Original Article

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The prevalence of erythrocyte alloimmunization in clinical practice: A hospital-based study

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Abstract:

BACKGROUND: One of the complications of blood transfusion is the development of red cell alloimmunization. Little published literature on the prevalence and actual significance of red cell alloantibodies among nonregularly transfused patients and the general population.

OBJECTIVES: This study aimed to estimate red cell alloantibodies' prevalence, specificity, and clinical significance in obstetric and medical practice in Iraq.

MATERIALS AND METHODS: A cross-sectional hospital-based study involving internal medicine patients and ladies in obstetric wards of Baghdad Teaching Hospital in Baghdad/Iraq, from January 2022 to May 2022. Demographic data were collected along with detailed medical, obstetric, and transfusion history. Alloantibody screening was performed, and samples with positive results were subjected to antibody titration and identification.

RESULTS: A total of 200 patients were enrolled. Indirect antiglobulin test was positive in 15% of patients in internal medicine wards and 23% of ladies in obstetric wards, with most of the identified alloantibodies being clinically significant, against Kidd and Duffy antigen groups. Blood transfusion of more than four units to patients at internal medical wards showed a significant association as a risk for developing red cell alloantibodies ($P = 0.025$). For ladies in obstetric wards, there was a significant association between pregnancy loss at the time of screening and alloimmunization ($P = 0.0164$).

CONCLUSION: High prevalence of red cell alloantibodies in comparison to what is published worldwide. Transfusion of more than four units of blood and pregnancy loss at the time of screening were statistically significant risks for alloimmunization of the medical and obstetric populations, respectively.

Keywords:

Alloantibodies, indirect antiglobulin test, indirect Coombs test, red blood cell alloimmunization

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Introduction

Although often lifesaving, blood transfusion is not risk free, it may have serious consequences for the transfused patients, including alloimmunization against donor red blood cell (RBC) antigens.[1] In transfusion medicine, alloimmunization most often refers to the development of antibodies to non-ABO RBC antigens following pregnancy, transfusion, transplantation, or other exposures such as intravenous drug use/needle sharing.[1]

RBC alloimmunization is a serious complication challenging the selection of compatible units for future transfusions.[2] More importantly, it can complicate patient care and increase the risk of acute and delayed hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDFN).[1]

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The incidence of alloimmunization varies markedly according to the clinical setting studied, study design, detection techniques, and condition of the patient, with widely variable prevalence rates among centers that might be related to the strategies used for antigen matching.[3]

The possibility of alloimmunization varies between populations based on the prevalence of blood group antigens within the population.[4] For an alloantibody to develop, an individual must be exposed to a non-self RBC antigen and have a human leukocyte antigen-binding motif capable of presenting a portion of the non-self antigen.[1]

Factors involved in alloimmunization are complex including the RBC antigenic differences between donors and recipients, the recipient's immune status, and the immunomodulatory effect allogeneic blood on the recipient's immune system.[5]

Patients with alloantibodies have a 20–25 times increased risk for additional antibodies after subsequent transfusions.[6] Hence, it is important to identify the antibody and provide the corresponding antigen-negative red cell component to the patient once indicated.

Materials and Methods

Patients

A cross-sectional hospital-based study involving 200 patients from Baghdad Teaching Hospital – Medical City Complex in Baghdad/Iraq, from January 2022 to May 2022. All patients were interviewed, and information was collected by questionnaire and from the patient's case sheets including demographical data (age and sex), the cause behind admission, full medical, surgical, and drug history in addition to detailed transfusion history (blood product type, number/frequency, time/duration, any presumed or documented complications, and their management).

For all female patients, a full obstetric history including any baby of neonatal jaundice with or without phototherapy or exchange transfusion and the ultimate neonatal fate.

Inclusion criteria

Patients 14 years of age and older from the wards of general medicine and hemodialysis unit, ladies from the obstetric ward and labor room, and mothers of neonates at the neonatal care units and antenatal outpatient clinic were included in the study.

Exclusion criteria

Exclusion criteria were Rh-D-negative mothers in obstetric wards; patients with known hemoglobinopathies, hematological or solid malignancies, bone marrow failure syndromes, autoimmune diseases, connective tissue disorders, or other immunological disorders; and those currently on immunosuppressive therapies or other immune-modulating agents whatever the cause.

Laboratory work

ABO blood grouping and Rh-D typing were performed for each patient using the Gel card method. RBC alloantibody screening, titration, and identification were performed by indirect antiglobulin test (IAT) using the standard tube method, and the steps are summarized in Figure 1.

Antibody screening

To develop screening cells from our local ethnic groups, in-house reagent RBCs were prepared, pooled from at least 10 random O+ fresh donors, screened to be direct antiglobulin test (DAT) negative, and suspended in saline. In the test tube, four drops of patient serum were mixed with 2 drops of a 5% (v/v) cell suspension, and then four drops of low ionic strength solution were added as an enhancing reagent.

After incubation, test tubes were centrifuged and inspected for hemolysis or agglutination, and then RBCs were washed 2–3 times with 0.9% fresh normal saline, to remove free unbound serum globulins, after each wash the tube was centrifuged.

Following the last wash, two drops of polyspecific antihuman globulin (AHG) reagent were added to RBCs, then another centrifugation at 1200 RPM for 30 s before

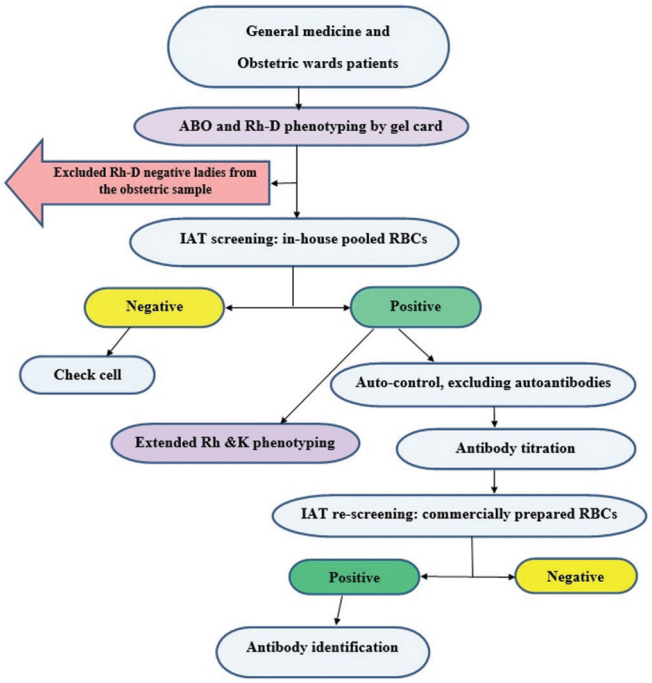


Figure 1: Flowchart demonstrating the procedure sequence of the study. IAT = Indirect antiglobulin test, RBC = Red blood cell

examining for agglutination under the light microscope to grade the results.

The screening test was repeated using once available a commercially prepared three-cell kit (NHSBT reagents, Lot. No. R123 3643 Tallinn, Estonia) containing three vials of RBC with known surface antigen (R1R1, R2R2, and rr).

Antibody titration

Serial doubling dilutions of plasma for each IAT-positive sample were tested with IAT using pooled reagent O-red cells till no agglutination was noted.

Extended phenotyping

Red cells of IAT-positive patients were further phenotyped for the full Rh (C, c, E, and e) and kell antigens by gel card method (Lot. 120016.18.01).

Antibody identification

Serum samples of IAT-positive patients were then stored at −20°C as antibody identification panels were unavailable until a few months after screening. Each sample was tested against a panel of 10 red cell samples of known antigen composition (NHSBT reagents, Lot. No. R146 3512 Tallinn, Estonia), steps were similar to an antibody screening test using AHG reagent.

The specificity of an antibody was assigned when it is reactive with at least three examples of reagent red cells carrying the antigen and nonreactive with at least three examples of red cells lacking the antigen, an example is illustrated in Figure 2.

Autocontrol and check cells

An autocontrol using the patient's cells and serum was performed to rule out autoantibodies. All IAT-negative results were rechecked using check cells (sensitized immunoglobulin G [IgG]-coated O+ RBCs).

Ethical consideration

This study was approved by the Ethical Committee of the Iraqi Ministry of Health (No.72\4\1\1-2022) to be carried out at the Department of Immunohematology of the National Blood Transfusion Center/Baghdad/Iraq. Informed consent was taken from all patients before enrollment, and privacy was protected for all of them.

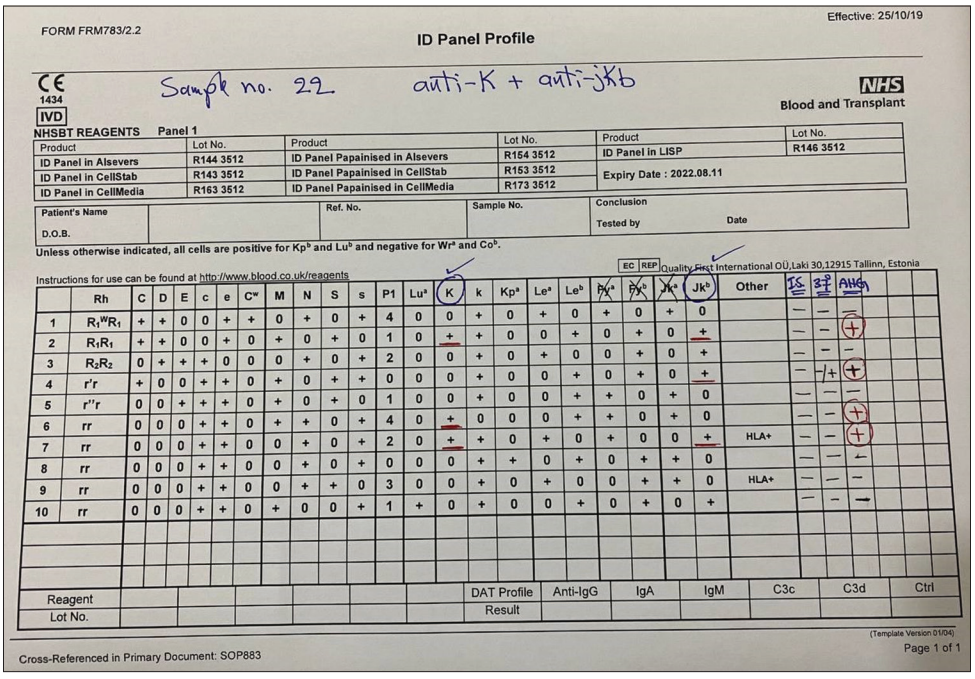
Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software (version 23) (IBM SPSS, Inc., Chicago, USA) was used for data entry and analysis. Interpretation for descriptive analysis was carried out for demographic and other parameters. Continuous variables were expressed as mean ± standard deviation and categorical variables as numbers and percentages. Analytic statistics as a Chi-square test to find an association between two categorical variables and Fisher's exact test when Chi-square was inapplicable due to a small sample size. $P \leq 0.05$ was considered to be statistically significant.

Results

Medical wards' sample

Among 100 patients in medical wards, the mean age was 52.9 ± 14.4 years (range: 14–89 years), with a male-


FORM FRM783/2.2

ID Panel Profile

Effective: 25/10/19

CE 1434 IVD

Sample no. 22 anti-K + anti-Jkb

NHS Blood and Transplant

NHSBT REAGENTS Panel 1

Product	Lot No.	Product	Lot No.	Product	Lot No.
ID Panel in Alsevers	R144 3512	ID Panel Papainised in Alsevers	R154 3512	ID Panel in LISP	R146 3512
ID Panel in CellStab	R143 3512	ID Panel Papainised in CellStab	R153 3512	Expiry Date : 2022.08.11	
ID Panel in CellMedia	R163 3512	ID Panel Papainised in CellMedia	R173 3512		

Patient's Name | Ref. No. | Sample No. | Conclusion
D.O.B. | Tested by | Date

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Wr^a and Co^b.

EC REP Quality First International OÜ,Laki 30,12915 Tallinn, Estonia

Instructions for use can be found at http://www.blood.co.uk/reagents

	Rh	C	D	E	c	e	C^w	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	P_1^a	P_1^b	Jk^a	Jk^b	Other	IS	37	AHG
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	+	4	0	0	+	0	+	0	+	0	+	0		−	−	+
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	1	0	+	+	0	0	+	0	+	0	+		−	−	−
3	R_2R_2	0	+	+	+	0	0	0	+	0	+	2	0	0	+	0	+	0	0	+	0	+		−	−/+	+
4	$r'r$	+	0	0	+	+	0	+	0	+	+	0	0	0	+	0	0	+	0	+	0	+		−	−	−
5	$r''r$	0	0	+	+	+	0	+	0	+	0	1	0	0	+	0	0	+	+	0	+	0		−	−	+
6	rr	0	0	0	+	+	0	+	+	0	+	4	0	+	0	0	0	+	+	0	+	0		−	−	+
7	rr	0	0	0	+	+	0	0	+	0	+	2	0	+	+	0	+	0	+	0	0	+	HLA+	−	−	+
8	rr	0	0	0	+	+	0	0	+	0	+	0	0	0	+	+	0	+	0	+	+	0		−	−	←
9	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	+	0	0	+	+	0	HLA+	−	−	−
10	rr	0	0	0	+	+	0	+	0	0	+	1	+	0	+	0	0	+	0	+	0	+		−	−	→

Reagent								DAT Profile	Anti-IgG	IgA	IgM	C3c	C3d	Ctrl
Lot No.								Result						

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Figure 2: Example for alloantibody identification, this patient had multiple warm alloantibodies (anti-K and anti-Jkb), they share reaction with cells 2, 4, 6, and 7 carrying the corresponding antigens

to-female ratio was 1.1:1. Most of the patients had end-stage kidney disease with regular hemodialysis visits [Figure 3].

A chronic medical illness was found in 90% of patients, whereas past surgical interventions were found in 60% of them. Rh-D antigen was positive in 89% while negative in 11% of them (seven males and four females). Transfusion history was reported in 74% of patients. IAT screening was positive in 15% (reaction strength: +1 in 12, +2 in three). At least one previous pregnancy was found in 26% of females [Tables 1 and 2].

Antibody titers of 1 / 16 and more were found in 40% of them. DAT was negative in all samples except one female who was transfused a month before, showing a reaction to IgG and C3d (mixed field appearance).

The only statistically significant association with the IAT result was with the blood transfusion of more than four units [$P = 0.025$, Table 3].

RBCs alloantibodies were identified in six out of 15 patients with positive IAT results, with the remaining nine samples no antibody can be determined. Nine alloantibodies were specified against six antigen groups: three anti-Jkb (33.3%), two anti-K (22.2%), another two against the Duffy antigen (anti-Fya and anti-Fyb) (22.2%) for both, and one (11.1%) for each of anti-M and anti-c antibodies [Figure 4].

Details of patients with positive IAT and identified alloantibodies are illustrated in Table 4.

Obstetric sample

Among 100 ladies from obstetric wards, the mean age was 29.3 ± 5.5 years (range: 19–41 years). Past medical, surgical, and transfusion histories were reported in 34%, 74%, and 37%, respectively [Tables 5 and 6].

Adverse obstetric history was present in 55% [Figure 5], a history of having a baby with neonatal jaundice was found among 20%, and multigravida status constituted 90%.

At the time of screening, 40% were still pregnant (of them 4% in their 1st, 11% in 2nd, and 25% in 3rd trimesters); 60% were in the puerperal period, two-thirds of them delivered their babies at term, and one third at preterm.

IAT screening was positive in 23 mothers (strength: +1 in 19, +2 in three, and +3 in one), with only one sample with a titer ≥1/16. DAT was negative in all of them.

Pregnancy loss was noted in 14% of mothers at the time of the screening, and it is the only statistically significant association with positive IAT results ($P = 0.0164$).

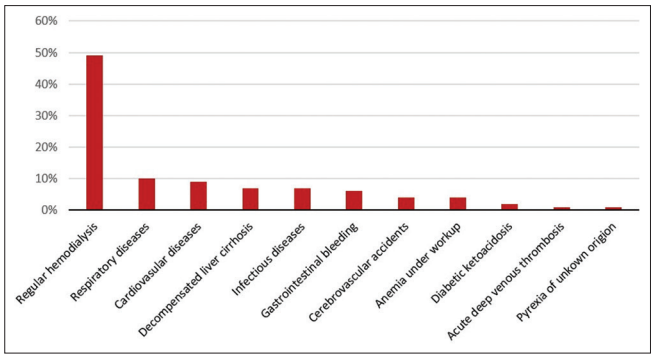

Figure 3: Cause behind hospital admission of patients in medical ward sample

Table 1: General characteristics and their association with indirect antiglobulin test results of the medical ward's population

Variable	IAT		Total (%)	*P*
	Negative, *n* (%)	Positive, *n* (%)		
Age groups (years)				
14–35	10 (83.4)	2 (16.6)	12	0.849*
>35–55	35 (87.5)	5 (12.5)	40	
>55	40 (83.3)	8 (16.7)	48	
Gender				
Male	47 (87.1)	7 (12.9)	54	0.53*
Female	38 (82.7)	8 (17.3)	46	
Chronic medical illness				
Positive	77 (85.5)	13 (14.5)	90	0.64*
Negative	8 (80)	2 (20)	10	
Past surgical history				
Positive	48 (80)	12 (20)	60	0.086*
Negative	37 (92.5)	3 (7.5)	40	
Rh-D antigen				
Positive	76 (85.4)	13 (14.6)	89	0.66*
Negative	9 (81.8)	2 (18.2)	11	
Transfusion history				
Negative	20 (76.9)	6 (23.1)	26	0.207*
Positive	65 (87.8)	9 (12.2)	74	
Total	85	15	100 (100)	

*Chi-square test, significant ≤0.05. IAT=Indirect antiglobulin test

Table 2: Association of being pregnant in the past and indirect antiglobulin test results among female patients admitted to the medical wards

Being pregnant in the past	IAT		Total, *n* (%)	*P*
	Negative, *n* (%)	Positive, *n* (%)		
Yes	11 (91.7)	1 (8.3)	12 (26)	0.66*
No	27 (79.4)	7 (20.6)	34 (74)	
Total	38 (82.6)	8 (17.3)	46 (100)	

*Fisher's exact test, significant ≤0.05. IAT=Indirect antiglobulin test

Twenty-five alloantibodies against 10 blood group systems were identified in 19 out of 23 mothers with positive IAT results. Six Anti-Fyb (24%), six anti-Jka (24%), four anti-Jkb (16%), two ant-Fya (8%), two anti-K

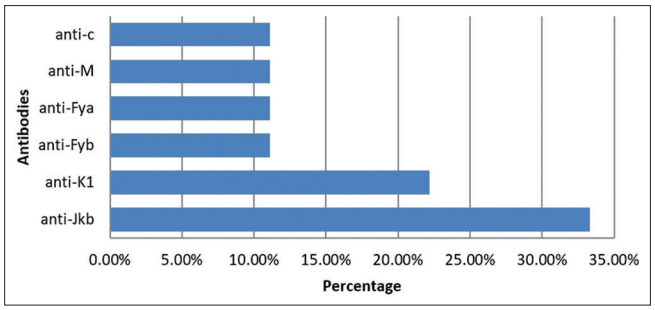

Figure 4: Identified alloantibodies and their respective percentage among patients with positive indirect antiglobulin test results admitted to medical wards

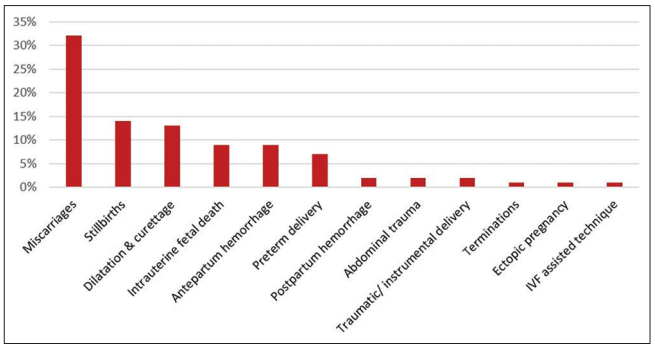

Figure 5: Adverse obstetric events among ladies of the obstetric population

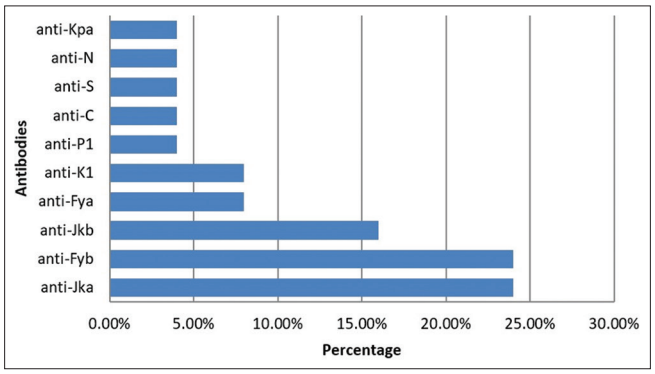

Figure 6: Identified alloantibodies and their respective percentage among ladies with positive indirect antiglobulin test results admitted to obstetric wards

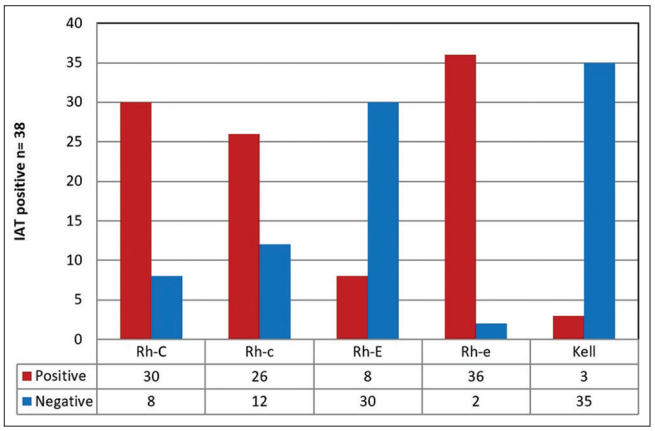

Figure 7: Rh-C, c, E, e, and kell phenotyping for samples with positive indirect antiglobulin test results of the whole study. IAT = Indirect antiglobulin test

(8%), and one (4%) for each of the anti-P1, anti-C, anti-S, anti-N and anti-Kpa1 antibodies [Figure 6].

Details of ladies with positive IAT and identified alloantibodies are illustrated in Table 7.

Phenotyping

Concerning the ABO blood group system, the most common phenotype was O 39.5% [Table 8]. For all positive samples, extended Rh and kell phenotyping was done, and most of them were kell and E-antigen negative [Figure 7].

Discussion

We found three studies done in Iraq over the last 12 years restricted to patients with hemoglobinopathies requiring multiple blood transfusions with the prevalence of RBC alloimmunization 15%, 4.5%, and 5.8%, respectively.[7-9] These records are within what is found in a review from the eastern Mediterranean region as overall frequencies of alloantibodies among patients of beta-thalassemia in the range of 2.87%–30%.[10] Our study, up to our knowledge, is the first one to assess the frequency of RBC alloantibodies among general hospital patients in Iraq.

Medical wards' population

Researchers from the general population of patients report RBC alloimmunization rates between 0.46% and 2.4%.[11] Close to this range, rates obtained from nonchronically transfused patients in Iran were 0.8%–0.92%,[12,13] India 1.4%,[14] and 2.9% among women in France.[15]

Table 3: Indirect antiglobulin test results among transfused patients admitted to the medical wards

Variable	IAT		Total, *n* (%)	*P*
	Negative, *n* (%)	Positive, *n* (%)		
Duration for the last transfusion (years)				
<1	32 (91.4)	3 (8.6)	35 (47.3)	0.48*
>1	33 (84.6)	6 (15.4)	39 (52.7)	
Transfusion reactions				
Yes	14 (77.8)	4 (22.2)	18 (25.4)	0.208*
No	51 (91.1)	5 (8.9)	56 (75.6)	
Number of units transfused				
1–4	53 (92.9)	4 (6.1)	57 (77)	0.025*
>4	12 (70.5)	5 (29.5)	17 (23)	
Total	65 (87.8)	9 (12.2)	74 (100)	

*Fisher's exact test, significant ≤0.05. IAT=Indirect antiglobulin test

In the current study, the prevalence of RBC alloimmunization among general medical ward patients was 15%. This is high compared to what is reported in the literature. Several factors might have

Table 4: Details of patients with positive indirect antiglobulin test and identified alloantibodies admitted to medical wards

Age/sex	Gravida	Admission cause	PSH	Number of blood units	Transfusion reactions	Antibody specificity	Antibody titer
65/female	6	Hemodialysis	Yes	10 in 3 years	TACO	Anti-K1, anti-Jkb	1/32
50/male	NA	Infected DLc, hemodialysis	Yes	6 in 8 months	TACO, febrile, allergic	Anti-c, anti-Fya	1/256
61/female	9	Complicated pneumonia	Yes	No	-	Anti-K1	1/8
70/female	4	Hemodialysis	Yes	No	-	Anti-M	1/16
61/female	4	Hemodialysis	Yes	Many	Allergic	Anti-Fyb, anti-Jkb	1/4
47/male	NA	Hemodialysis	Yes	4 in 5 years	No	Anti-Jkb	1/2

PSH=Past surgical history, DLc=Double lumen catheter, TACO=Transfusion-associated circulatory overload, NA=Not available

Table 5: General characteristics and their association with indirect antiglobulin test results of the obstetric ward's population

Variable	IAT		Total (%)	*P*
	Negative, *n* (%)	Positive, *n* (%)		
Maternal age (years)				
19–25	22 (91.6)	2 (8.4)	24	0.094*
26–35	42 (70)	18 (30)	60	
>35	13 (81.25)	3 (18.75)	16	
Chronic medical illness				
Positive	24 (70.6)	10 (29.4)	34	0.27*
Negative	53 (80.3)	13 (19.7)	66	
Surgical intervention				
Positive	58 (78.4)	16 (21.6)	74	0.58*
Negative	19 (73.1)	7 (26.9)	26	
Transfusion history				
Negative	51 (81)	12 (19)	63	0.22*
Positive	26 (70.3)	11 (29.7)	37	
Gravida status				
Primigravida	7 (70)	3 (30)	10	0.415*
Multigravida	70 (77.8)	20 (22.2)	90	
Maternal adverse obstetric history				
Positive	39 (71)	16 (29)	55	0.109*
Negative	38 (84.5)	7 (15.5)	45	
History of having a baby with neonatal jaundice				
Positive	16 (80)	4 (20)	20	0.54*
Negative	61 (75.2)	19 (24.8)	80	
Status at the time of screening				
Pregnancy loss	7 (50)	7 (50)	14	0.0164*
Viable fetus/baby	70 (81.4)	16 (18.6)	86	
Total	77	23	100 (100)	

*Chi-square test, significant ≤0.05. IAT=Indirect antiglobulin test

contributed to explaining this discrepancy, including but not limited to small sample size, no regular use of prestorage leukodepleted blood, and the use of in-house pooled screening cells. Iraqi population comprises a true mosaic of different ethnicities; Arabs, Kurds, Turkmens, Assyrians, and Yazidis among others.[16] This large racial mix partly might explain the high rate of alloimmunization, probably caused by the antigenic difference between blood donors and recipients.[17]

Ameen *et al*. documented that the female gender is a risk factor for alloimmunization may be due to more exposure to immunizing events through pregnancy.[18] Saverimuttu *et al*. found that the prevalence of alloimmunization increases with age.[15] However, our data did not find a significant relationship between alloimmunization and age or gender.

Similar to our results, Sood *et al*. and Yusoff *et al*. found no significant association between the number of alloimmunized cases and patients' diagnoses.[19,20] Munzer *et al*. reported that test tube contamination by some bacterial species may result in false-positive IAT reactions.[21] This is to be kept in mind as two of our male patients with positive IAT and no previous transfusion were screened while admitted for infectious diseases.

Table 6: Association of the number of units transfused, duration of last transfusion, and transfusion reaction with indirect antiglobulin test status among transfused ladies admitted to the obstetric wards

Variable	IAT		Total, *n* (%)	*P*
	Negative, *n* (%)	Positive, *n* (%)		
Number of units transfused				
1–4	22 (71)	9 (29)	31 (83.7)	0.59*
>4	4 (66.6)	2 (33.4)	6 (16.2)	
Duration for last transfusion history (years)				
<1	20 (76.9)	6 (23.1)	26 (70.3)	0.166*
>1	6 (54.5)	5 (45.5)	11 (29.7)	
Transfusion reactions				
Yes	20 (71.5)	8 (28.5)	28 (75.5)	0.54*
No	6 (66.6)	3 (33.4)	9 (24.5)	
Total	26 (70.3)	11 (29.7)	37 (100)	

*Fisher's exact test, significant ≤0.05. IAT=Indirect antiglobulin test

Table 7: Details of ladies with positive indirect antiglobulin test and identified alloantibodies admitted to the obstetric wards

Age/gravida	Adverse obstetric history	Current pregnancy loss	Number of blood units	Transfusion reactions	Antibody specificity	Antibody titer
32/3			2		Anti-Jka, anti-Fya	1/4
26/4	1 stillbirth, 1 blunt trauma				Anti-P1, anti-K1	1/4
22/1		IUFD			Anti-Jka	1/2
32/9	4 missed abortions, 1 D&C		1		Anti-Fyb	1/8
28/5			5	Allergic, febrile	Anti-S, anti-Jkb	1/2
29/4	1 missed abortions		2		Anti-Jka	1/8
29/5					Anti-C	1/8
29/5			2		Anti-Jka	1/8
26/3	2 stillbirth	Abortion	1		Anti-Fyb	1/2
34/6					Anti-Jka	1/2
28/6	2 preterm delivery				Anti-Fyb	1/4
21/4			2	Allergic	Anti-Fyb	1/4
29/1	1 NNJ		1		Anti-Kpa	1/4
39/1	2nd IVF	Abortion			Anti-Jkb, anti-Fyb	1/4
29/2	2 NNJ				Anti-N, anti-Fyb, anti-Jkb	1/8
27/4	1 stillbirth, 1 NNJ				Anti-Jkb	1/16
32/4	1 APH	Stillbirth	10		Anti-Jka	1/4
38/13	3 missed abortions, 1 stillbirth, 1 NNJ, 3 D&C		1		Anti-K1	1/8
34/6	1 stillbirth				Anti-Fya	1/2

IUFD=Intrauterine fetal death, D and C=Dilation and curettage, NNJ=Neonatal jaundice, IVF=*In vitro* fertilization, APH=Antepartum hemorrhage

Table 8: ABO blood groups and their association with indirect antiglobulin test result of the whole study

ABO blood groups	IAT		Total, *n* (%)	*P*
	Negative, *n* (%)	Positive, *n* (%)		
O	68 (86.1)	11 (13.9)	79 (39.5)	0.459*
B	43 (78.2)	12 (21.8)	55 (27.5)	
A	37 (75.6)	12 (24.4)	49 (24.5)	
AB	14 (82.3)	3 (17.7)	17 (8.5)	
Total	162	38	200	

*Chi-square test, significant ≤0.05. IAT=Indirect antiglobulin test

Again, no association was demonstrated between alloimmunization and history of surgical intervention in the current study. There were two splenectomized male patients, both with negative IAT results, similar to what is seen in repeatedly transfused patients in India[22] and multitransfused thalassemia patients in Sulaymaniyah.[9] Ghorbani *et al*. and Reyhaneh *et al*. found a statistically significant association with previous surgeries in Iran, probably due to surgery-related transfusion.[13,23]

Similar to what we had observed, Yusoff *et al*. showed no significant association between alloimmunization and history of blood transfusion.[20] Furthermore, Suresh *et al*. found no correlation with transfusion history in pregnant

ladies.[24] In agreement with our results, Makroo *et al*. reported that a higher number of transfusions were a statistically significant factor affecting alloimmunization rates, with ABO blood groups, did not show significant differences.[25]

Al-Mousawi *et al*. found that Rh-D status and history of previous transfusion reactions showed a statistically significant risk of alloimmunization,[8] in contrast to what is observed in our study.

All transfused patients in the current study had received unfiltered blood, and we did not know exactly whether the units were leukodepleted, as this procedure was introduced to our national blood center at the end of 2012. None of our screened patients had reported a presumed or documented hemolysis after being transfused. One of our IAT-positive patients was transfused last month and had her DAT positive to both IgG and C3d (mixed-field) without evidence of anemia or hemolysis. This might indicate alloantibody reacting on antigens of recently transfused donor red cells.[26]

We did not find a statistically significant association between the history of pregnancy or parity status and the IAT result. This is the opposite of what is found by Reyhaneh *et al*. in Iran,[23] Yusoff *et al*. in Malaysia,[20] and Makroo *et al*. in India[25] as the rate is higher with increasing pregnancies.

Among 15 patients with positive IAT in the general medical ward population, nine alloantibodies were identified in six patients, half of them encompass multiple antibodies. Anti-Jkb was the most frequently encountered (33.3%), followed by anti-K and Duffy group antibodies (anti-Fya and anti-Fyb) (22.2%) for each [Figure 4 and Table 4]. These findings come in contrast to those reported in transfusion-dependent thalassemia patients in Baghdad,[7] Kurdistan of Iraq,[8,9] Tehran,[27] and Egypt[17] where antibodies against the Rh and kell antigens were the most frequent. The same is noticed in general patient population studies in India[14,25] and Kuwait.[18] Factors contributing to the higher prevalence of some alloantibodies over others are the higher prevalence of the related antigens in the population and higher antigenicity power.[13]

Obstetric population

The overall prevalence of erythrocyte alloantibodies in pregnant females reported in various studies ranges from 0.89% to 5.98%.[24,28] Although the risks of adverse pregnancy outcomes associated with anti-D antibodies are well recognized, much less is known concerning alloimmunization with other RBC antibodies detected during routine maternal screening.

One of the main strengths of our study lies in the fact that it evaluated the non-D alloimmunization among the obstetric population, as their actual rate in Iraq is not well studied if we know that routine antibody screening to all antenatal women is not done. Our study includes only Rh-D-positive ladies in the obstetric sample, as Rh-D-negative mothers in our country are routinely checked and given Rh-D immunoglobulin once indicated.

Gündüz *et al*. found the incidence of non-D antibodies to be 1.21% in antenatal women in Turkey.[29] An even lower rate for non-D Rh alloimmunization of 0.33% was noticed by Healsmith *et al*. among Australian pregnant ladies.[30] In our study, IAT was positive in 23% of Rh-D-positive ladies, which is much higher than what is reported in literature worldwide and as most of them were in the low titers so may be overrepresented given the tertiary nature of Baghdad Teaching Hospital.

Among antenatal women in India, Dholakiya *et al*. and Naik *et al*. demonstrated a significant correlation between the adverse obstetric history, gravida status, and history of newborns with neonatal jaundice with the rate of alloimmunization,[31,32] whereas Sidhu *et al*. did not find a significant association with bad obstetric history,[22] supporting our results. Healsmith *et al*. found that non-D Rh alloimmunization can lead to significant fetal/neonatal morbidity and may lead to mortality.[30]

In our study, however, as we have no registry data to identify which baby was hydropic and which one with hyperbilirubinemia was actually due to HDFN, our results found clinically but not statistically significant association of poor obstetric events with RBC alloantibodies [Tables 5 and 7]. In our study, the only significant association with alloimmunization in the obstetric population was pregnancy loss at the time of screening ($P = 0.0164$).

In the obstetric sample of the current study, 23 ladies were found to have positive antibody screening, and 19 of them were found to possess single or multiple antibodies determined. Twenty-five alloantibodies were identified; the most common ones were anti-Fyb and anti-Jka (24%) for each, followed by anti-Jkb (16%) [Figure 6 and Table 7]. Only one mother had clinically insignificant antibody (anti-N), but this was not neglected as it coexisted with anti-Fyb and anti-Jkb antibodies.

The opposite of our results, alloimmunization due to Rh system antigen was the most commonly reported antibody among the obstetric population worldwide; however, the spectrum of antibodies was variable among countries. For example, anti-E antibodies were the most common among pregnant ladies in Australia[33] and

Michigan, followed by anti-K.[34] Again in India, the least common alloantibodies among antenatal women in India were those of the Duffy blood group.[31]

Indirect antiglobulin test positive, identification negative

Ahmed *et al*. revealed that alloantibody types can be specified in 75% of thalassemia patients with positive alloantibody screen.[7] A study in India among those with multiple blood transfusions[19] and another one for women in France[15] showed that RBC alloantibodies were identified in about two-thirds of cases with positive antibody screen. Similarly, we could not identify types of antibodies in 34.2% of patients with positive IAT screens in the two populations. Explanations for this might either be due to the presence of antibodies against a low-incidence antigen, an antigen not typed in the identification cell panel,[14] false-positive IAT screening results, or storage effect and time laps between sampling and identification process, due to technical issues in our national blood bank.

Conclusion

This study showed a high prevalence of alloimmunization (15%), among nonregularly transfused patients in general medicine and (23%) of Rh-D-positive ladies in obstetric wards in comparison to what is published worldwide. Most detected alloantibodies were clinically significant, against Kidd and Duffy antigen groups. Transfusion of more than four units of blood and pregnancy loss at the time of screening were significant risks for alloimmunization in the medical and obstetric populations, respectively. In the two study populations, there was no statistically significant correlation between alloimmunization with the patient's age, ABO blood groups, transfusion reactions, chronic medical condition, previous surgery, or poor obstetric history.

Recommendations

- Real and more representative data must be obtained from broader studies among different Iraqi cities
- It is necessary to formulate a strict transfusion policy to minimize unnecessary liberal transfusion practices, transfuse all repeatedly transfused patients and females of reproductive ages with Rh and kell-matched blood products, in addition to doing double ABO/Rh-D checks
- It is also important to review local protocols to screen and identify RBC alloantibodies, expand the technical expertise with antibody identification panels available among different centers, and spread orientation to follow and manage such patients from various medical specialties in collaboration with experts in transfusion medicine.

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Conflicts of interest

There are no conflicts of interest.

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