Frequencies of Allo-antibodies In Pregnant Women

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Abstract:

Background and Aim:

Hemolytic disease of the fetus and newborn (HDN) is a condition in which the lifespan of an infant's red blood cells (RBCs) is shortened by the action of specific IgG antibodies directed against Rhesus or other blood group antigens on fetal RBCs that are inherited from the father but are not expressed by the mother. The aim of the present study was to provide data on the type and frequency of maternal RBC alloimmunization in pregnant women attending the ante-natal Clinics, King Khalid University Hospital between January 2010 to December 2010

Materials and Methods:

A retrospective review of medical records of all pregnant women with RBCs allo-antibodies who were followed and delivered in King Khalid University hospital between January 2010 and December 2010. All samples were tested for blood grouping and screened for irregular antibodies using DiaMed ID Micro Typing system (Gel Test).

Results:

4224 samples of pregnant women were tested, 60 samples were found positive with frequencies of 1.4% as follows:
anti-D 11 (18%),anti-K 7 (11.7 %),anti-E 14 (23.3 %),anti-c 5 (8.3%), anti-E,c 3 (5%),anti-D,C 2 (3.3 %), anti-Lua 1 (1.7%),anti-Fya,Fyb,E 1 (1.7 %), non specific 4 (6.7 %) ,anti-C 2 (3.3 %),anti-E,c,K 1 (1.7%),anti-S 1(1.7%),anti-Leb,Lea,E1 (2.3%),anti-E,S,K 1(2.3%), anti-Jka 1 (1.7%) anti-M 1(1.7%), anti-Lea 1 (1.7%)anti-D,K,Fya,Jka 1(1.7%),anti-D,C,E 1 (1.7%), anti-E,Lea,Leb 1 (1.7%) and anti-C,e 1 (1.7%).

Conclusion:

Primary prevention by using K-negative, Rh c-, Rh E-, and RhC-compatible red blood cell transfusion for women younger than 45 years may prevent up to 40% of cases of haemolytic disease of the newborn Allo-antibodies detected among the pregnant females were due to pregnancy and not blood transfusion.

Key words: HDN, Alloimmunization, Rh(D) immunoglobulin, Hydrops fetalis .

1-Introduction:

Hemolytic disease of the fetus and newborn (HDN) is recognized in which the lifespan of fetal's red blood cells (RBCs) is shortened by the action of specific IgG antibody/ (ies) inherited from the father but are not expressed by the mother (Bidyut *et al*, 2010; Koelewijn et al,2008;2009 Mona *et al*,2011; and Bondagji *et al*,2011). The most common routes of maternal sensitization are via blood transfusion or feto-maternal hemorrhage, associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy, or invasive obstetric procedures. These antibodies may be directed against Rhesus or other blood group antigens on fetal RBCs HDN can exhibit different clinical forms, from a mild anemia with neonatal hyperbilirubinemia to a major fetal damage with stillbirth due to hydrops fetalis, a high-output cardiac failure syndrome, generalized edema and death may occur in the untreated cases (Nordvall et al,2009; Ngoma *et al*, 2016; Davutoglu et al,201). Despite the development and implementation of Rh(D) immune globulin prophylaxis, maternal Rh alloimmunization remain a cause of erythroblastosis fetalis and hemolytic disease of the newborn (Moise 2008 and Carbonne 2010). However, with appropriate monitoring and intervention, hemolytic disease of the fetus and newborn can be treated successfully in almost all pregnant women with no long-term sequelae in offspring. Rh negative women with large feto-maternal haemorrhage (FMH) from Rh (D) positive fetus are at risk for anti D alloimmuniztion if they do not receive the adequate Rh immune globulin (RhIG) (Bricca *et al,2011;* Lubuský *et al* 2010 and DeHaas *et al*,2014). Determination of the RhIG dose for these women is a critical laboratory procedure for protecting their future Rh D positive children, other Rhesus (C,c ,E,e),K,MNSs and Duffy antigens, and the corresponding maternal antibodies may cause severe HDN (Karagol *et al,2012* and Wamelen *et al,2007*). Primary resources against HDN consist of extended matching of red blood cell (RBC) transfusions and the administration of Rh D immunoprophylaxis. In addition to ABO and Rh D matching of RBC transfusions, for example Dutch guidelines recommend matching for K, Rh c and Rh E for women below 45 years of age (Dutch guidelines, 2011). Rh D immunoprophylaxis has been licensed in Europe and North America since 1968. As a consequence, Rh D immunization rate decreased from 13.2% to 0.14% per Rh D positive pregnancy (Bowman,2003). With effective antenatal care, fetuses at risk are identified by maternal antibody screening and titration, determination of paternal antigen status, and determination of fetal antigen status (Geaghan *et al*,2011 and Jophy *et al* 2013) the latter can also be done by amniocentesis or noninvasively using free fetal DNA in maternal plasma. This noninvasive fetal blood group typing was first reported by Lo *et al* (1998). However, currently noninvasive detection of fetal anemia by Doppler assessments of peak systolic velocity in the fetal middle cerebral artery is used predominantly, by measuring the speed of the blood flowing through a blood vessel in the baby's brain (Moise *et al*,2008*;* Oepkes *et al*,2006 and , Zipursky *et al,2011*).Treatment of fetal anemia by intrauterine intra-peritoneal RBC transfusion was introduced in 1963 (Liley,1963). In the 1980s intravascular transfusion via umbilical vessels became practice Rodeck *et al* (1984). Other proposed antenatal treatment options include maternal or fetal administration of intravenous immunoglobulin and maternal administration of Phenobarbital, Trevett *et al* (2005).After birth, diagnosis of HDN can be confirmed by blood group and Rh typing, measuring antibodies and bilirubin, and performing direct antibody (Coombs') test. Postnatal management includes

intense phototherapy and exchange transfusion to reduce hyperbilirubinemia and blood transfusion to treat anemia (Smits *et al*, 2008 and Rath *et al*, 2010).

2- Materials and Methods:

The study group included all pregnant women registered and delivered in King Khalid University Hospital between January 2010 and December 2010 .Age range between (19-44 years). All ante-natal women were tested for blood group, Rhesus factor and antibody screening in their first visit as part of their routine antenatal care. Anti body identification was done manually for samples with positive antibody screening using Gel Test Method, (Micro Typing System Cressier sur Morat, Switzerland) (Lapiere,1990**) .**Titer was done on samples after antibody identification**.**

3- Results:

4224 samples of pregnant women were tested, 60 samples were found positive with frequencies of 1.4%.
The most frequent and potentially significant non-anti-D antibody in our study was anti-E 14 (23.3 %), followed by anti-D 11 (18.3%) anti anti-K 7 (11.7 %) , anti-c 5 (8.3%), anti-S 1(1.7%),anti-Jka 1 (1.7%) anti-M 1(1.7%), anti-Lea 1 (1.7%),anti Lua 1 (1.7%) , non specific 4 (6.7%) and anti-C 2 (3.3%).
Other antibodies of more than one specificity was found as follows : anti-E,c 3 (5%),anti-D,C 2 (3.3 %), ,anti-Fya,Fyb,E 1 (1.7 %) ,anti-E,c,K 1 (1.7%) anti-D,K,Fya,Jka 1(1.7%),anti-D,C,E 1 (1.7%), anti-E,Lea,Leb 1 (1.7%) and anti-C,e 1 (1.7%) anti-Leb,Lea (2.3%),anti-E,S,K 1(2.3%) (Table 1, 5) and (Graph 1).
The distribution of antibodies within different blood groups of pregnant women was as follows:
O positive 27 (45%),O negative 10 (16.7 %),A positive 9 (15 %), A negative 5 (8.3 %), B positive 8 (13.3 %) and B negative 1 (1.7 %) (Table 2, 5) .
6 pregnant women showed antibodies with high titers,anti-K 1:512 in (A pos), anti-K 1:512 in (O pos) anti-Jka 1:16 in (A pos), anti-D 1:128 in (O neg),anti-D 1:512 (O neg) and anti-D,C(C- 1:16,D-1;512) in (A neg) (Table3) .
out of 60 pregnant women 12 received blood transfusion while 48 not received blood transfusion as shown in (Table4) and (Graph 2).

3-1 Statistical Analysis:

Data generated was subjected to the statistical analysis using SPSS (social package for social science) version 23 (Table 6, 7).

4- Discussion:

HDN is a condition caused by maternal antibodies to fetal red cell antigens, which cross the placenta and cause haemolysis. The antibodies can be natural or immune. In the latter case, the sensitizing event is frequently a previous pregnancy or a transfusion, where the mother was exposed to the relevant antigen. Some antibodies

(including anti-D, anti-K (Kell) and anti-c) confer significant fetal and neonatal risks such as anemia requiring intrauterine or neonatal transfusion, jaundice or perinatal loss. There are many antibodies that are unlikely to significantly affect the fetus but can cause neonatal anemia and hyperbilirubinaemia, while others may cause problems for the screening and provision of appropriate blood or blood components to the mother or fetus/neonate when required (Koelewijn,2009).This study showed that the frequencies of red cell alloimmunization among the pregnant women was 1.4% (Table 2).

Irregular RBC antibodies found in the sera of pregnant women have been studied in many parts of the world where prenatal immunohematologic care is given due priority. In this study, the frequency of irregular antibodies in maternal serum was 1.4%%. This appears high when compared with values from developed countries, such as Sweden (0.4%) ,(Gottvall, 2008) and a study done in China with a prevalence of 0.79 % ,Lee et al,(2003).

Our study was lower than the studies in Port Harcourt, Nigeria with the frequency of 3.4% (Zazzheaus,2011), in Uganda 2.1%, in Tunisia 3.7%, in Sudan 10% and in Cameron was 6.7% (Ngoma,2016).

Our study is in accordance with the findings of several other studies, such as those by Koelewijn, et al (2008) tested 305.000 samples with prevalence of (1.2%), Pahuja, et al (2011) tested 3.577 samples 45 was positive for antibodies with prevalence of 1.25% and Howard, et al(1998) tested 22,264 samples,45 samples were found positive with prevalence of 1%.

The prevalence of minor RBCs antibodies alloimmunization among Rhesus positive pregnant was 2.7% in a study in Oman. 33 out of 1160 Rh positive women alloimmunized with minor RBCs antibodies that gave a prevalence of 2.7%.

The most frequent antibody was anti-E 14%, followed by anti-D 11% and anti-kell 17 (Tamima, 2015). Our study also showed that the most frequent antibody was anti-E which is 14 (23.3%).

In a Croatian study, clinically significant non-D antibodies produced HDN in approximately 55% of alloimmunized pregnancies, and severe HDN, defined by perinantal transfusion requirement or death, in approximately 25% (Dajak, 2011).

Our results (1.4%) were similar to results conducted in Tanzania (Africa), among 77.949 pregnant women tested, 855 samples was found positive for antibodies identification with frequencies of 1.1% (Ngoma, 2016).

Deliveries of non-primiparous women are more affected by alloimmunization since the immunization event is often the previous delivery (Afra,2013). The parity distribution is important, as each pregnancy and delivery increases the cumulative risk

for FMH. For obvious reasons the prevalence is lower in countries with a low fertility index.

Our study showed that most of women with abortion got, anti E and anti E combined with other antibodies.

Anti-E alloimmunization can cause fetal anemia, and the incidence could be underestimated**,** MacKenzie et al, (1992) Unlike anti-D alloimmunization, anti-E titer is less sensitive in detecting severity of hemolysis in the subsequent pregnancy.

Of 269 potentially relevant articles reviewed in Africa 12 studies representing 93 871 pregnant women. Overall proportions of RBC alloimmunization exhibited a wide variation ranging from 1·1 (95% CI: 1·0, 1·2) to 12·1 (95% CI: 9·8, 14·7) per 100 pregnant women. Among clinically relevant antibodies, anti-D ranked as the most common, followed by anti-K and anti-E (Ngoma, 2016).
In populations at high obstetric risk, the prevalence of immunization also will be higher, as most obstetric risk factors directly or indirectly are related to FMH in the past. Also the antigen distribution differs between populations. For example in the Chinese population D immunisation is rare since the frequency of D-negativity is low (<1%) (Lee, 2003).

5-Conclusion & Recommendations:

Primary prevention by using K-negative, Rh c-, Rh E-, and RhC-compatible red blood cell transfusion for women younger than 45 years may prevent up to 40% of cases of haemolytic disease of the newborn Allo-antibodies detected among the pregnant females were due to pregnancy and not blood transfusion.

- All test procedures must be well established and validated in compliance with published guidelines .
- Ideally testing should be performed on automated equipment which ensures positive sample identification and with electronic transfer of results to the woman's computer record. By this way errors will be reduced.

- Knowledge of the distribution of red cell antigens can help to prevent alloimmunisation by facilitating the provision of antigen negative blood for pregnant women .
- Detailed phenotyping for all clinically significant red cell antigens be carried out routinely among all pregnant women as this will facilitate the optimum obstetric management of HDN in pregnant women who have a clinically significant alloantibody. When selecting blood for transfusion to such patients, it would be useful if we have access to already phenotyped RBCs of donor population so that particular antigen typed blood can be given to such patients to prevent alloimmunization

- Women should be screened for atypical red-cell allo-antibodies in early pregnancy and again at 28 weeks, regardless of their Rhesus D status.

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7- Appendices:

Table (1): Antibodies.

Antibodies	Frequency	Percent
D	11	18.3
K	7	11.7
C	5	8.3
C	2	3.3
E	14	23.3
Lua	1	1.7
non specific	4	6.7
D,C	2	3.3
E,Fya,K	1	1.7
cE	3	5.0
S	1	1.7
cEK	1	1.7
M	1	1.7
Lea,Leb,E	1	1.7
Fya,Fyb,E	1	1.7
JKa	1	1.7
D,K,Fya,Jka	1	1.7
D,C,E	1	1.7
Lea	1	1.7
Ce	1	1.7
Total	60	100.0

Table (2): Blood groups.

Blood groups	Frequency	Percent
O pos	27	45
O neg	10	16.7
B pos	8	13.3
B neg	1	1.7
A pos	9	15
A neg	5	8.3
Total	60	100.0

Table (3): Ante-natal with high titer.

Blood grouping	Antibody	Titer
A neg	D,C	D 1:512 C 1:16
O pos	K	512
A pos	K	K1:512
A pos	Jka	Jka 1:16
O neg	D	D 1:128
ONEG	D	D 1:512

Table (4): Blood Transfusion.

	Frequency	Percent
Yes	12	20.0
No	48	80.0
Total	60	100.0

Table (5) : Crosstab.

Antibodies	Blood group						Total
	O pos	O neg	B pos	B neg	A pos	A neg	
D	0	8	0	1	0	2	11
K	4	0	0	0	3	0	7
c	4	0	1	0	0	0	5
C	2	0	0	0	0	0	2
E	8	0	1	0	4	1	14
Lua	0	0	1	0	0	0	1
non specific	1	0	2	0	1	0	4
D,C	0	1	0	0	0	1	2
E,Fya,K	1	0	0	0	0	0	1
cE	2	0	1	0	0	0	3
S	1	0	0	0	0	0	1
cEK	0	0	0	0	1	0	1
M	0	0	1	0	0	0	1
Lea,Leb,E	1	0	0	0	0	0	1
Fya,Fyb,E	1	0	0	0	0	0	1
JKa	0	0	0	0	1	0	1
D,K,Fya,Jka	0	1	0	0	0	0	1
D,C,E	0	1	0	0	0	0	1
Lea	0	0	1	0	0	0	1
Ce	0	0	0	0	1	0	1
Total	25	11	8	1	11	4	60

Statistical Analysis

Table (6): Chi-Square Tests.

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	112.699[a]	95	.104
Likelihood Ratio	104.792	95	.231
Linear-by-Linear Association	.156	1	.693
N of Valid Cases	60		

a. 119 cells (99.2%) have expected count less than 5. The minimum expected count is .02.

Table (7): Symmetric Measures.

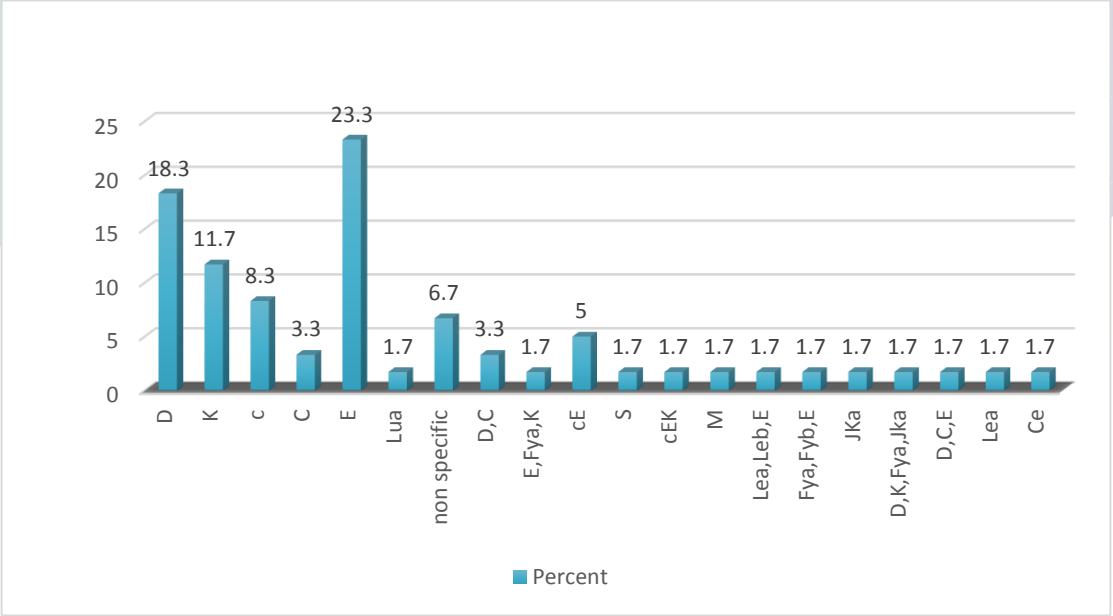
		Value	Asymptotic Standardized Error [a]	Approximate T [b]	Approximate Significance
Interval by Interval	Pearson's R	0.051	0.120	0.392	0.696[c]
Ordinal by Ordinal	Spearman Correlation	0.000	0.122	0.000	1.000[c]
	N of Valid Cases	60			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Graph (1) showing percentage of alloantibodies antibodies



Graph (2) showing (20%) of pregnant women received blood transfusion