

Original Article

Red Blood Cell Alloimmunization among Saudi Pregnant Women in the Central Province of Saudi Arabia

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ABSTRACT

Objectives: To determine the incidence of alloimmunization among pregnant women in Saudi Arabia

Design: Prospective study

Setting: King Khaled University, Hospital, Riyadh, Saudi Arabia

Subjects: One thousand one hundred and ninety five pregnant women

Main Outcome Measures: The rates of alloimmunization among pregnant women subjects by analyzing the blood type of both mother and neonate

Results: The largest fraction of alloimmunization involved Rh antigens (52.38%), while other groups such as Kell & Duffy play a less common role. Alloantibodies identified five types of alloantibodies in addition to nonspecific-autoantibodies. The most frequent (52.38%) were against Rhesus 2.38%; Kell 2.38%; Duffy 2.38%; 4.76% were non-

specific antibodies and 33.3% were autoantibodies.

Alloimmunization are: anti-D 28.57%, anti-C 4.76% anti-E 14.28% and anti-e 4.76%; only one 2.38% developed anti-K; anti-Jk, one 2.38%; one had anti-Le 2.38%; there was one 2.38% with anti-Fy. 1.84% of the total number of study subjects were alloimmunized by antigens of Rh while 0.08% were alloimmunized to antigens either from Kell, Kidd, Lewis or Duffy.

Conclusions: The relative importance of antigens other than Rh D have increased since the introduction of Rh D prophylactic treatment. Alloimmunization to E, c and Kell antigens can reach significant proportions of studied populations and can result in deleterious effects on fetus. The actual risk of alloantibody production during pregnancy is unknown but stimuli for antibody production are feto-maternal bleeds that occur throughout pregnancy.

KEY WORDS: alloimmunization, ABO, autoimmune hemolysis, erythroblastosis fetalis, IgG, IgM, Rh

INTRODUCTION

The overall incidence of hemolytic disease of the newborn (HDN) varies in different places ranging from as low as 7.2/10,000 births to as high as 14.4/10,000 births. Despite prophylactic use of rhesus immune globulin, anti-D is still a common antibody identified in 20% of pregnant women^[1]. The incidence of anti-D alloimmunization in the weak D (Du) population is closer to that of the D-negative than the D-positive population because few Du patients are of the 'partial' D or Du type.

There are more than 43 other Rh red blood cell antigens which have been implicated in hemolytic disease of the newborn. Anti-C and anti-D are the most frequent antibodies identified in gravid women. Seventy-four percent of infants born to C-alloimmunized women mated to C-positive men show serologic evidence of HDN^[2]. Pregnancies where the maternal anti-C titer remains at or below 1:16 proceed to final term. Fifty percent of alloimmunized pregnant women need blood

transfusion. Anti-C, Ce alloimmunization is relatively rare. A study in Manitoba, Canada spanning a period of 37 years recorded 11 cases of anti-Cw, and in a review of 131,898 Rhesus positive pregnant women screened for irregular antibodies, the overall incidence was 1:330. Thirty infants in the series required exchange transfusions for HDN^[3].

Mothers subjected to unnecessary blood transfusions can produce alloantibodies such as anti-E and anti-Jkb and develop delayed transfusion reactions. Anti-E is found frequently in pregnancy^[4].

Majority of Kell sensitization cases are secondary to incompatible red blood cell transfusions since blood is not routinely cross-matched for the Kell antigen. Anti-Kell antibodies are encountered in reproductive women about 60% as often as Rho antibodies but the risk of disease is less than 5% when such antibodies are detected. Anti-k alloimmunization is very rare^[5].

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Lewis antibodies are the commonest single cause of positive results in pre-transfusion antibody screening tests. However, the strength of Lewis antibodies is clinically important: Lewis antigens in the plasma of incompatible blood will completely neutralize antibody of low titer. In addition, Lewis antibodies are mostly IgM and red blood cells of newborn infants react only weakly or not at all with Lewis antibodies^[6].

Young-Owens and co-workers in 1997^[7] showed that the prevalence of anti-M isoimmunization may be increasing; the incidence of severe HDN due to anti-M is extremely low and the obstetric concern for the presence of detectable anti-M is only reserved for rising titers. Both anti-M and anti-N react significantly only when the target antigen is present in double dose, *i.e.*, MM or NN.

Antibodies to S, s and U occur following RBC stimulation and are capable of hemolytic transfusion reactions and HDN. Anti-S occurs as infrequently as anti-N while anti-s is rare. Anti-U hemolytic reaction is a diagnosis of exclusion.

Lutheran system antibodies are very rare and can occur in the absence of RBC stimulation. However, they are poorly developed at birth and have not been reported as a cause of HDN. Anti-Fya and anti-Fyb cause HDN and HTRs and are common in endemic areas where natural selection of persons with the Fya-b- phenotype is favored due to resistance to *Plasmodium vivax* infection^[8].

Anti-Jka and anti-Jkb are rare and may cause delayed HTR and HDN. Anti-Yta and anti-Ytb are extremely rare and are of no clinical significance. Pregnant women with antibodies to high incidence blood group antigens should be diagnosed as early as possible. An indirect Coombs test should be obtained in all pregnant patients at their first prenatal visit, after which titers are made if found positive.

There are several *in vitro* tests that attempt to mimic fetal conditions that produce red blood cell hemolysis such as the ADCC assay, the monocyte monolayer assay and the monocyte chemiluminescence test all of which try to predict the need for intrauterine fetal blood transfusion. Percutaneous umbilical blood sampling plays a major role in treating hemolytic disease of the fetus, and is an alternative to serial spectrophotometric measurements of fetal blood specimens to identify fetuses at high risk of having antenatal anemia^[9].

MATERIAL AND METHODS

The study involved 1195 consecutive samples from pregnant women consulting at King Khaled University Hospital, Riyadh over a period of

two years. Data were acquired by standardized methodology using forms that were completed during antenatal care. Demographic data were collected from patient files, and from our knowledge that no data was available for the incidence of HDN in this hospital. Blood samples were collected from pregnant women by trained nurses and submitted to the blood bank. All samples were centrifuged 3000 x g for five minutes and separated. Samples were either done at the same time or frozen at -2 °C and assayed later.

ABO blood grouping was done using Diaclon typing cards (DiaMed-ID, Cressier/Switzerland). All blood group tests were confirmed with the reverse serum test with known test red cells. Negative controls were included in all tests. ABO blood group tests were performed only at room temperature. Reverse group cells were supplied by Gamma Biologicals. Rh grouping was done using Diaclon anti-D monoclonal (Diamed Cressier/Switzerland). Rhesus controls were applied in all tests. When applicable, the weak D (Du) procedure was performed by adding Diaclon Rhesus control to patients' red cell suspension and incubating it for 15 min at 37 °C. After washing with isotonic saline Diamed AHG was added and immediately spun. The cells were re-suspended gently and observed macroscopically for agglutination. Negative reactions were confirmed with Diamed Coombs control cells.

Antibody screening was done using the Diamed ID Micro typing system-antibody (Cressier/Switzerland) and screened by the ID Card combined with Coomb's and enzyme test which offers complete antibody screening for one donor in an easy single step using ID Dia Cell I+II+III test cell reagents for the indirect antiglobulin test procedure and ID DiaCell I+II+III papainized test cell reagent for the enzyme technique. Human group O red cells from single donors in a buffered suspension medium at 0.8% were used during testing.

Test cell reagents were allowed to reach room temperature before use. 50ul of ID Dia Cell I+II+III and ID DiaCell I+II+IIIP were pipetted to the appropriate microtubes (1-3 and 4-6) respectively. Serum (25 ul) was added to each microtube. The cards were incubated for 15 min. at 37 °C in the ID incubator. The ID cards were centrifuged for 10 minutes in the ID centrifuge.

The interpretation for all tests done in glass tubes was performed using grades of agglutination ranging from + to +++++, whereby the result is recorded as positive, or negative when any sign of agglutination is absent. For all card tests, a positive result means agglutinated cells form a red line on the surface of the gel in the microtube or agglutinates dispersed the gel. A negative result means the presence of a compact button of cells on the bottom

Table 1: Distribution of blood groups among Saudi pregnant women in the study

	O	A	B	AB
Rh +	524	284	177	41
Rh + %	43.9	23.8	14.8	3.4
Rh -	70	44	44	11
Rh - %	5.9	3.7	3.7	0.9
Total	594	328	221	52
Total %	49.7	27.4	18.5	4.4

of the microtube.

Antibody titration was obtained by testing serial dilutions of a serum against selected cells from Diamed. The results were expressed as the reciprocal of the highest serum dilution that causes macroscopically apparent agglutination. Titration scores can provide information about the amount of antibody in a serum or the amount of antigen present on red cells. Titration ranged from 1:1 to 1:2048, when applicable, using the patients' serum and test cells which were incubated at 37 °C for one hour after which AHG method was done when required. The end point is reported as the reciprocal of the test tube that last shows macroscopic agglutination. Titration was performed in the following situations:

1. Prenatal studies: the mother's serum is tested at intervals during pregnancy.

2. Antibody identification: some antibodies cause universal agglutination when undiluted serum is used but comparing titration results may indicate specificity.

The ABO and Rhesus blood groups were determined, screened for alloantibodies after which, the type of alloantibodies present were determined. Alloimmunized patients' files were reviewed for the history of blood transfusion, medical history, number of pregnancies, husband's blood group, presence of alloantibodies among their children, whether the latter were given treatments such as blood transfusion and exposure to ultraviolet light and if they were diagnosed to have hemolytic disease during the neonatal period.

STATISTICAL METHODS:

All analysis was performed using the Instat (Instat Biostatistics, Graphpad Package USA). Normal distributed data were analyzed using student t-test and values calculated. Mann-Whitney test was also used. The two-sided p-value was applied and a value less than 0.05 was considered significant

RESULTS

The results of blood grouping are shown in Table 1. Type O had the highest prevalence at 594 (49.7%) out of the total, with 524 Rh (D) positive (43.9%)

Table 2: Distribution of red blood cell antibodies in alloimmunized pregnant subjects

Antigen System	Antibody positive cases	Percentage	Total percentage
Rh			
D	12	28.57	
C	2	4.76	
Cw			
C			52.38
E	6	14.28	
E	2	4.76	
Kell			
K	1	2.38	2.38
K	0		
Kidd			
Jka	1	2.38	2.38
Jkb	0		
Lewis			
Lea	1	2.38	2.38
Leb	0		
Duffy			
Fya	0		
Fyb	1	2.38	2.38
MNS			0
Lutheran a & b	0	0	0
Non-specific	2	4.76	4.76
Autoantibodies	14	33.33	33.33
Total	42	100	

and 70 subjects Rh (D) negative (5.9%). Blood type A was seen in only 328 (27.5%) among whom Rh (D) positive were 284 (23.8%) and Rh (D) negative were only 44 (3.7%). Blood type B subjects numbered 221 (18.5%), among whom Rh (D) positive were 177 (14.8%) and Rh(D) negative 44 (3.7%). Blood type AB subjects numbered 52 (4.4%), among whom Rh(D) positive (3.4%) and Rh(D) negative 11 (0.9%). Generally, Rh (D) positivity was present in 1026 (85.9%) of subjects against 169 (14.1%) Rh negative.

Screening for alloantibodies identified five types of alloantibodies in addition to non-specific and autoantibodies. Table 2 shows specificities of identified antibodies in immunized pregnant women. The most frequent (52.38%) were against the Rhesus system, against the Kell system, 2.38 %; against the Duffy system 2.38%; 4.76% were non-specific antibodies and 33.3% were autoantibodies.

The frequency of clinically significant alloimmunization causing hemolytic disease or hemolytic transfusion reactions were: anti-D, 12 (28.57%), anti-C, 2 (4.76%), anti-E, 6 (14.28%) and anti-e, 2 (4.76%); only one (2.38%) developed anti-K; anti-Jk, one (2.38%); one had anti-Le (2.38%); there was one (2.38%) with anti-Fy (Table 2).

In summary, 1.84 % of the total number of study subjects were alloimmunized by antigens of the Rh system while 0.08% were alloimmunized to antigens

Table 3: Percentage of pregnant women with alloantibodies according to the antigen systems

Antigen System	Rhesus	Kell	Kidd	Lewis	Duffy
Frequency of antibodies	22	1	1	1	1
Percentage of antibodies	1.67	0.08	0.08	0.08	0.08
Percentage of alloimmunized	1.84	0.08	0.08	0.08	0.08

Table 5: The incidence of alloimmunization in Rh D positive and Rh D negative women

	O		A		B		AB	AB
	Rh+	Rh-	Rh+	Rh-	Rh+	Rh-	Rh+	Rh-
Rh								
D		5		3		3		1
C		1		1				
Cw								
C								
E	3		2					1
e	1				1			
Kell								
K	1							
K								
Kidd								
Jka	1							
Jkb								
Lewis								
Lea	1							
Leb								
Duffy								
Fya								
Fyb	1							
MNS								
Lutheran a & b								
Non-specific	1							
Auto-antibodies	10	1	2			1		
Total	19	7	4	4	2	4	1	1
	26		8		6		2	
%	45.2	16.7	9.5	9.5	4.8	9.5	2.4	2.4
	61.9		19		14.3		4.8	

either from the Kell, Kidd, Lewis or Duffy system (Table 3). The number of pregnant women negative for alloantibodies was 1171 (98%), those with only one alloantibody 22 (1.8%), while only two women had a combination of two kinds of alloantibodies (0.2%), (Table 4).

Most alloantibodies were detected in patients with blood group O - 26 (61.9%) followed by blood group A - 8 (19%), blood group B - 6 (14.3%), and blood group AB - 2 (4.8%). Anti-D antibodies were present in five women with blood group O, Rh negative; in three women with group A, Rh negative; in three women with group B Rh negative, and one with group AB, Rh negative blood. Anti-C antibodies were present in one woman who was group O, Rh negative and in one woman with group A, Rh negative blood. Anti-E antibodies were present in three women with group O Rh positive blood, and also in two women

Table 4: Number of pregnant women in relation to alloantibodies

No. of pregnant women	Percentage	No. of alloantibodies developed
1171	98	0
22	1.8	1
2	0.2	2

with group A Rh positive blood. Only one AB, Rh positive woman showed anti-E antibodies. Anti-antibodies were present in one woman with group O, Rh positive blood and in one woman with group B, Rh positive blood. There was only one incident of anti-K antibodies in one patient with group O, Rh positive blood. There was one incident each of anti-Jk anti-Le and, anti-Fy, all of whom had group O, Rh positive blood. Non-specific antibodies were detected in one patient with group O, Rh positive blood and in one for group B, Rh positive blood. Lastly, autoantibodies were present in 10 women with group O, Rh positive blood, in one with group O, Rh negative blood, in two with group A, Rh positive blood and in one with group B, Rh negative blood (Table 5). 1.8% were blood group A, four (2.4%) were blood group B and one (0.6%) had blood group AB blood.

The files of women in whom alloimmunization was detected were reviewed for history of previous pregnancies, transfusions, abortions, dilatation and curettage procedures or cesarean section (Table 6). Demographically old, most of the subjects' age ranged from 24 to 43 years. Most of the detected alloantibodies were of low titer. Ten of alloantibodies identified in the alloimmunized pregnant women were weak in titer- 4 with titer 2, 2 with titer 4, 2 with titer 8, one with titer 16, 2 with titer 32 and 2 with titer 256. Alloantibodies were present in seven live-born infants. Five of these needed treatment. One infant needed phototherapy only, while two needed a combination of ultraviolet phototherapy and blood transfusion. One needed to be admitted to the neonatal intensive care unit (NICU) while the last one received blood transfusion. In addition there were two infants who needed exchange transfusion due to hyperbilirubinemia but no antibodies were detected (Coomb's test negative).

All the alloimmunized Rh negative patients with anti-D had husbands who were Rh positive and gave children with positive Rhesus blood groups. One of the alloimmunized patients had a previous history of anti-D immunization, one had six previous pregnancies and the others had no known history of exposure to antigens. One woman with anti-D alloantibodies gave birth to seven babies with the following consequences: five needed treatment, one needed phototherapy, two

Table 6: The clinical data of pregnant women who were alloimmunization positive and their husband and baby

Clinical data of pregnant women who were alloimmunization positive							Husband		Baby			Treatment
No	Age	ABO	Rh	Ab type	Ab titer	Previous history	ABO	Rh	ABO	Rh	Ab Detection	
1	30	O	+	anti-K	1:2	Grav. 5, para 3+0	B	+	O	+	NO	NO
2	31	O	+	anti Jka	1:1	unknown	A	+	A	+	NO	NO
3	40	O	+	anti E	1:1	P6+ve	O	+	O	+	NO	bilirubin high, BT
4	30	O	-	anti D	1:16	known case Rh	B	+	B	+	YES	UV
5	42	O	-	anti D	1:4	six gravida	A	+	A	+	YES	NO
6	38	O	+	anti E	weak	two abortions	O	+	O	+	NO	NO
7	38	A	-	anti D	1:32	unknown	A	+	A	+	YES	UV & BT
8	32	B	+	anti e	weak	two gravida, hemorrhage	O	+	O	+	NO	NO
9	18	O	-	anti D	weak	unknown	B	+	B	+	NO	NO
10	43	A	+	anti E	Weak	P6+ve	O	+	O	+	NO	BT
11	41	O	-	anti D	1:32	unknown	A	+	A	+	YES	BT
12	25	O	+	anti Lea	weak	unknown	A	+	O	+	NO	NO
13	30	A	+	anti E	1:4	blood transfusion	O	+	O	+	NO	BT
14	28	O	+	anti E	1:1	blood transfusion	O	+	O	+	NO	BT
15	40	AB	-	anti D	1:8	cesarean,	A	+	A	+	YES	NICU
16	39	O	-	anti C+D	1:16	cesarean, diabetes mellitus	O	+	O	+	NO	NO
17	37	B	-	anti D	1:8	diabetes, RA	O	+	O	+	YES	NO
18	24	A	-	anti D	1:1	abortion, rubella+ve	A	+	A	+	NO	NO
19	39	AB	+	anti E	weak	5 gravida	O	+	O	+	NO	NO
20	33	O	+	anti Fyb	weak	cesarean, abortion	O	+	O	+	NO	NO
21	31	B	-	anti D	1.256	recurrent fetal loss	O	+	-	+	-	-
22	31	B	-	anti D	1.256	unknown	B	+	B	+	YES	bilirubin high, BT
23	24	A	-	anti C+D	weak	unknown	O	+	O	+	NO	NO
24	33	O	+	anti e	weak	unknown	O	+	O	+	NO	NO

Ab: antibodies, UV: ultraviolet treatment, BT: blood transfusion, RA: rheumatoid arthritis
The remaining women, not included in this table were normal

were given phototherapy and blood transfusion, one had to be admitted to NICU and the last one received blood transfusion. Two women with anti-C antibodies were shown to have concurrent anti-D antibodies (anti- C + D) but these antibodies were not detected in their respective neonates. Six cases with anti-E antibodies had titers which ranged from weak up to 4. Two of them had a previous history of G6PD deficiency, one had a history of two abortions and two had a history of blood transfusion. None of the alloimmunized patients gave birth to neonates who were positive for anti-E antibodies. Two babies were given blood exchange transfusion due to hyperbilirubinemia but no antibodies were detected. Two patients with anti-e antibodies gave weak reactions. One patient had two previous pregnancies and an episode of puerperal hemorrhage, while the others had unknown history of immunization (Table 6). Respective neonates of these mothers also gave no reaction indicative of alloimmunization.

There was one case with an anti-Kell titer of 2, who had five previous pregnancies but no antibody was detected in all of them. There was one case of anti Jk with a titer of 2 but no identifiable alloantibodies were found in the offspring. There was one case with anti-Le antibodies with weak titer; offspring

was negative for any alloantibodies. One patient with anti-Fy antibodies (with weak reaction) had a previous cesarean section, transfusion and abortion but alloantibodies were not detected in the newborn.

DISCUSSION

The study of blood groups of women in the Riyadh region indicated that the highest blood group is O (49.71%), followed by A (27.45%), B (18.49%) and AB (4.35%). The high frequency of O blood group is not unique in women in the Riyadh region as similar findings were seen in Tabuk, Madina Munawwara and Eastern Province which indicates that blood group O is not related to the Riyadh region but that it is most likely common to Saudi Arabia.

The blood groups in the Tabuk area were (53.02%) for O, (30.12%) for A, (12.04%) for B and (4.02%) for group AB while the blood groups in Madina Munawwara were group O (44.8%), group A (28.9%) group B (20.7%) and (5.5%) for group AB^[10]. Blood group distribution of women in the Eastern province showed group O, 45.3%, group A 27.3%, group B, 20.8% and 6.6% for group AB^[11]. Therefore the chance of ABO incompatibility

occurring among the offspring of mothers of the O blood group should be taken into consideration more often if the father's blood group is not O.

In contrast blood group O and B are more frequent in women of Arabic speaking populations in Turkey. Blood group A is reported to be highest (49%) in Turkish women^[12].

In the Central Province Rh positivity was 85.86% against 14.14% Rh negative, while Rh negatives in the Eastern province reached only 1.9%^[11]; Rh negative in Madina Munawwara and Tabuk was 11 and 8% respectively^[10]. This study supports the finding that the highest prevalence of Rh negative women is in the Riyadh region. Prevalence of this group in England is 17% and 14.7% in Turkey. In Sweden approximately 17% of all mothers were Rh D negative - this implies an immunization frequency of 1.1% in this population^[13].

The Rh blood group comprises more than 40 individual antigens of which five are routinely identified: DCcE and e. The first Rh antigens discovered were the D+ and the D- phenotypes. Because of the light linkage between the D and C/cE/e loci however these five antigens are inherited. Certain genotypes are more common than others although gene frequencies vary from one population to another. The frequency of Rh negativity is lower in Saudi Arabia suggesting prevalence of more African blood group markers^[14].

During birth, blood cells from the fetus can escape into the mother's bloodstream. These cells are recognized as foreign if they are a different blood type from the mother and a natural rejection process will ensue with the formation of antibodies. The process is known as red blood cell alloimmunization. This event typically occurs after the delivery of a baby at the end of pregnancy but other pregnancy related events such as abortion can result in antibody formation. The British Committee for Standards in Hematology and Blood Transfusion Task Force (Chairman P. Kelsey, 2003)^[15] reported that intrauterine transfusion in the alloimmunized women increased the incidence of additional alloantibodies.

Most (98%) of the Saudi pregnant women who participated in this study showed no antibodies, *i.e.*, they were non-immunized. Most of the immunized women (n = 22, 1.8%) developed only one type of alloantibodies, while 0.2% developed two types of antibodies.

The study shows that immunization due to antibodies belonging to the Rhesus system is about 1.84% of all examined Saudi pregnant women. The Anti-D group formed 28.57% of the alloimmunized cases. Despite the use of rhesus immunoglobulin anti-D is still a common antibody identified. Monchamont *et al*^[5] reported that the percentages

of HDN with anti-D alloimmunization was 98.4, 93.5 and 68.1% respectively. Holtzman *et al*^[11] demonstrated that anti-D accounts for less than 20% of all identified red blood cell antibodies. Vietor *et al*^[16] found that women with anti-D were generally considered to have high responses in producing red blood cell antibodies outside the Rh system. Ten percent of women with pre-existing anti-D had more than one alloantibody other than Rh system antibodies. Mayne *et al*^[17] reported that the gene for the partial D or Du is Ce-linked in most families and only rarely with cE or ce.

Anti-C antibody was found in about 4.76% of the immunized patients. Baker *et al*^[18] reported one case on a group A Rh positive, C negative woman in whom anti-C was developed as a result of blood transfusion in childhood.

Hardy and Napier^[19] in their review of red blood cell antibodies among Rh positive women in South and Mid-Wales over a 30 - year period, described two infants with hemolytic disease caused by anti-C who died, one after an exchange transfusion and one without undergoing exchange transfusion.

Bowman *et al*^[20] mentioned that the prevalence of anti-C and anti-Ce alloimmunization ranged from 8.7 to 185 / 100,000 pregnancies. The incidence of affected babies requiring treatment compared with the total number of anti-C and anti-Ce alloimmunized pregnancies ranged from 2.6 to 22.2%.

No anti-Cw or anti-c was detected in the current study. Anti-Cw is a rare antigen occurring in about 2% of the white population. The specific antibody, anti-Cw either occurs together with anti-C or more rarely alone like most Rh antibodies. Anti-Cw can cause hemolytic transfusion reactions and HDN. But anti-c is one of many atypical antibodies that can be produced by Rhesus D positive patients during pregnancy. Maternal anti-c alloimmunization appears to be a rare cause of fetal death. However multiple transfusions on different occasions or transfusion plus pregnancy may contribute to the development of clinically apparent c-hemolytic disease. The great proportion of previously transfused mothers may partly account for the greater number of cases of severe c-hemolytic disease. Furthermore, the titer of maternal antibody correlated poorly with severity of hemolytic disease except that very low titers were not associated with either moderate or severe disease according to Denomme *et al*^[21].

In this study, the anti-E and anti-e were found to be 14.28 and 4.76% respectively among Saudi immunized pregnant women. Vietor *et al*^[16] reported only one case who developed anti-E antibodies.

In summary, the number of antibodies against antigen of the rhesus system formed the highest

percentage (52.38%) of the alloantibodies detected.

The Kell system antibodies were found only in one pregnant woman forming 2.2% of the total antibodies detected in the study group. HDN due to anti-k alloimmunization of the mother is very rare. Moncharmont *et al*^[5] collected 3000 cases of HDN over thirty years. Among these 273 (9.1%) were due to alloantibody other than anti-D and only three cases of anti-k HDN (1.09%) were found; two of them required exchange transfusion after delivery. Bowman *et al*^[22] reported 3426 cases of alloimmunization in about 175,000 women and found only one due to anti-k (0.03%). While in 1992^[20], he explained that the rarity of severe Kell hemolytic disease is almost entirely due to the rarity of any degree of Kell hemolytic disease. When antibody screening of Rh positive pregnant women was universal, only eight of 324 (2.5%) Kell – immunized pregnancies ended in delivery of affected infants during the twenty year study period.

Kell hemolytic disease is rare because Kell immunization is usually produced by blood transfusion. In addition only 9% husbands of the women studied were Kell-positive out of whom 98% are heterozygous for Kell. It is observed that when Kell hemolytic disease occurs it can be as severe as Rh (D) hemolytic disease. Wenk *et al*^[2] reported that Kell antibodies were encountered in reproductive women about 60% as often as Rh o antibodies but the risk of Kell disease is less than 5% when such antibodies are detected. Holtzman *et al*^[1] studied 121 cases (22%) of Kell alloimmunization. This frequency is even higher than that of D alloimmunization (101, 18.4%).

Immunization against the Kidd system is also very rare. We detected only one anti-Jka case (2.38%) among the immunized Saudi pregnant women. Vietor *et al*^[16] reported one case of anti Fya as a result of intrauterine transfusion. Center for Disease Control^[23] reported a case complicated by the presence of anti-Fyb with a titer of 32 due to blood transfusion.

In this study, the percentage of Lewis system was 2.38% of the alloimmunized Saudi pregnant women in the form of anti-Lea. Lewis antibodies are the commonest single cause of positive results in pre-transfusion antibody screening tests. They occur without obvious stimulation by transfusion or pregnancies and so can be found the first time a serum is tested. Occasionally anti-Lea but not anti-Leb can cause severe transfusion reactions with hemoglobinemia and hemoglobinuria. Lewis antibodies do not cause HDN because they are almost always IgM and because newborn infants group as Le a – b –.

This study indicates that the percentage of alloimmunization among Saudi pregnant women

is low. Alloimmunization to the Rhesus system was 1.84% in the study population. This low incidence is mostly due to the early detection of Rh negativity of the mothers before or as early as possible during pregnancy. Also, giving D-immune globulin after delivery for the Rh negative mothers lowered alloimmunization rates. In the United States, with the widespread use of D-immune globulin, it was once thought that Rh alloimmunization in pregnancy could be eradicated. The rate of HDN due to Rh antibody declined from 45.1 cases/10,000 total births in 1970 to a rate of 15.6 cases/10,000 total 1983, (Center for Disease Control, 1985)^[23]. Though not entirely eradicated, Rh alloimmunization has dropped markedly due to immune globulin administration and improved transfusion practices.

We detected most cases of alloimmunization in blood group O (61.9%) women, followed by group A (19%) then group B (14.3%) and lastly group AB with 4.8% of all immunized pregnant women. Alloimmunization in Rh (D) negative women was 8.3%. The incidence of Rh alloimmunization could be reduced to 0.18% if a single dose of Rh immune globulin was administered at 28 weeks gestation. Also they proposed that the routine use of antenatal RhIg in Rh negative women would be 88% effective in preventing sensitization.

All immunized mothers with anti-D in the serum were rhesus negative and their husbands were Rh positive and gave birth to babies who were rhesus positive. The titer of anti-D ranged from weak to high. One alloimmunized patient had a previous history of anti-D immunization; one gave six live births, another had a history of recurrent fetal loss possibly due to alloimmunization with anti-D and the rest had unknown history of immunization with anti-D. There were two cases of anti-C linked with anti-D but was not detected in the neonate.

In six cases of anti-E the titer ranged from weak up to 4. Two of the anti-E cases have a previous history of G6PD deficiency, one has a previous history of two abortions, and two have a history of blood transfusion and the last one had unknown history of immunization. Two babies given blood exchange due to hyperbilirubinemia may have been due to G6PD deficiency because no antibodies were detected.

The two patients with anti-e gave weak reactions. One patient gave history of live births and puerperal hemorrhage and the other had unknown history of immunization. Neonatal blood examinations gave no indication of alloimmunization.

There was one case of anti-kell with a titer of 2. She had five live births but the antibody was not detected in the most recent offspring. There was only one case of anti-Jka with a titer of 1. The baby

of the immunized patient gave no evidence of alloantibody in his blood. Anti-Le was detected in one patient with weak titer and gave no reaction in the newborn. There was only one case with detected anti-Fyb but the reaction was weak. The mother had previous cesarean section, transfusion and one abortion. The alloantibody was not detected in the newborn.

CONCLUSION

Even if Rh D alloimmunization is still a dominant problem giving rise to hemolytic disease and transfusion reactions, the relative importance of antigens other than Rh D have increased since the introduction of Rh D prophylactic treatment. Alloimmunization to E, c and Kell antigens can reach significant proportions of studied populations and can result in deleterious effects on the fetus. Forty-six percent (46%) of alloimmunization cases and 22% of exchange transfusions were in patients with antibodies to these three antigens.

The actual risk of alloantibody production during pregnancy is unknown but stimuli for antibody production are feto-maternal bleeds that occur throughout pregnancy but occur most frequently at the time of delivery.

Screening for irregular antibodies during pre-transfusion reduce the sensitization of red blood cells and reduce the risk of occurrence of hemolytic disease due to antibodies other than to the Rh group of antigens.

Blood to be transfused to women planning future pregnancies should be compatible not only with the D antigen status of the patient but also with her Kell and other Rh antigen status.

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