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Original article

Alloimmunization in Patients with Sickle Cell Disease and Thalassemia: Experience of a Single Centre in Oman

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Abstract. ***Background:*** **Blood transfusion is an integral part of the supportive care for patients with sickle cell disease (SCD) and thalassaemia. The hazard of red cell alloimmunization, however, is one of the main complications of this therapy.**
Objectives: **The aim of this study was to evaluate the prevalence of red cell alloimmunization in Omani patients with sickle cell anaemia and thalassemia.**
Methods: **This study included 262 patients whose historical transfusion records were available. One hundred and twenty-nine patients with thalassaemia who were attending the day care unit for regular transfusions, and 133 SCD patients admitted at our hospital were included in this study. The Diamed® gel system was used for the screening and identification of atypical antibodies.**
Results: **The rate of alloimmunization in SCD patients was 31.6% (n=42, 95%CI, 24.87-40.66), whereas in patients with thalassaemia it was 20% (n=26; 95%CI, 13.9-27.6). Antibodies to E, e, C, c, D, K, S, Fy^a, Kp^a, Jk^a and C^w were observed; 85% of the patients were also immunised with Rh and Kell antigens. Considering the two groups together, 8 developed nonspecific antibodies and 12 developed more than one antibody.**
Conclusions: **Red cell transfusions were associated with a significant risk of alloimmunization. It is, therefore, imperative to perform an initial extended red cell phenotyping for both donors and recipients, and carefully select ABO, Rh and Kell matched donors. The higher incidence of alloimmunization in SCD patients is related to the inherent SCD-specific inflammatory state.**

Keywords: Multitransfused; Alloimmunization; Antibodies; Blood Transfusion; SCD; Thalassaemia.

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Introduction. Sickle cell disease and thalassaemia are the most frequent genetic disorders in Oman with a combined carrier frequency rate of about 6%.[1-3] Furthermore, in these congenital haemolytic disorders, there are limited curative options. Thus, long-term blood transfusion remains an integral treatment option for these conditions, in order not only to save life but more importantly to improve the quality of life.[4]

Development of anti-RBC antibodies (alloantibodies and autoantibodies) can significantly complicate transfusion therapy.[5-7] Furthermore, some of these alloantibodies being haemolytic, can cause haemolytic transfusion reactions, and thereby limit the utility of further transfusion, whereas others are clinically insignificant.[8] Erythrocyte autoantibodies appear less frequently, but they can result in clinical hemolysis and difficulty in cross-matching compatible blood units.[9] Patients with autoantibodies may have a higher transfusion rate and often require immunosuppressive drugs, splenectomy or alternative treatments to maintain an adequate level of haemoglobin.

Despite the recognition of antibodies as a transfusion-associated risk, [7,10-13] little is known about the extent and causes of these phenomena among thalassaemia and sickle cell disease patients from the Sultanate of Oman or the most appropriate methods of prevention. Approaches for prevention or treatment of alloimmunizations are under debate and include the provision of RBCs matched for all the major antigens associated with clinically significant antibodies, or to only give blood matched for antibodies that have already been detected. The reason for such a controversy may lie in the fact that many alloantibodies are not harmful and that expensive prevention methods may, therefore, benefit only some patients.[14] In addition, donor feasibility and the cost of RBC matching could impact on these approaches as also the own local guidelines regarding this issue. Furthermore, a better knowledge basis of the potential harmful antibodies among the thalassaemia and sickle cell disease patients can assist in considering the appropriate transfusion strategy to use. Our objective was to assess the prevalence of alloimmunization among our multiply transfused patients with thalassaemia and sickle cell anaemia.

Materials and Methods. Diagnosis of homozygous thalassaemia major and sickle cell disease was initially made by high-performance liquid chromatography [HPLC] profiles. However, it was further confirmed with family member studies [parents] and where necessary, by DNA studies using Sanger sequencing.

Thalassemia patients: Clinical features and transfusion records of 129 thalassaemia patients, aged 5-32 years, 44 males, 85 females, who received regular transfusion were analysed. These patients were attending the day care unit at SQUH for regular transfusions.

Sickle cell anaemia patients: 133 sickle cell disease patients [113 SS and 20 S-beta thal] who were admitted to SQUH haematology wards (30 males and 103 females) and who received regular transfusion were analysed. The transfusion records of all the patients including those transfused for their first time were examined for the presence of alloimmunization and antibody specificity, age, gender and ethnicity.

Donors: Blood donors from the SQUH blood bank were identified for their racial background, and RBC phenotype was performed for the following antigens C, c, D, E, e and Kell. The donor's ethnic origin was classified into Arabs and non-Arabs.[Data not shown]

Laboratory protocol. Antibody screening: Detection of alloantibodies was performed on a fresh blood sample using the indirect antiglobulin test by the column agglutination method. The gel card centrifugation technique was used (DiaMed AG, Cressier sur Morat, Switzerland). All patients were screened before any transfusion.

Antibody identification: Antibody specificity was determined using a standard panel of red cells reacting to known antigens using column agglutination and gel centrifugation (ID-DiaPanel and ID-DiaPanel-P, DiaMed AG). The indirect antiglobulin test and enzymatic papain–treated RBC test at 37^0C were performed when necessary and elution of antibodies was done to help in identification. Detection of alloantibodies masked by autoantibodies required the use of adsorption techniques using Polyethylene glycol [PG], or albumin or low-ionic strength saline [LISS] to identify the underlying antibody by the indirect antibody test [IAT].

Results. *Thalassaemia:* 26(20%) of the 129 patients had positive antibody screening, in whom 34 IgG alloantibodies were detected (95%CI, 13.9-27.6). 18(69%) patients developed one antibody; 6(23%) developed two antibodies and one (4%) developed three antibodies. One of the patients presented with a non-specific antibody (NSA). **Table 1** and **Figure 1b** shows the specificities and

Table 1. Type and frequency of antibodies identified in Thalassemia and SCD patients

Disease	Thalassemia		Sickle cell disease	
Antibody	No. of patients	Frequency %	No. of patients	Frequency %
K	7	26.9	5	12.2
E	6	23.1	11	26.8
C	0	0	2	4.9
D	1	3.8	1	2.4
e	1	3.8	1	2.4
c	2	7.7	1	2.4
NSA*	1	3.8	7	17.1
Kp^a	1	3.8	1	2.4
Fy^a	0	0	1	2.4
$E+Kp^a$	1	3.8	0	0
$K+Kp^a$	0	0	1	2.4
E+K	1	3.8	3	7.3
C+D	2	7.7	2	4.9
C+e	1	3.8	0	0
E+S	0	0	1	2.4
E+Cw	1	3.8	0	0
$C+D+Jk^a$	1	3.8	0	0
C+D+E	0	0	1	2.4
C+D+K	0	0	1	2.4
Jk^a+Fy^a	0	0	1	2.4
$E+Fy^a$	0	0	1	2.4
$E+Jk^a$	0	0	1	2.4
Total	26	100	42	100

* Non-specific antibody

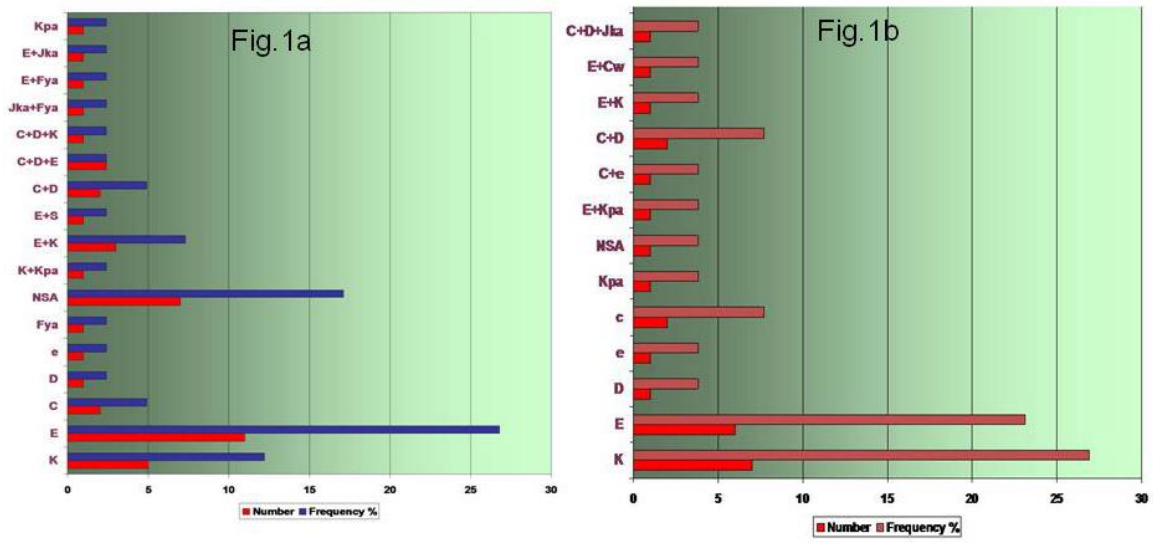

Figure 1. Number (%) of specific antibodies in SCD (Figure 1a) & Homozygous Thalassaemia Major patients (Figure 1b).

frequencies of the alloantibodies; 30(88%) of the alloantibodies were against the Rh and Kell antigens. The rate of alloimmunization among males was 19% and females 21%.

The rate of alloimmunization in adults (aged 13-32 years) was higher at 14.4% as compared to 4% in children (aged 5-12 years).

Sickle cell anaemia: Out of 133 patients, 42(31.5%) developed positive antibody screen in whom 46 IgG alloantibodies were detected (95%CI, 24.87-40.66). Seven patients (16.6%) showed NSA, 23(54.7%) developed one antibody; 10(24%) developed two antibodies, and two patients had developed three antibodies. The specificities and frequencies of the alloantibodies in Omani patients with SCD are shown in **Table 1** and **Figure 1a**. 38(83%) of the alloantibodies were Rh and Kell antibodies. The rate of alloimmunization among males was 30% and among females 33%.

Furthermore, in the eight patients who had a non-specific antibody, we observed that PG-IAT detected clinically significant antibodies like anti-E, anti-C, anti-D, anti-Jk(a), anti-c, anti-e, anti-s that were masked by an autoantibody in our cohort of multi-transfused patients. PG-IAT was superior in detecting clinically significant allo- antibodies

in the presence of masking autoantibodies as compared to the other techniques employed.

Discussion. The factors involved in alloimmunization are complex and includes at least three main contributing elements: the RBC antigenic differences between the blood donor and recipients, the recipient's immune status and the immunomodulatory effect of the allogeneic blood transfusions on the recipient's immune system.[15,16]

This study shows that the prevalence of alloimmunization in Omani homozygous thalassaemia patients was 20% (n=26; 95%CI, 13.9-27.6). Comparing the rate of alloimmunization in Omani thalassaemia patients with that of other populations, it was similar to several countries namely, 16.32% from Iran,[17] and 22% from California[18] and 19% from the CDC data in the USA on Asian and Caucasians patients.[19] But in general, there is a reduction of the frequency of alloimmunization when the patient receives blood from the same ethnic groups like those living in Hong Kong[20] and in Saudi Arabia.[21] The low incidence of immunization found in an old Italian cooperative study of 1984 (5/68;5.4%), could have the same explanation since this study included only thalassaemic patients living in Italy and receiving blood from the same ethnic group.[22] The higher rate of alloimmunization in adults as compared to children is in keeping with other studies where age is a significant factor.[11] The incidence of alloimmunization in transfused Omani patients with SCD was found to be 31.5% (n=42, 95%CI, 24.87-40.66). This rate is similar to that reported in USA, France, Holland, and patients of Asian descent in Brazil.[23-27] It was also noticed that most of the alloantibodies were to Rh and Kell, antigens and that the E antibody had a higher rate.

The Omani population, for historical reasons, is known to be a mixture of more than one ethnic group. So it was expected to detect some antigenic differences among the Omani donors themselves. Also, 10% of donors are non-Omani, so patients receiving blood transfusions will be further exposed to "foreign" antigens. The frequency of alloantibodies may be reduced by limiting the transfusion from donors with the same ethnic origin.[21]

It was noticed that patients with sickle cell anaemia showed a slightly higher rate of alloimmunization (31.5%) than thalassaemia patients (20%). This datum is consistent with observations by other studies as well. One reason for this observation could be because thalassemia patients are usually transfused at a younger age and regular intervals. The immune system response will be affected by the patient's age at their first transfusion and number of blood units the patient received.[27] It is believed that transfusions at an early age may offer some immune tolerance and protection against alloimmunization. The relation between the number of blood units transfused and antibody formation is unknown in thalassemia, but it is a major factor for increased alloimmunization in patients, including SCD, who receive multiple transfusions. However it should be taken into account that SCD is a chronic inflammatory state, and pro-inflammatory stimuli promote alloimmunization.[28,29] Furthermore, age is a significant factor, so children with SCD, who are chronically transfused, might have less inflammation, which could explain their lower rate of alloimmunization.[30,31] However, some antigen-negative patients may not produce antibodies at all or may form only one antibody despite exposure to antigen-positive cells. Studies have suggested at least in SCD patients, that genetic makeup is very relevant to the development of antibodies mainly altered Rh or Kell alleles, and perhaps acquiring these antibodies may be genetically driven.[32,33] In Omani population further studies will be needed to assess the effect of the number of transfusions on the immune response, the effect of the age at which the patient is first transfused, and the genetic makeup of recipients and donors on alloimmunization.

One of the biggest problems in a conventional hospital blood bank is finding the appropriate antigen-negative blood for the allo-immunised patients. Numerous reports show that transfusion of phenotype-matched RBCs (Rh and Kell) can reduce the risk.[19,34] However, there are still few reports revealing that the risk of alloimmunization is still high even when the donor blood is Rh and Kell matched with the recipient.[12,13]

BCSH transfusion guidelines also state that all patients with sickle cell disease and thalassaemia have their full phenotype tested at diagnosis and are given matched blood for C, c, E, e and K.[35,36] Moreover, extended red cell phenotype matching, although useful in preventing the formation of most alloantibodies, may prove impractical to

provide adequate and timely donors for these patients.[37] At present, we too follow this standard recommendation and hope to decrease the rate of alloimmunization. In our hospital it is estimated that the cost of one year of phenotyping for Rh and Kell antigens is about 12,500 OMR ($32,400) for all the donated units in our blood bank, raising the question whether it is cost effective to phenotype all of these units routinely. Nevertheless, DNA-based phenotyping can overcome certain limitations of serological studies and is beneficial in patients recently transfused or with interfering allo- or autoantibodies.[38]

Conclusions. Red cell transfusions are the cornerstone in the management of homozygous thalassaemia major but remain underutilised in SCD patients for fear of complications, although they can be lifesaving in the context of SCD complications. Nonetheless, they are associated with a considerable risk of alloimmunization as well as iron overload. The current BCSH transfusion guidelines recommend the initial extended red cell phenotyping for both donors and recipients. Thus with careful selection of donated units, coupled with further elaborative studies of the genetic diversity of patients and donor pool will certainly go a long way in reducing the prevalence of red cell alloimmunization. Therefore it will be cost-effective in the long term to choose the appropriate blood donor

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