

# Frequency of Red Blood Cells Alloimmunization in Thalassemia Patients at King Abdulaziz University Hospital in Jeddah, Saudi Arabia

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## Abstract

The aim of the study is to assess the alloimmunization rate to red blood cell in thalassemia patients at King Abdulaziz University Hospital. Thalassemia is the most common genetic disorder worldwide that represents a major public health problem and requires long life blood transfusion to the patients as the main treatment. Alloimmunization to the transfused red blood cell can cause hemolytic transfusion reactions and significantly complicate transfusion therapy. Screening and identification of alloantibodies and transfusion of extended phenotyped blood can minimize these risks. A retrospective study was conducted on 134 thalassemia patients at King Abdulaziz University Hospital in Jeddah. Patients' samples were subjected to red cell typing, antibody screening and identification of red blood cell antibodies. Alloimmunization in thalassemia patients was 20.15%; antibodies were mainly to the Rh and Kell blood group systems, the highest rate was for anti-E (32.4%) followed by anti-K (21.6%). Alloimmunization rate was the highest in the age group from > 10 – 20 years (40.7%). Red cell alloimmunization is a frequent event among thalassemia patient. A national protocol for screening and identifying of the red cell alloantibodies and transfusion of phenotype blood is required for proper management of these patients.

## Keywords

Transfusion; Thalassemia; Alloimmunization; Phenotyping

## Introduction

The thalassemia major is the most common inherited hemoglobin disorder that results from diminished production of beta globin chains, resulting in increase of alpha globin chains. Beta ( $\beta$ ) thalassemia is characterized by ineffective erythropoiesis and eventually shortened red blood cells (RBCs) life span<sup>[1]</sup>.

Beta ( $\beta$ ) thalassemia major is commonly discovered within the first year of life in the majority of the patients. The disorder is mainly concentrated across the Middle East, India and Southeast Asia, with over 250 million carriers worldwide<sup>[2]</sup>. The disease is treated by life-long regular blood transfusions (every 2-3 weeks) to increase patients' hemoglobin levels<sup>[3]</sup>. Although these transfusions were proven to be successful measures for the survival and development of thalassemia patients, it was also associated with the risk of alloimmunization<sup>[4,5]</sup>, a status where the recipient develops alloantibodies against the transfused RBCs and becomes at risk of developing catastrophic hemolytic reactions<sup>[6-8]</sup>. This presents a serious challenge for the pre-transfusion testing procedures and the overall safety of transfusions<sup>[6]</sup> and has mobilized many researchers to estimate the prevalence of alloimmunization in endangered thalassemia patients. The reported rates of alloimmunization ranged between 4% to 50%<sup>[3,9-11]</sup>. These variations are attributed to several elements thought to be responsible for the rate of alloimmunization, including the ethnic variations for the RBC antigens among blood donor and the recipients and the response of the immune system of the patient and the immune-modulatory effects of the allogenic transfusions on the recipient's immune status<sup>[5]</sup>.

Exploring the responsible factors for alloimmunization can aid in determining a suitable approach to minimize alloimmunization frequency.

This paper aimed to determine the rate of RBC alloimmunization in an ethnically diverse population of thalassemia patients attending a tertiary care hospital in Saudi Arabia, to detect the prevalence of RBCs typing and to identify the specificities of alloantibodies among the patients. This is done to ensure that appropriate action is taken to reduce alloimmunization and minimize the risk of hemolytic transfusion adverse events in thalassemia patients.

## Materials and Methods

A retrospective study was conducted on 134 thalassemia patients at King Abdulaziz University Hospital (KAUH), from the year 2014 to 2017 that included diagnosed patients with thalassemia major, followed at KAUH hematology outpatient clinics, irrespective of their age or gender. Data of the patients was collected according to the ethical approval at KAUH; which was obtained from KAUH Ethical and Technical Committee (No. 1316-13). All patients provided written informed consent and were assured about the confidentiality of their personal information. A data extraction sheet was designed to collect patients' information from their medical records including demographics: age, gender and nationality; hematological profile: such as their blood group, transfusion history, history of splenectomy; and lastly data on antibody formation including the rate of alloimmunization against the different blood group antigens. Patients were classified into: Males and females, Saudi and non-Saudi and into five age groups; from 0 to 10 years, from > 10 to 20 years, from > 20 to 30 years, from > 30 to 40 years and > 40 years old.

Patients planned for transfusion were targeted and then were tested for alloimmunization according to the protocols followed by KAUH. Ethylenediaminetetraacetic acid (EDTA) blood samples (3 ml each) were drawn from the patients prior to transfusion, and RBCs were serologically phenotyped before transfusion. After that, the separated plasma of the patients was subjected to screening for the presence of RBCs alloantibodies with polyspecific antihuman globulin. Gel Column Agglutination Technology at 37°C was used for detection of antibodies. Alloantibody screening was performed using three red cells' screening panels (automated Ortho machine); positive screening samples were tested manually to identify the specificity of antibodies; to be against Rh system, Kell, Duffy, Kidd, Lewis, MNSs, P1 or others<sup>[14]</sup>. Antibody identification was performed using commercial 11 red cells' panels (Bio-Rad Laboratories, Inc., Hercules, CA USA).

Screening and identification panels of red cells were the commercially available O cells that were fulfilling the international specification for proper detection of antibodies<sup>[14]</sup>.

## Transfusion Protocol

All patients were transfused at KAUH according to local transfusion policy. patients were transfused with ABO/RhD compatible blood after cross matching. Phenotypically matched units were issued whenever known. In case a patient was detected to have alloantibodies; antigen negative units that were compatible on full serologic cross match were issued<sup>[14]</sup>.

## Statistical Analysis:

The IBM Statistical Package for Social Sciences (SPSS) was used for Windows, Version 20. (IBM Corp., Armonk, NY USA). Data were described in counts and frequencies, while continuous data were presented in means and standard deviations. Comparative analysis was performed between alloimmunized and non alloimmunized patients, and across all measured parameters. Chi-squared test and Fisher's exact test have been used to test the difference between groups. A P-value ( $\geq 0.05$ ) was significant.

## Results

The alloimmunization rate among 134 patients was 20.15% (27 cases). Males represent 72 (53.7%) cases and females represent 62 (46.3%) cases; 13 male patients were alloimmunized representing 48.1% of the alloimmunized patients, while 14 female patients were alloimmunized representing 51.9% of the alloimmunized patients (Table 1, P-value was 0.514).

The Saudi thalassemia patients were 35 (26.1%) cases while the non-Saudi were 99 (73.9%) cases. Four cases of the Saudi patients were alloimmunized representing 14.8% of the alloimmunized patients, while 23 cases of the non-Saudi patients were alloimmunized representing 85.2% of the alloimmunized patients (Table 2, P-value was 0.134).

According to the age groups; patients from 0 to 10 years were 5 (3.7%) cases, from > 10 to 20 years were 48 (35.8%) cases, from > 20 to 30 years were 48 (35.8%) cases, from > 30 to 40 years were 29 (21.7%) cases and > 40 years old were 4 (3%) cases. The alloimmunization

**Table 1.** The rate of alloimmunization according to the gender of the patients

Gender	Number (%)	Alloimmunized
Male	72 (53.7%)	13 (48.1%)
Female	62 (46.3%)	14 (51.9%)
Total	134	27

**Table 2.** The rate of alloimmunization according to the nationality of patients

Nationality	Number (%)	Alloimmunized
Saudi	35 (26.1%)	4 (14.8%)
Non-Saudi	99 (73.9%)	23 (85.2%)
Total	134	27

**Table 3.** The rate of alloimmunization according to the age groups of the patients

Age Group	Number (%)	Alloimmunized
0 – 10	5 (3.7%)	1 (3.7%)
> 10 – 20	48 (35.8%)	11 (40.7%)
> 20 – 30	48 (35.8%)	8 (29.7%)
> 30 – 40	29 (21.7%)	7 (25.9%)
> 40	4 (3.0%)	0
0 – 10	5 (3.7%)	1 (3.7%)

in the patients of the age group 0 to 10 years was 1 case representing 3.7% of the alloimmunized patients, 11 cases in the age group > 10 to 20 years representing 40.7% of the alloimmunized patients, 8 cases in the age group > 20 to 30 years representing 29.7% of the alloimmunized patients, 7 cases in the age group > 30 to 40 years representing 25.9% of the alloimmunized patients and there were no alloimmunized patients above 40 years old (Table 3, P-value was 0.610).

The ABO/D blood grouping frequencies among patients were: O-positive: 55 (41%) cases, A-positive: 42 (31.3%) cases, B-positive: 24 (17.9%) cases, AB-positive: 5 (3.7%) cases, O-negative: 6 (4.5%) cases and A-negative: 2 (1.5%) cases. Eleven cases of blood group O-positive were alloimmunized which represent 40.7% of the alloimmunized patients, 10 cases of blood group A-positive were alloimmunized which represent 37% of the alloimmunized patients, 4 cases of blood group B-positive were alloimmunized which represent 14.8% of the alloimmunized patients, 1 case of blood group AB-positive was alloimmunized which represent 3.7% of the alloimmunized patients and 1 case of blood group A-negative was alloimmunized which represent 3.7% of the alloimmunized patients. Blood group O was the most common followed by group A. (P-value was 0.225).

The prevalence of alloantibodies found in these patients was 37 antibodies; which have a rate of anti-E: 12 (32.4%), anti-K: 8 (21.6%), anti-C: 7 (18.9%), anti-c: 2 (5.4%), anti-Fya: 2 (5.4%); moreover anti-e, anti-D, anti-Fyb, anti-Jka, anti-Jkb and anti-Lua were found one time (2.7%); (Fig. 1).

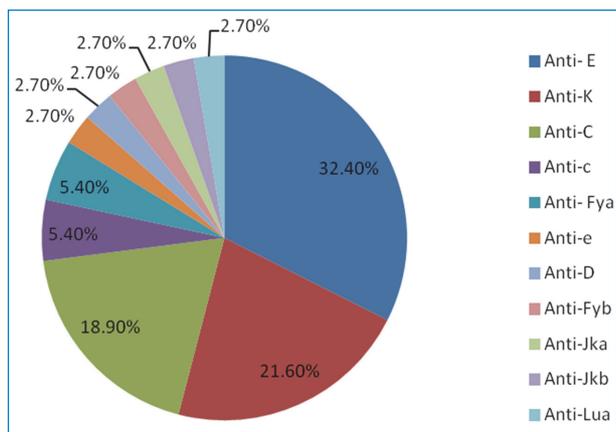


Figure 1. The prevalence of red blood cells alloantibodies.

## Discussion

Frequent blood transfusion is the main treatment for thalassemia patients, in order to minimize the complications of severe anemia. The beneficial effects of transfusions over the long term must be weighed against the risks of hemosiderosis, viral infections and alloimmunization against RBCs.

This paper reported an alloimmunization prevalence of 27/134 (20.15%) in the thalassemia patients, belonging mainly to the Rh and Kell systems. The frequency of alloimmunization depends on the heterogeneity of the donor-recipient population and the RBC administration matching process. Numerous studies have reported a wide frequency range of alloimmunization in thalassemia patients: 30% (57/190) in Kuwait, 4.97% (8/161) in India, 5% (72/1435) in Italy, 26.25 % (84/320) in Egypt, 22.06% (16/68) in Saudi Arabia and 3.7% (44/1200) in Greece<sup>[10-13,15-21]</sup>.

In this study, the most common antibody implicated was Anti-E followed by Anti-K and Anti-C. The highest rate of alloimmunization was in the age group > 10 – 20 years that represents 40.7% of the alloimmunized patients.

The rate of alloimmunization in the thalassemia patients was shown to be high in this study (20.15%), as most of those patient were referred to this hospital after they had already developed alloantibodies; they were receiving frequent transfusions of non-phenotypically matched blood units in other centers that do not follow local or international guidelines regarding the management of chronically multi-transfused patients, as there is no clear national protocol for this. Those

patients who developed antibodies are now receiving phenotyped blood in this hospital to prevent hemolytic transfusion reactions.

Gaining knowledge of the frequency of RBC antigen phenotypes in this population will allow establishment of a comprehensive donor and patient blood database that can be used to match those patients with phenotypically similar donors, to minimize the risks of alloimmunization in thalassemia patients.

Molecular bases of almost all the major blood group antigens has been determined enabling development of DNA-based methods for determining blood group genotype. DNA-based blood group typing provides a valuable adjunct to traditional methods of pre-transfusion testing especially for recently transfused thalassemia patients for whom phenotyping of RBCs is difficult or irrelevant.

Alternative treatment options to blood transfusion for thalassemia patients may also be considered, such as stem cell transplantation.

Other promising therapeutic strategies for thalassemia patients have recently been developed, which include genetic and pharmacological approaches to ameliorating ineffective erythropoiesis<sup>[22]</sup>.

## Conclusion

Alloimmunization to red cell antigens is a frequent complication among thalassemia patients in developing countries. Rh and Kell blood group antibodies are most commonly expressed with other RBC antibodies also clinically implicated from this report.

This study re-emphasizes the need for typing RBCs antigens for thalassemia patients prior to first transfusion and finding the most suitable blood, especially for Rh and K antigens. Proper documentation of the results and investigations done for thalassemia patients is essential for systemic follow up and treatment that would ultimately lead to safe blood transfusion and better quality of life.

## Recommendations

- A national protocol for screening and identifying of the red cell alloantibodies is required for all thalassemia patients

- Extended phenotyped matched PRBC transfusions should be implemented in all hospitals as a feasible method to minimize the risk of alloimmunization.

### Conflict of Interest

The authors have no conflict of interest.

### Disclosure

The authors did not receive any type of commercial support either in forms of compensation or financial for this study. The authors have no financial interest in any of the products or devices, or drugs mentioned in this article.

### Ethical Approval

The study design was reviewed and approved by the Unit of Biomedical Ethics Research Committee at King Abdulaziz University.

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### References

- [1] Srinoun K, Svasti S, Chumworathayee W, Vadolas J, Vattanaviboon P, Fucharoen S, Winichagoon P. Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice. *Haematologica* 2009; 94(9): 1211-1219.
- [2] De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, Daar S, Wali Y, Yassin M, Soliman N, Sobti P, Al Jaouni S, El Kholy M, Fiscina B, Angastiniotis M.  $\beta$ -thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. *Mediterr J Hematol Infect Dis* 2017; 9(1): e2017018.
- [3] Wang LY, Liang DC, Liu HC, Chang FC, Wang CL, Chan YS, Lin M. Alloimmunization among patients with transfusion dependent thalassemia in Taiwan. *Transfus Med* 2006; 16(3): 200-203.
- [4] Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis* 2010; 5: 11.
- [5] Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, Marwaha RK, Arora S. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. *Asian J Transfus Sci* 2014; 8(2): 84-88.
- [6] Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. *Br J Haematol* 2012; 159(4): 394-404.
- [7] Pandey H, Das SS, Chaudhary R. Red cell alloimmunization in transfused patients: A silent epidemic revisited. *Asian J Transfus Sci* 2014; 8(2): 75-77.
- [8] Kruatrachue M, Sirisinha S, Pacharee P, Chandarayyong D, Wasi P. An association between thalassaemia and autoimmune haemolytic anaemia (AIHA). *Scand J Haematol* 1980; 25(3): 259-263.
- [9] Sirchia G, Zanella A, Parravicini A, Morelati F, Rebulli P, Masera G. Red cell alloantibodies in thalassemia major. Results of an Italian cooperative study. *Transfusion* 1985; 25(2): 110-112.
- [10] Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood* 2000; 96(10): 3369-3373.
- [11] Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian origin. *Hematology* 2010; 15(3): 174-177.
- [12] el-Danasoury AS, Eissa DG, Abdo RM, Elalfy MS. Red blood cell alloimmunization in transfusion dependent Egyptian patients with thalassemia in a limited donor exposure program. *Transfusion* 2012; 52(1): 43-47.
- [13] Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. *Transfusion* 2003; 43(11): 1604-1610.
- [14] Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. *Transfus Med Rev* 2007; 21(1): 58-71
- [15] Gader AG, Al Ghumlas AK, Al-Momen AK. Transfusion medicine in a developing country - alloantibodies to red blood cells in multi-transfused patients in Saudi Arabia. *Transfus Apher Sci* 2008; 39(3): 199-204.
- [16] Keramati MR, Shakibaei H, Kheiyami MI, Ayatollahi H, Badiei Z, Samavati M, Sadeghian MH. Blood group antigens frequencies in the northeast of Iran. *Transfus Apher Sci* 2011; 45(2): 133-136.
- [17] Thakral B, Saluja K, Sharma RR, Marwaha N. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in North Indian blood donors. *Transfus Apher Sci* 2010; 43(1): 17-22.
- [18] Bilwani F, Kakepoto GN, Adil SN, Usman M, Hassan F, Khurshid M. Frequency of irregular red cell alloantibodies in patients with thalassemia major: a bicenter study. *J Pak Med Assoc* 2005; 55(12): 563-565.
- [19] Alboog AS, Tayeb TM, Alsager MO, AlNajjar SA, Damanhour GA, Jarullah JS, Hindawi SI. Red blood cell alloimmunization in sickle cell disease patients in Jeddah, Saudi Arabia: a pilot study. *J KAU Med Sci* 2015; 22(3): 17-22.

- [20] Ghasemi A, Abbasian S, Ghaffari K, Salmanpour Z. Prevalence of alloantibodies and autoantibodies in transfusion dependent thalassemia patients. *IJBC* 2016; 8(3): 80-85.
- [21] Shah S, Shah M, Gajjar M, Bhatnagar N, Soni S, Shah M. Frequency of alloantibody in multiple-transfused thalassemia major patients and factors influencing on alloimmunization: Alloimmunization in thalassemia patient. *Nat J Integr Res Med* 2016; 7(1): 40-46.
- [22] Cappellini MD, Porter JB, Viprakasit V, Taher AT. A paradigm shift on beta-thalassemia treatment: how will we manage this old disease with new therapies? *Blood Rev* 2018; 32(4): 300-311.

## معدل تكوين مضادات خلايا الدم الحمراء في مرضى الثلاسيميا في مستشفى جامعة الملك عبد العزيز بجدة ، المملكة العربية السعودية

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**المستخلص.** بهدف دراسة معدل تكوين مضادات كريات الدم الحمراء لمرضى الثلاسيميا بمستشفى جامعة الملك عبد العزيز الذين يتم نقل الدم لهم بشكل منتظم.

يعتبر مرض أنيميا البحر المتوسط أكثر الاضطرابات الموروثة في أنحاء العالم. ويمثل مشكلة صحية عامة وجسيمة تتطلب نقل دم للمرضى مدي الحياة كعلاج أساسي. ان تكوين المضادات لكريات الدم الحمراء المنقولة قد يؤدي إلى رد فعل انحلاي للدم مما يجعل العلاج بنقل الدم معقد للغاية. الكشف عن المضادات وتحديد أنواعها و نقل وحدات دم متطابقة لفصائل الممتدة للمريض يقلل من تلك المخاطر.

تم إجراء دراسة مرجعية لعدد ١٣٤ مريض ثلاسيميا بمستشفى جامعة الملك عبد العزيز بجدة، تم من خلالها فحص فصائل الدم للمرضى والكشف عن مضادات الكريات الحمراء وتحديد أنواعها.

بلغت نسبة المناعة المكتسبة لمرضى الثلاسيميا ٢٠,١٥٪ وكانت الأجسام المضادة بشكل رئيسي ضد فصائل الدم Rh و Kell ، أعلى معدل anti-E ٣٢,٤٪ يليه anti-K ٢١,٦٪ وبلغ أعلى معدل لتكوين المضادات في الفئة العمرية أكثر من ١٠ سنوات الي ٢٠ سنة ٤٠,٧٪.

ان تكوين مضادات لفصائل كريات الدم الحمراء يعد أمرا واردا بشكل كبير في مرضى الثلاسيميا. مما يتطلب وجود بروتوكول وطني لفحص وتحديد الأجسام المضادة لخلايا الدم الحمراء و نقل وحدات دم متطابقة لفصائل الممتدة للمرضى لإدارة سليمة لهؤلاء المرضى.

