh and Kell antigens; we expect matching for these antigens is justified and highly-recommended.

Acknowledgement

We would like to thank all the patients and their families for their enthusiastic enrollment in this study. We are very grateful for the medical, laboratory, and nursing staffs at the 3 centers which participated in this study, especially Dr. Wafaa Ahmad.
REFERENCES


ائيات الأضداد المناعية تجاه مستضدات الكريات الحمر لدى مرضى فقر الدم المنجلي في اللاقية

سراء بدوري - محمد أيمن عوامة - سوزان الشمالي
قسم الكيمياء الحيوية والأحياء الدقيقة، كلية الصيدلة، جامعة تشرين، اللاذقية، سوريا

إن فقر الدم المنجلي من ا栾لالات الخضاب الوراثية شائعة الانشار في بلادنا. نقل الدم هو إجراء داعم للحياة ومستخدم بشكل كبير في تدبير هذه الأمراض. من المخالفات المترتبة على نقل الدم تشكل الأضداد الخفيفة، تجاه مستضدات الكريات الحمر المنقوطة. الهدف من هذه الدراسة هو تحديد معدل شيوخ التصريح الخفيف لدى مرضى فقر الدم المنجلي المتزمنين نقل الدم المتكرر في اللاقية، وكذلك تحديد نوعية هذه الأضداد ودراسة العوامل التي قد تؤثر على حدوث التصريح المستضدات الكريات الحمر لدى هؤلاء المرضى.

شملت هذه الدراسة 100 مريضًا من مراكز مختلفة. تم إجراء اختبار مسح الأضداد وتحديد هوتها باستخدام اختبار كومس اللامباشر بالدرجة 37 ° ففي بطاقات الهلام. تم إجراء التصريح الموسع واختبار كومس المباشر لجميع المرضى المعنيين.

طور 10% من المرضى أضداداً ذاتياً. لم يشكل أي من المرضى المشاركين في الدراسة أضداداً ذاتياً. كانت الأضداد التي تم تحديدها anti-E(1/1)anti-K(2/3)anti-C(1/1)anti-D(1/2)anti-D(1/1)anti-D(1/1)anti-D(1/1)anti-D(1/1)anti-D(1/1)

سيخفض معدل التصريح بشكل هام لدى مرضىهم؛ ربما

سيمنع تشكيل حوالي 90% من الأضداد لديهم.