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Frequency and Types of Red Cell Alloantibodies in Pregnant Females

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ABSTRACT

Introduction: Maternal isoimmunization, also called alloimmunization, occurs when immune system of a pregnant female is sensitized to foreign RBC surface antigen producing immune process. This maternal blood when goes to fetal circulation, causes immune reaction and disease in case of maternal and fetal blood group differences. **Aims & Objectives:** To find out the frequency and types of red cell alloantibodies among females presenting to antenatal care and recognize risk factors for alloimmunization. **Place and duration of study:** This cross-sectional descriptive study was carried out at Antenatal Clinic of Shaikh Zayed Hospital, Lahore from 1st January 2013 to 31st August 2013. **Material & Methods:** Pregnant females with at least one previous pregnancy were typed for ABO and Rh antigens. They were screened and typed for red cell alloantibodies. Detailed history was taken to explore for the risk factors. SPSS version 20.0 was used for data analysis, frequencies of different alloantibodies, blood groups and risk factors reported as percentages, age and gravidity in mean±s.d. **Results:** Out of 200 enrolled cases, 6(3%) had alloantibodies. Of the positive cases, anti-D was found in 3(50%), anti-C in 2(33.33%) and anti-Kell in 1(16.67%). Commonest risk factors were history of peripartum hemorrhage and gynecological procedures. In Rh-negative cases, disparity of spouse Rh group was also main factor. **Conclusion:** The most common culprit antibody for alloimmunization was anti-D followed by anti-Kell and anti-C. Commonest risk factor for alloimmunization was pregnancy related bleeding and gynecological procedures. Large population-based studies are required to assess true magnitude of the problem.

Key words: Alloimmunization, Hemolytic Disease of Fetus and Newborn (HDFN), Pregnancy, Red Cell Alloantibodies.

INTRODUCTION

Maternal red cell alloimmunization occurs due to exposure of foreign red cell antigen inherited by fetus from father[1,2] to antenatal immune system and subsequent sensitization. Sensitization can also occur following blood transfusion.[1] More than 50 antigens can cause sensitization and alloimmunization. Rhesus is commonest followed by ABO, while Kidd, Kell and Duffy are less common causes.[2]

Rh alloimmunization and hemolytic disease of fetus and newborn (HDFN) was first described by Levine and later by Dr. Louis. Incidence of Rh alloimmunization and subsequent HDN was highest among whites i.e. 15-16 % and lowest among Asians (<1%).[1]

Most important general risk factor for alloimmunization is repeated blood transfusions.[3] Other risk factors include age, gravidity/parity and eventful/bad obstetric and gynaecological history.[4]

ABO incompatibility occurs usually during first pregnancy because previous antigen exposure is not required. ABO antibodies are usually IgM type and do not cause HDFN because these cannot cross placenta. However ABO related HDFN can take place in group O mothers with high titers of naturally occurring Ig G anti A or B.[5]

For all other blood groups causing HDFN, antigenic exposure by transfusion or fetomaternal hemorrhage (FMH) is necessary. Immunization response after FMH depends on its volume, ABO incompatibility, RhD phenotypes and gender of the fetus.[2]

In case of incompatibility with Rh and other blood, the primary immune response is weak and the

antibodies produced are of IgM type which do not cross placenta, hence chances of HDFN are scarce.[6] In subsequent exposure, IgG are produced that cross placenta and cause HDFN. Chances of HDFN increase in subsequent pregnancies.[7]

IgG antibodies cross placenta and attack the fetal red blood cells causing hemolysis. This leads to increased bilirubin in fetal circulation. Fetal liver is incapable of conjugation, so extra bilirubin is conjugated and excreted by mother. Neonates develop hyperbilirubinemia.

Both mother and the fetus should undergo proper tests for the timely diagnosis of HDFN and its management. All Rh negative blood group women having Rh D positive husbands should be screened for alloimmunization at their booking visit to antenatal care unit. If the pregnant woman is found to be positive for alloimmunization at any point in time, then antibody titer must be done. The fetus should be screened for anemia with the help of Middle Cerebral Artery-Peak Systolic Velocity.[8]

The dose of anti-D after delivery is 1500 IU to all Rh negative women. Additional dose of anti-D may be required in females with large fetomaternal hemorrhage.[9]

Aim of this study is to find out the frequency and types of red cell alloantibodies among females presenting to antenatal care and associated risk factors.

MATERIAL AND METHODS

This cross-sectional descriptive study was conducted at Department of Hematology and Blood Bank at Shaikh Zayed Medical Complex, Lahore. 200 pregnant females between 16th to 18th week of gestation and having second and subsequent pregnancies presenting to Antenatal Clinic of Gynecology and Obstetrics Department, SZH were selected for this study. Pregnant females with history of multiple transfusions, autoimmune diseases, autoimmune hemolytic anemia, malignant disease, immunomodulation and immuno-suppressive therapy and those women who tested positive for Direct Coombs Test (DAT) were excluded from the study.

After written informed consent, data were collected by interviewing participants of study and evaluating their medical record on predesigned proforma. 5ml of peripheral venous blood sample was collected under aseptic measures. Clear serum was used for blood grouping, Direct Antiglobulin Test (DAT) and Indirect Antiglobulin Test (IAT). For antibodies screening, three-cell panel and for antibodies detection, extended cell panel by DiaMed was used. Blood grouping and DAT was done on same day while other tests were done later after thawing preserved sera.

Statistical analysis:

All the data were analyzed using SPSS version 20.0. The frequencies of different alloantibodies, blood groups and risk factors were reported as percentages. Age and gravidity were reported as mean and standard deviation.

RESULTS

The study was conducted on 200 pregnant women of reproductive age with at least one previous pregnancy. Age of the women included in the study ranged from 20-40 years with a mean of 29.36±4.18 years. (Fig-1)

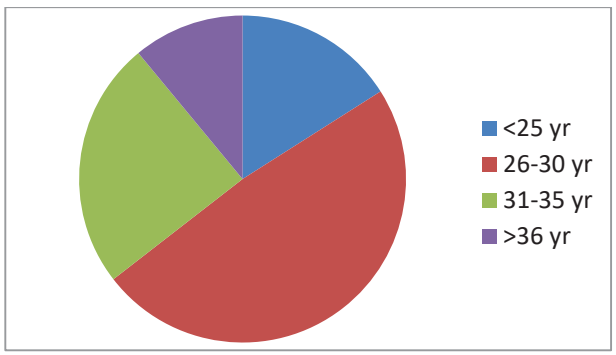

Fig-1: Distribution of women by Age

The gravidity of our cases ranged from 2-7 with a mean of 3.2±1.8 and median of 3.

23 (11.5%) had no previous live births, 15 (7.5%) had experienced one or more still births whereas 73(36.5%) had experienced one or more abortions.

Most common blood group was B+ve in 80 (40.0%) women and in 69 (34.5%) of their spouses.

The ABO blood group of 70 women(35.0%) and Rh blood group of 169 (84.5%) was the same as that of their spouses while it was different in rest of them.

History of APH was present in 54 (27.0%) women, IPH in 12 (6.0%) and PPH in 9 (4.5%).

History of gynecological risk factors was present in 18 (9.0%) women and out of them, 16 (8.0%) had previous ectopic pregnancy while 2 (1.0%) had molar pregnancy.

History of obstetrical maneuvers and high risk events was available in 11 (5.5%) women. External Cephalic Version had been performed in 5 (2.5%), manual removal of placenta in 6 (3.0%), Blunt abdominal trauma in 8 (4.0%) and 2 (1.0%) had a history of manual removal of placenta along with abdominal trauma.

60 (30.0%) women had the history of gynecological procedures related to previous pregnancies.

Previous transfusion history was present in 38 (19.0%) women. Amongst them 33 (16.5%) had packed red cell transfusion, 2 (1.0%) had platelet transfusion and 3 (1.5%) had been transfused by more than one blood product.

IAT was performed in all cases. 6 (3.0%) women had a positive result showing the presence of antibodies. Red cell alloantibodies were found in all 6 of them. Anti-D was the most common alloantibody and was present in 3 (50%) cases followed by anti-Kell 2(33.3%) and anti-C 1(16.7%) Blood group status of alloantibody positive cases is shown in Table-1.

		Blood Group of Spouse						
		A+ve	**B+ve**	**O+ve**	**A-ve**	**B-ve**	**O-ve**	**Total**
Blood Group of Case	**A+ve**	0	0	0	0	0	0	**0**
	B+ve	0	0	0	0	0	0	**0**
	O+ve	1	1	0	0	0	0	**2**
	A-ve	0	1	0	0	0	0	**1**
	B-ve	1	0	0	0	0	0	**1**
	O-ve	2	0	0	0	0	0	**2**
	Total	**4**	**2**	**0**	**0**	**0**	**0**	**6**

Table-1: Blood group of women with positive alloantibodies and their spouses

Among these, two were gravida 2, one each was gravida 3, 4, 6 and 7.

Risk factors in previous pregnancies in alloantibody positive cases are shown in Table-2.

Risk factors	**N**	**Percentage**
Peripartum Bleeding		
APH	2	33.3
IPH	1	16.7
Nil	3	50.0
Total	6	100.0
Gynaecological		
Ectopic	1	16.7
Nil	5	83.3
Total	6	100.0
Obstetrical Risk factors		
Manual Removal of Placenta	1	16.7
Abdominal Trauma	1	16.7
Nil	4	66.7
Total	6	100.0
Procedures		
E&C	1	16.7
D&C	2	33.3
Nil	3	50.0
Total	6	100.0
Transfusion History		
Packed RBCs	2	33.3
Nil	4	66.7
Total	6	100.0

Table-2: Risk Factors in Previous Pregnancies in Alloantibody Positive Cases

Summary of alloantibody positive cases is shown in Table-3.

Sr. No	Age	Gravida	Blood-Group self	Blood-Group spouse	Live Births	Still Births	Abortions	Pregnancy bleeding history	Gynaecological risk factors	Obstetrical procedures/ high risk event	Gynecological procedures	Transfusion history	Red cell Alloantibody
1	25	2	O-	A+	1	0	0	Nil	Nil	Nil	Nil	Nil	Anti D
2	27	3	O-	A+	1	0	1	Nil	Ectopic pregnancy	Blunt/concealed abdominal trauma	Dilatation & curettage	Nil	Anti D
3	40	7	O+	A+	4	0	2	APH	Nil	Nil	Dilatation & curettage	Nil	Anti Kell
4	23	2	A-	B+	0	0	1	Nil	Nil	Nil	Evacuation & curettage	Nil	Anti D
5	27	4	O+	B+	3	0	0	IPH	Nil	Nil	Nil	Packed RBCs	Anti Kell
6	37	6	B-	A+	3	2	0	APH	Nil	Manual removal of Placenta	Nil	Packed RBCs	Anti C

Table-3: Cumulative status of alloantibody positive cases

DISCUSSION

In the present study, the frequency of red cell alloantibodies and risk factors for alloimmunization were determined in the pregnant women visiting antenatal clinic of Shaikh Zayed Hospital, Lahore.

Most severe form of alloimmunization is against Rh-D in which IgG antibodies develop due to sensitization to D antigen in D-negative women. In the developed countries, its incidence is low due to

anti-D prophylaxis, but in developing countries like Pakistan, there is a significant risk of Rh incompatibility due to lack of anti-D prophylaxis.[10,11]

The frequency of red cell alloimmunization in pregnant women in the current study is found to be 3%. The frequency studies of red cell alloimmunization from different parts of the world have reported variable results in different countries, regions and centers.

The frequency reported in an Indian study conducted in Delhi was 1.25%. However it was 0.5% in Sweden and 2.7% in Netherlands.[10,11] A frequency of 1.92% was reported from large scale study in Saudi Arabia whereas it was 10.2% in Mexico and 3.4% in Nigeria.[10,12]

These variations in frequencies may possibly be due to difference in racial, environmental, socioeconomic status as well as status of anti-D prophylaxis. The frequency of red cell alloantibodies is low in developed countries because of good antenatal services and regular anti-D prophylaxis.

With the advent of anti-D prophylaxis, incidence of HDFN due to ABO and other red cell alloantibodies has relatively increased internationally.[13]

Antenatal services in Pakistan are not equivalent in various regions of the country. Limited research has been done in this field and there are no local guidelines.

In our study, anti-D was detected as the commonest alloantibody among the positive cases. The next frequent alloantibody was anti-Kell followed by anti-C.

In Sweden, anti-Rh were reported as most frequent type of alloantibodies followed by antibodies against Lewis, MNS and Kell system antigens.[11] A study conducted in central province of Saudi Arabia also showed anti-Rh(anti-D being most frequent) to be most common followed by anti-Kell, anti-Kidd, anti-Lewis and anti-Duffy [12]. Similarly, in India, anti-D was reported to be the most common alloantibody, other less frequent being anti-C, anti-M and anti-S.[14]

The results of our study are comparable with most international studies, where anti-D is the most frequent type of alloantibody, other less frequent types being anti-Kell and anti-C. Our results are also comparable to two local studies of Pakistan. The study conducted in Rawalpindi General Hospital showed similar results to our study where anti-D was reported to be most frequent in pregnant females , second most frequent being anti-Kell.[15] Anti-C was found to be least common among the positive cases in the present study. Our results are therefore consistent with another study conducted in Karachi which also reports anti-C to be less frequent than anti-D.[16]

The most common risk factors for alloimmunization in our study was pregnancy related bleeding in 3(50.0%) and gynaecological procedures in 3(50.0%), the next most common risk factor being blood transfusion in 2(33.3%) and 2(33.3%) gave history of eventful pregnancies whereas 2(33.3%) had history of disparity in Rh blood group with husbands. Risk factors were overlapping among the cases. The results were comparable with a study carried out in Western Uganda where history of peripartum hemorrhage was significantly associated with alloimmunization.[4]

Another bicentric scientific study in India showed transfusions and bad obstetrical history playing a remarkable role in alloimmunization.[17]

CONCLUSION

Shaikh Zayed Hospital is a tertiary care hospital, located amidst the effluent localities of Lahore. Hence it is likely to be serving relatively higher socioeconomic group of patients in comparison to the other public institutions. So results of our study cannot be extrapolated on overall population of Lahore or on the country as a whole. Therefore large scale and multicenter studies need to be conducted in various regions of the country to assess the magnitude of the problem and to observe its pattern.

In light of results of the present study, the alloantibody screening should be adopted as a routine antenatal parameter for all the pregnant females and should not be restricted to the mothers with Rh-D negative blood group married to Rh-D positive spouses. This will help in prevention and early management of hemolytic disease of fetus and newborn.

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