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Red Cell Alloimmunization and Autoimmunization in Transfusion-Dependent Sickle Cell Disease Patients in Sana'a, Capital of Yemen

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Abstract

Objectives: Red cell alloimmunization is a major complication in patients with sickle cell disease (SCD) , resulting in delayed hemolytic transfusion reactions (HTR) and hyperhemolysis. The rate of SCD alloimmunization in Arabic countries varies from 4.3% to 65.5%. This study is the first research that aimed to determine the prevalence and phenotypes of red cell allo/autoimmunization in transfusion-dependent SCD patients at the Yemeni Society of Thalassemia and Genetic Blood Disorders (YSTGBD) in Sana'a city, Yemen.
Materials and methods: This cross-sectional study was conducted on a total of 153 patients with SCD who had undergone multiple blood transfusions between 2023 and 2024. Data was collected using a standard-designed questionnaire. Blood samples were used for blood group determination, Rh typing, and RBC alloantibody/autoantibody detection. Data was analyzed using SPSS version 26.
Results: Among 153 SCD patients, there were 88 males and 65 females, aged 1-56 years. The most common blood group was O (58.2%), followed by A (30.7%), B (9.8%), and AB (1.3%). There were 86.3% of patients who had RhD+, while 13.7% had RhD-. The prevalence of alloantibodies and autoantibodies was 9.15% and 1.3%, respectively. Anti-E alloantibody had the highest prevalence (35.7%), followed by anti-K alloantibody (28.6%), anti-c antibody (21.4%), and anti-C and anti-e alloantibodies (7.1% each).
Conclusion: Alloimmunization and autoimmunization were found with a low rate among transfusion-dependent SCD patients in Sana'a City, Yemen. The most frequent alloantibodies identified were anti-E, anti-K, anti-c, anti-C, and anti-e antibodies. We recommended the use of extended phenotyping and molecular genotyping in transfusion practice for patients with SCD .

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1. INTRODUCTION

Sickle cell disease (SCD) is a genetic autosomal recessive disorder caused by abnormal hemoglobin (Hb) synthesis. It affects millions of people around the world, especially in South Asian, Mediterranean, Middle Eastern, and African [1, 2]. Regular blood transfusions are necessary for SCD patients to maintain appropriate hemoglobin levels, which enhance blood's ability to carry oxygen and lessen the severe problems linked to the condition [3]. The goal of transfusion is maintaining Hb of 10-12 g/dL or HbS levels $\leq$ 30% [4] . Chronic transfusions in SCD are indicated in the prevention of primary and secondary stroke and recurrent vaso crisis [5, 6]. Red cell alloimmunization and/or autoimmunization are major complications in SCD patients, which result in delayed hemolytic transfusion reactions (HTR) and hyperhemolysis [6, 7]. Alloantibodies linked to blood transfusions, however, can have a detrimental effect on these patients' health and make transfusion therapy more difficult. Patients with SCD have an alloimmunization rate of 20% to 30%, but this high rate is significantly decreased when transfusions are performed using blood

from racially homogeneous populations [8, 9, 10, 11] . SCD alloimmunization rates in Arabic nations range from 4.3% to 65.5% [12, 13, 14, 15, 16, 17, 18, 19, 20, 21]. The reported alloimmunization rate in Saudi Arabia is 12.8%-39-42% [12, 13, 14, 15, 16], Oman (31.6%) [17], Kuwait (25.6-65.5%) [18], Egypt (10.0%) [19], Palestine (7.67%) [20] and Sudan (4.3%) [21]. In Yemen, the prevalence of sickle cell trait (Hb AS) is 2.2%, with higher rate observed in midwestern and western coastal regions compared to the central mountainous and eastern desert regions. In some places, the rate of impacted homozygous births (Hb SS) might be as high as 20/10,000 [22]. There is no information or prior research addressing the prevalence of autoimmunization and alloimmunization in patients with transfusion-dependent SCD . Investigating the frequency and characteristics of RBC antigens that result in hemolytic transfusion responses is crucial. The purpose of this study was to determine the prevalence and phenotypes linked to these findings. By employing expanded phenotype matching to find more compatible blood, an understanding of these phenotypes can assist in reducing the occurrence of unfavorable transfusion outcomes.

2. MATERIALS AND METHODS

2.1. Study population

This cross-sectional study was conducted on a total of 153 SCD patients who received multiple blood transfusions at the Yemeni Society of Thalassemia and Genetic Blood Disorders (YSTGBD) in Sana'a city, Yemen. The patients included 88 (57.5%) males and 65 (42.5%) females, aged between 1 and 56 years. This study was conducted during the period from December 1, 2023, to April 30, 2024.

2.2. Data collection

Data was collected using a standard-designed questionnaire that included sociodemographic and transfusion characteristics. It involved age, sex, age of initial transfusion therapy, transfusion frequency, total number of blood units transfused, blood type, and frequency of acute transfusion reactions.

2.3. Ethical Statement

All the participants provided written informed consent. The study objectives and procedures were explained to each participant. Approval for this study was obtained from the Faculty of Medicine and Health Sciences Committee on Postgraduate Studies and Scientific Research and the Yemeni Society for Thalassemia and Genetic Blood Disorders (YSTGBD).

2.4. Sample collection

Each patient provided a five-milliliter (5 ml) EDTA blood sample, which we used for blood group determination, Rh typing, and RBC alloantibody/autoantibody detection using a tube serological technique (Lorne Laboratories, United Kingdom).

3. LABORATORY ANALYSIS

3.1. Blood group typing

Anti-A, Anti-B, and Anti-AB monoclonal for tube techniques. Blood group typing was done manually using tube serological technique (Lorne Laboratories, United Kingdom).

3.2. Rh typing

Anti-C, Anti-D, Anti-E, Anti-c, and Anti-e monoclonal for tube techniques. Rh typing was done manually using tube serological technique (Lorne Laboratories, United Kingdom).

3.3. Direct Antiglobulin Technique (DAT)

Direct Antiglobulin Technique (DAT) was done manually using AHG Elite (Clear or Green) for the Antiglobulin Test (Lorne Laboratories, United Kingdom).

3.4. Indirect Antiglobulin Technique (NISS IAT)

Normal Ionic Strength Saline Indirect Antiglobulin Technique (NISS IAT) was done manually using AHG Elite (Clear or Green) for Antiglobulin Techniques (Lorne Laboratories, United Kingdom).

3.5. Anti-K (Cellano) monoclonal blood grouping

Anti-Kell alloantibody was detected using anti-K (Cellano) monoclonal for the indirect antiglobulin technique (Lorne Laboratories, United Kingdom).

3.6. Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Inc., New York, USA). Descriptive analysis was used to determine the frequency and specificity of red cell alloantibodies as well as the frequency of sociodemographic and transfusion characteristics.

4. RESULTS

As shown in Table (1) , there were 88 (57.5%) males and 65 (42.5%) females with an M:F ratio of 1.35:1. The ages of the SCD patients ranged from 1 to 56 years, with the majority of these patients in the 1-14 years' age group with 85 (55.5%), followed by the 15-29 years' age group with 55 (36.0%), followed by the 30-44 years' age group with 9 (5.9%) and the 45-56 years' age group with 4 (2.6%).

Table 1. The sociodemographic characteristics of sickle cell disease patients (n=153)

Characteristic		SCD	
		No.	%
Gender	Male	88	57.5
	Female	65	42.5
	Total	153	100.0
Age (Years)	1-14	85	55.5
	15-29	55	36.0
	30-44	9	5.9
	45-56	4	2.6
	Total	153	100.0

As shown in Table (2), the most common blood group was O, which was detected in 89 (58.2%) patients, followed by A blood group in 47 patients with a percentage of 30.7%, B blood group in 15 patients with a percentage of 9.8%, and AB blood group in 2 patients with a percentage of 1.3%. RhDpositive was detected in 132 patients with a percentage of 86.3%, while RhD negative was detected in 21 patients with a percentage of 13.7%.

Table 2. Blood groups of sickle cell disease patients (n=153)

Characteristic		SCD	
		No.	%
Blood group	A	47	30.7
	B	15	9.8
	AB	2	1.3
	O	89	58.2
	Total	153	100.0
Rh D typing	Positive	132	86.3
	Negative	21	13.7
	Total	153	100.0

As shown in Table (3), the majority of patients, 107 (69.9%), took blood transfusions more than 15 times, while 20 (13.1%) patients took 1-5 blood transfusions, followed by 11-15 (11.8%) and 6-10 (5.2%). On the other hand, 133 (86.9%) took less than 12 blood units per year, while 20 (13.1%) patients took more than 12 blood units per year. There were 98 (64.1%) patients without transfusion reactions, while 55 (35.9%) patients had transfusion reactions. Among 153 SCD patients, 14 (9.15%) alloantibodies had been detected in 6 patients, including 4 males (66.7%) and 2 females (33.3%), while 2 (1.3%) patients had developed autoantibodies.

As shown in Table (4), 14 alloantibodies were identified. Among them, anti-E alloantibodies had been detected in 5 (35.7%) patients, followed by anti-K alloantibodies in 4 (28.6%) patients, anti-c alloantibodies in 3 (21.4%) patients, and anti-C and anti-e alloantibodies both detected in 1 (7.1%) patient. Table 4: As shown in

Table 3. Blood transfusion characteristics of sickle cell disease patients (n=153)

Characteristic		SCD	
		No.	%
No. transfusion / Times	1-5	20	13.1
	6-10	8	5.2
	11-15	18	11.8
	>15	107	69.9
	Total	153	100.0
No. blood unit / year	<12	133	86.9
	≥12	20	13.1
	Total	153	100.0
Transfusion reaction	Yes	55	35.9
	No	98	64.1
	Total	153	100.0
No. allo/autoantibodies	Alloantibodies	14	9.15
	Autoantibodies	2	1.3
	Total	153	100.0

Table 4. Frequency of alloantibodies in sickle cell disease patients (n=153)

Blood groups	Alloantibody	No.	%
RH	Anti-C	1	7.1
	Anti-c	3	21.4
	Anti-E	5	35.7
	Anti-e	1	7.1
Kell	Anti-K	4	28.6
Duffy	Anti-Fy^a	NA*	-
Kidd	Anti-Jk^a	NA*	-
MNS	Anti-S	NA*	-
Lutheran	Anti-Lu^a	NA*	-
Total		14	100.0

NA* Not available

Table (5), the rate of SCD alloimmunization in our study compared to other Arabic studies. In our study, the alloimmunization rate was 9.15%, while the autoimmunization rate was 1.3%.

5. DISCUSSION

RBC alloimmunization and autoimmunization are potential side effects of transfusion. RBC alloimmunization and autoimmunization can result in significant morbidity through delayed hemolytic transfusion reactions, even though not all transfusion recipients develop alloantibodies or autoantibodies. In this study, RBC alloimmunization and autoimmunization frequencies were investigated in SCD patients who attended the YSTGBD in Sana'a city, Yemen. The most common blood groups in SCD patients were O (58.2%), followed by A (30.7%), B (9.8%), and AB (1.3%). The prevalence of RhD+ was 86.3%, while RhD- was 13.7%. The alloimmunization rate in patients with SCD ranges between 20% and 30%; however, this high rate was considerably reduced when blood from racially homogenous populations was uti-

Table 5. Rate of sickle cell disease alloimmunization in our study compared to other Arabic studies

Country	Reference	SCD	Alloimmunization	
		No.	No.	%
Yemen	Sana'a city (our research)	153	14	9.15
	Jeddah [13]	234	30	12.8
	Jazan [14]	385	50	12.98
	Eastern [15]	350	48	13.7
	Al-Ahsa [16]	78	13	16.7
	Jeddah [17]	104	41	39.42
Oman	[18]	133	42	31.6
Kuwait	Group 1 [19]	110	72	65.5
	Group 2 [19]	123	29	23.6
Egypt	[20]	60	6	10.0
Palestine	[21]	116	9	7.76
Sudan	[22]	210	9	4.3

lized for transfusion [8, 9, 10, 11]. Previous studies have reported varying rates of alloimmunization in Arab countries from 4.3% to 65.5% [12, 13, 14, 15, 16, 17, 18, 19, 20, 21] and in the Middle East, from 12.98% to 39.42% in SCD patients [13, 17, 23, 24]. The present study reported that alloimmunization rate amongSCD patientswas 9.15%, which is in line with that reported in studies conducted in Saudi Arabia, including Jeddah (12.8%) [12], Jazan Province (12.98%) [13], and Eastern Province (13.7%) [14]; in Egypt (10.0%) [19];and in Palestine (7.67%) [20]. Conversely, the alloimmunization rate in our study was lower than that reported in studies conducted in Saudi Arabia, including the Al-Ahsa region (16.7%) [15] and Jeddah (39.42%) [16], Oman (31.6%) [17], and Kuwait group 1 (65.5%) and Kuwait group 2 (23.6%) [18]. Conversely, the alloimmunization rate in our study was higher than that reported in a study conducted in Sudan (4.3%) [21].The low rate of alloimmunization observed in SCD patients could be explained by the homogeneity of RBC antigens between blood donors and recipients [8, 9, 10, 11]. The majority of donors and transfusion-dependent patients at Sana'a City's National Blood Transfusion and Research Center (NBTRC) are locals. One of the main reasons for the low prevalence of alloantibodies may be the homogeneity between local patients and donors. Another possible explanation for the low alloimmunization rates in SCD patients is that antigen avoidance via phenotyping is employed at NBTRC for at-risk patients, including SCD and β-thalassemia patients, prior to RBC transfusions [25]. There were 14 alloantibodies identified among 6 SCD patients. Among them, anti-E alloantibody had the highest prevalence of being detected (35.7%), followed by anti-K alloantibody (28.6%), anti-c antibody (21.4%), and anti-C and anti-e alloantibodies (7.1% each). Similar findings were reported in studies conducted in Saudi Arabia [12, 13, 14, 15, 16]. The autoimmunization rate in SCD patients (1.3%) was lower than the rate reported in Saudi Arabia by Kuriri et al. (5.3%) in the Al-Ahsa region [15] but higher than the rate reported by Halawani et al. (0.52%) in Jazan Province [13]. In order to give safe and compatible blood, serological compatibility testing may be complicated by autoantibodies, which are commonly found during pre-transfusion testing and might result in false positive results.

LIMITATIONS OF STUDY

Our study, along with those of others, has demonstrated the imperative for strategies to prevent alloantibody development and maximize transfusion benefits while avoiding complications. To minimize the prevalence of RBC alloantibodies and hemolytic transfusion responses in SCD patients, it is important to profile newly diagnosed individuals for routine testing for Duffy, Kidd, MNS, and Lutheran blood type antigens and to transfuse matching blood components.

6. CONCLUSION

Alloimmunization and autoimmunization were found with a low rate among transfusion-dependent sickle cell disease patients in Sana'a City, Yemen. The most frequent alloantibodies identified were anti-E, anti-K, anti-c, anti-C, and anti-e antibodies.We recommended the use of extended phenotyping, including ABO, RH, Kell, Duffy, Kidd, and MNS blood group systems, in transfusion practice for patients with sickle cell disease. Furthermore, molecular genotyping, either alone or in combination with extended phenotyping, may provide a more accurate and comprehensive assessment of a patient's antigen profile, further minimizing the risk of alloimmunization and improving transfusion outcomes.

ABBREVIATIONS

AHG: Antihuman Globulin; DAT: Direct Antiglobulin Technique; DHTR: Delayed Hemolytic Transfusion Reactions; EDTA: Ethylene Diamine Tetra Acetic Acid; Hb: Hemoglobin; Hb S: Hemoglobin S; Hb SS: Hemoglobin SS; HTR: Hemolytic Transfusion Reactions; IAT: Indirect Antiglobulin Technique NBTRC: National Blood Transfusion and Research Center; NISS IAT: Normal Ionic Strength Saline Indirect Antiglobulin Technique; SCD: Sickle Cell Disease; SD: Standard Deviation; SPSS: Statistical Package for the Social Sciences; YSTGBD: Yemeni Society of Thalassemia and Genetic Blood Disorders.

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