Frequency of Red Blood Cell Alloantibody in Kuwaiti Population

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Key Words

Red blood cells · Alloimmunization, frequency · Arabs · Kuwait

Abstract

Objective: The aim of this study was to analyze the alloimmunization against red blood cells (RBC) among Kuwaiti population. **Materials and Methods:** Retrospective analysis of RBC alloimmunization among 179,045 Kuwaiti patients, pregnant women, and allogeneic blood donors was conducted for the period of 1992–2001. The frequency of 24 alloantibody specificities from 1,278 alloimmunized Kuwaitis was estimated. **Results:** The prevalence of positive antibody screen in Kuwait was 0.49%. The five most frequently identified alloantibodies were anti-D (27.3%), anti-E (18.5%), anti-K (15.6%), anti-Le^a (8.7%), and anti-Le^b (6.6%). Frequency of alloimmunization was greater among Kuwaiti female than male patients (3.2 F to 1 M). Anti-D ($p < 0.001$), anti-E ($p = 0.05$), anti-K ($p = 0.04$) were also more frequently identified alloantibody among Kuwaiti females. **Conclusion:** The results show higher predominance of RBC alloimmunization in females than males. Anti-D, anti-E, and anti-K were the most common clinically significant alloantibodies identified in the Kuwaiti population and were also more frequent among Kuwaiti females.

Introduction

The incidence of red blood cell (RBC) alloimmunization depends on the demography of the population being studied. Previous studies of the frequency of RBC alloimmunization have focused on Western societies [1–4]. Reports on the frequency of RBC alloimmunized patients or allogeneic blood donors are limited in populations from the Arabian Gulf region.

The state of Kuwait is located at the northeast extremity of the Arabian Peninsula with a total population of 2.2 million in 2001. Kuwaitis comprised 38% of all residents, consisting of 18.8% males and 19.3% females. Non-Kuwaitis comprised the remaining 62% with higher predominance of males (42%) compared to females (20%) [5]. The main reason for the large percentage of non-nationals in the country is due to immigrant workers from many different countries [6], some of whom are accompanied by their family.

The Kuwaiti national population is genetically homogeneous because of the high rate of consanguinity [7]. Hence the Kuwaiti population is ideal for studying RBC alloimmunization. Virtually, all RBC alloantibody identifications are performed at the Kuwait Central Blood Bank, as it is the only referral center and local supplier of blood and blood components in Kuwait. Equally, a centralized system of RBC alloantibody records is available,

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and thus provides an opportunity to evaluate the frequency of alloimmunization to RBC antigens.

This retrospective study sets out to evaluate the frequency of alloimmunization to RBC antigens in the Kuwaiti population.

Subjects and Methods

Subjects and Samples

The data set included 179,045 Kuwaiti patients, pregnant women, and allogeneic blood donors who underwent antibody screening between 1992 and 2001. Each alloimmunized subject record was unique, and no subject was entered twice. Although some patients or pregnant women might have multiple antibody investigations only one was entered into the database. ABO, Rh typing, and gender were available for alloimmunized Kuwaitis. The alloimmunized subjects were divided into the following subgroups: patients, pregnant women, and allogeneic blood donors.

Serology Test

Antibody screening was performed as a routine immunohematologic investigation in support of the transfusion service. A standard two-cell panel (Immucor, Calif., USA, and Ortho, N.J., USA) was used to screen for RBC alloantibodies using tube test method. A low ionic strength solution was used for indirect antiglobulin method (Gamma Biological Inc., Houston, Tex., USA). A mixture of two parts serum, one part RBCs, and two parts low ionic strength solution was incubated for 15 min at 37°C. The cells were then washed 3 times in phosphate-buffered saline (Inverclyde Biological, Scotland, UK), and polyspecific anti-human globulin serum (Gamma Biological Inc, Houston, Tex., USA) was then added before spinning and final reading. Any pretransfusion sera that had a positive antibody screen were investigated to identify the specificity of the alloantibody. Antibody specificity was determined by using a commercial RBC panel tested against the patient's serum in the same phases as antibody screening, including enzyme (ficin) treatment (Ortho, N.J., USA, and Gamma Biologicals Inc., Houston, Tex., USA). The criteria for antibody specificity were based on American Association of Blood Banks recommendations [8]. In case of difficulty in identifying RBC alloantibody locally, the patient samples were referred to the International Blood Group Reference Laboratory (IBGRL) at Bristol, United Kingdom, for further investigations. Nine such samples were referred to IBGRL for serologic investigations.

Statistical Analysis

The frequency of each alloantibody was assessed based on the study population, as well as gender. Fisher's exact test was performed to determine the difference in antibody distribution by gender; p value ≤ 0.05 was considered significant. The analyses were performed using SPSS software version 10.5.

Results

The 179,045 subjects were divided as follows: 5,916 patients (without inclusion of pregnant women), 2,932 pregnant women, and 170,197 allogeneic blood donors. Of the screened subjects, 1,278 (0.7%) had alloantibodies with clinical significance: 836 (65.4%) patients, 412 (32.2%) pregnant women, and 30 (2.3%) donors (table 1). The prevalence of alloimmunization among Kuwaiti blood recipients and donors (without the inclusion of pregnant women) was 0.49% (210 male, 1,068 female). Among the patient group there were 201 men and 635 women, while among the donor group there were 9 men and 21 women.

Twenty-four of the RBC alloantibody specificities were identified (table 1). In general, the five most frequently identified alloantibodies were anti-D (27.3%), anti-E (18.5%), anti-K (15.6%), anti-Le^a (8.7%), and anti-Le^b (6.6%).

A total of 72.6% of the study population had one alloantibody, 22.2% two alloantibodies, 3% three alloantibodies, 2.1% had between four and six alloantibodies.

The frequency of developing of RBC alloantibody was higher among Kuwaiti females than males in patients and allogeneic blood donors. Antibodies to D, E and K were more frequent among females with positive antibody screen. Anti-D was identified in the sera of 177 (90.3%) alloimmunized women compared to only 19 (9.7%) men who formed anti-D. Anti-E was identified in the sera of 123 (75%) alloimmunized women compared to only 41 (25%) males. Anti-K was present in the sera of 109 (70.3%) females, compared to 46 (29.6%) males (tables 1, 2). However, the remaining clinically insignificant alloantibodies such as anti-Le^a and anti-Le^b showed no gender difference (data not shown).

Alloantibodies against high frequency antigens (HFA) were identified in 6 subjects of whom 2 had anti-Ge², 1 had anti-Lan, and 3 unidentified antibody against HFA.

Discussion

The incidence of alloimmunization against RBC antigen depends on the demography of the population being studied. Characteristics such as race and sex of the individual and the population being studied (patients, obstetric patient, and allogeneic blood donor) make it difficult to relate the experience of one study to another. Most of the previously described data [2, 3, 9,10] are related to Western society, and/or a particular transfusion indica-

Table 1. Total RBC alloantibody identified frequency in Kuwaitis from 1992 to 2001

Antigen system or collection	Antigen	RBC alloantibodies identified			Total of all subjects with alloantibody (n = 1,278)
		patients (n = 836, 65.4%)	pregnant women (n = 412, 32.2%)	donors (n = 30, 2.3%)	
Rh	D	188 (22.5%)	153 (37%)	8 (26.7%)	349 (27.3%)
	C	38(4.3%)	20 (4.9%)	–	58 (4.5%)
	c	33 (4%)	26 (6.3%)	2 (6.7%)	61 (4.8%)
	E	159 (19%)	72 (17.5%)	5 (16.7%)	236 (18.5%)
	e	9 (1.1%)	5 (1.2%)	–	14 (1.1%)
	C ^w	5 (0.6%)	3 (0.7%)	–	8 (0.6%)
	V	15 (1.8%)	4 (1%)	–	19 (1.5%)
Kell	K	154 (18.3%)	45 (11%)	1 (3.3%)	200 (15.6%)
	Kp ^a	4 (0.5%)	–	–	4 (0.3%)
	Js ^a	4 (0.5%)	–	–	4 (0.3%)
Duffy	Fy ^a	21 (2.5%)	8 (2.0%)	–	29 (2.3%)
	Fy ^b	4 (0.5%)	1 (0.2%)	–	5 (0.4%)
Kidd	Jk ^a	18 (2.1%)	7 (1.7%)	–	25 (2.0%)
	Jk ^b	9 (1.1%)	5 (1.2%)	–	14 (1.1%)
Lewis	Le ^a	62 (7.4%)	46 (11.2%)	3 (10%)	111 (8.7%)
	Le ^b	49 (5.8%)	31 (7.6%)	4 (13 %)	84 (6.6%)
P	P1	35 (4.2%)	21 (5.1%)	3 (10%)	59 (4.6%)
Luthern	Lu ^a	3 (0.4%)	1 (0.2%)	–	4 (0.3%)
	Lu ^b	2 (0.2%)	–	–	2 (0.2%)
MNSs	M	13 (1.5%)	4 (1.0%)	–	17 (1.3%)
	N	3 (0.4%)	1 (0.2%)	–	4 (0.3%)
	S	7 (0.8%)	6 (1.5%)	3 (10%)	14 (1.1%)
	s	–	3 (0.7%)	–	3 (0.2%)
HFA	HFA	2 (0.2%)	–	1 (3.3%)	3 (0.2%)

Total number of alloimmunized patients 1,278, total number of alloantibodies 1,329. Antibodies against high frequency antigens (HFA) identified were anti-Lan and anti-Ge².

Table 2. Sex distribution of the three most common clinically significant alloantibodies among Kuwaiti patients and donors

Alloantibody	Male	Female	p value
D	19 (9.7%)	177 (90.3%)	<0.001*
E	41 (25%)	123 (75%)	0.05*
K	46 (29.6%)	109 (70.3%)	0.04*
Total	106	409	

tion such as sickle cell disease [11]. However, little information is available regarding the alloimmunization among Arabs from the Arabian Gulf region. Furthermore, most of the previously mentioned studies did not include antibodies that react at room temperature (RT) because they are considered clinically insignificant. However, these RT reactive antibodies do interfere with the

pretransfusion laboratory investigations, and their presence should be considered in determining the incidence of positive antibody screening within a population [4]. Alloantibodies that react at RT were included in the current study.

The overall incidence of alloimmunization among transfusion recipients and blood donors (without the inclusion of pregnant women) was 0.49% in this study, which was lower than previously reported, 0.8–1.6% among transfusion recipients and 0.32–0.89% among donors [3, 4, 12].

In this study, the incidence of alloimmunization among female patients and donors was more than 3 times higher than among male subjects. Several reports have shown that the male:female ratio ranges from 1:2 to 1:2.7 [3, 4, 10, 12, 13]. Several possible reasons may account for the high female predominance observed in our study. First the fertility rate in Kuwait is higher than in Western societies; the estimated fertility rate among Kuwaiti women

is 4.2 [14]. Therefore, immunization through pregnancy [10, 12] could be the main reason for the greater incidence of RBC alloimmunization among Kuwaiti females. Second, it is known that postpartum administration of anti-D immunoglobulin is expected to significantly lower the percentage of developing anti-D among Rh-D-negative pregnant women [15]. However, administration of anti-D immunoglobulin may not prevent immunization if the quantity of D-positive fetal red cells in the mother's circulation exceeds the immunosuppressive capacity of anti-D immunoglobulin. Historically, Kleihauer acid elution test, which detects fetal hemoglobin (HbF), has been used to estimate the volume of fetal red cells that have entered the mother's circulation. Unfortunately, in Kuwait the majority of the hospitals do not routinely use the Kleihauer test or any other alternative technique to quantify the amount of fetomaternal hemorrhage (FMH). Most of women usually receive only a single postnatal dose (1,500 IU or 300 µg) of anti-D immunoglobulin without prior assessment of FMH. Furthermore, anti-D immunoglobulin prophylaxis is not usually administered as a routine antenatal prophylaxis to Rh-D-negative women, that in addition to the lack of proper intervention in some of the cases following potential sensitization events such as abortion, ectopic pregnancy, transfusion of blood products. That could have particularly influenced the incidence of developing anti-D observed in all of the subgroups in the current study. Most of the previously published reports in Western societies have shown a decline in the frequency of developing anti-D combined with the increase of the prevalence of anti-K and anti-E since the introduction of anti-D immunoglobulin [1, 3, 4, 9, 10, 16]. The differences observed in this study compared to other published reports further support the importance of adequate assessment of the amount FMH and therefore adequate dosage of anti-D immunoglobulin. National policy to screen the sera of Kuwaiti pregnant women for AB0, Rh, and antibody screen is currently underway. The main objectives of this policy are to identify pregnancy cases at risk of developing hemolytic disease of the newborn, and more importantly to identify Rh-D-negative women who need anti-D immunoglobulin prophylaxis.

This study also showed that anti-E was the second most common specificity among Kuwaiti females, followed by anti-K. Currently no policy exists that fertile women should receive AB0, Rh-compatible, and Kell-negative blood at the government or private hospitals. This could explain the obvious sex difference associated with anti-E and anti-K.

Most of the previously mentioned studies that described the high female predominance have included pregnant women (with the exception of Blumberg et al. [13]), while those studies that included transfused patients only found no difference between the genders. In our study, the alloimmunization rate among female patients was more than 3 times higher than male (3.2:1). This further suggests the great incidence of alloimmunization among Kuwaiti females compared to males. Further, RBC phenotypic study is currently performed on Kuwaiti blood donors in order to identify the RBC antigenic profile among the Kuwait population.

Conclusion

The results show higher predominance of RBC alloimmunization in females than males. Anti-D, anti-E, and anti-K were the most common clinically significant alloantibody identified in the Kuwaiti population and were also more frequent among Kuwaiti females.

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