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Study of red cell alloimmunisation in multigravida in RIMS hospital,Imphal

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***Abstract**: Red cell alloimmunisation is the development of antibodies in response to foreign red cell antigens through transfusion or pregnancy. Females can be alloimmunised to red cell antigens by previous transfusions or transplantations and/or previous or current pregnancy.Red cell alloimmunisation in pregnancy is an immune disorder due to an incompatibility between maternal and fetal red blood cell antigens.In pregnant women,these antibodies may cross the placenta and cause haemolytic disease of the foetus and newborn(HDFN).Timely detection of such antibodies in antenatal women is essential for early management of HDFN.A prospective cross-sectional study was carried out on 200 multigravida attending antenatal clinic or admitted at the Department of Obstetrics and Gynaecology,RIMS,Imphal to find out the prevalence of red cell alloimmunisation and to study other factors which may contribute to alloimmunisation namely age,gravida,blood group and ethnicity.The women were grouped and typed for ABO and rhesus(Rh) D antigens by tube method and screened for alloantibodies by column agglutination technology.The medical history and detailed obstetric history of these women were reviewed and information recorded on any prior haemolytic disease of the foetus and newborn among siblings and/or blood transfusions.The overall prevalence of alloantibodies was 1%.There was a statistically significant difference between alloimmunisation rates in the Rh D-antigen negative and D-antigen positive women (20% versus 0.5%).The antibodies detected in this study were anti-E and anti-Le^a.Red cell alloimmunisation is prevalent in around 1% of the multigravida women and significantly associated with gravida and ethnicity.As the other Rh and non-Rh group of antibodies were also identified,routine antibody screening and identification is recommended for all antenatal women.However,large-scale studies on pregnant women need to be done in order to collect sufficient evidence to be able to formulate guidelines regarding testing and interventional modalities for alloimmunisation in pregnancy.*

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I. Introduction

Red cell alloimmunisation is the development of antibodies in response to foreign red cell antigens through transfusion or pregnancy. Females can be alloimmunised to red cell antigens by previous transfusions or transplantations and/or previous or current pregnancy.Haemorrhage of fetal red cells into the maternal circulation can occur spontaneously during pregnancy or with trauma, amniocentesis, cordocentesis, abortion, and other manipulations[1].The development of the alloantibody is due to the anamnestic response. With the first exposure to the foreign antigen, the B lymphocyte clones are invoked which will result in a moderate production of IgM or IgG antibodies. It is the secondary exposure that elicits a more rapid production of large quantities of significant IgG-class antibody which can cross the placenta. Destruction of the fetal red cells occurs when the maternal antibody binds to the fetal red cell antigen, causing attachment to the Fc receptor of the macrophages in the spleen of the fetus and leads to a condition called haemolytic disease of the fetus and newborn (HDFN)[2.] Approximately 1% of pregnant women are found to have clinically significant red cell antibodies and there are more than 50 red cell antigens that can elicit antibodies that have been found to cause HDFN. Of these, the commonest specificity is anti-D, although universal introduction of routine antenatal anti-D prophylaxis has reduced this sensitization rate. However, with the introduction of antenatal anti-D prophylaxis, there has been a significant rise in positive antibody screening results, due to the detection of the prophylactic anti-D. Other alloimmune antibodies that can result in HDFN include other Rh (E, e, C, c), Kell (K and k), Duffy (Fy^{a}andFy^b), Kidd (Jk^a and Jk^b), and MNSs (M, N, S and s). Multiparous women and mothers with bad obstetric history (history of abortion, neonatal death and stillbirth) have been found to have higher chance of alloimmunisation due to multiple exposures to the incriminating foreignantigens2.Alloimmunisation in pregnant women has been extensively studied in different areas of the world, with the frequency being found to range from 0.4% to 2.7%

worldwide. According to the guidelines of the British Committee for Standards in Haematology, all pregnant women should be ABO and D antigen typed and screened for the presence of red cell antibodies. However, no such guidelines are followed in developing countries like India. Moreover, published data show wide variation in alloimmunisation rates between different geographic areas. It is universally considered that there should be evidence-based guidelines for screening of alloantibodies in pregnant women in developing countries such as India for proper management of child birth[3]..

II. Material And Methods

This study was planned to assess the prevalence of red cell alloimmunisation in multigravida women attending antenatal clinic or admitted at the Department of Obstetrics and Gynaecology, Regional Institute of Medical Sciences,Imphal. This prospective study was carried out at the Department of Transfusion Medicine,Regional Institute of Medical Sciences,Imphal over a period of two years,from October 2014 to September 2016. Written informed consent was obtained from the participants.Ethical approval was taken from the Institutional Ethics Committee,RegionalInstitue of Medical Sciences,Imphal.

The study was conducted on 200 multigravida women irrespective of their period of gestation and obstetric history.

Inclusion criteria:

1. All multigravida

Exclusion criteria:

1. Primigravidas
2. Women who had received anti-D prophylaxis in the current pregnancy
3. Women with previous history of blood transfusion
4. Women with history of autoimmune haemolytic anaemia
5. Women with any systemic diseases or haematological malignancies
6. Women with history of organ transplant before pregnancy

For each patient,name,age,ethnicity and other demographic details,obstetrichistory,history of blood transfusions were recorded and entered in a predesigned proforma prepared for the study prior to taking the blood samples.

Blood samples for the tests:

Six (6) ml of venous blood was collected, 4 ml of which was added to plain vials and 2 ml to EDTA vial. In case of delay more than 2 hours, the sample were stored at 2-6°C and were tested within 48 hours. The serum was separated by centrifugation.

Before proceeding to antibody screening,the subject's ABO and Rh group were determined as per the standard operating procedure(SOP) followed in the department. All the Rh D-negative samples were subjected to weak-D testing by an indirect antiglobulin test and Rh-D positive and negative results were recorded.

Antibody screening and identification was done using column agglutination technology in Coombs' phase. A commercially available three cell panel (Dia Cell I+II+III; DiaMed GmbH, 1785 Cressier FR, Switzerland of Bio-Rad Laboratories Pvt. Ltd.) was used for antibody screening procedure in which the patient's serum was reacted with panel of red cells using low ionic strength saline (LISS) Coombs gel card (DiaMed GmbH, 1785 Cressier FR, Switzerland of Bio-Rad Laboratories Pvt. Ltd.). The cards were incubated at 37°C for 15 minutes and then centrifuged for 10 minutes. The samples which were positive on antibody screen were frozen at -40°C for antibody identification, which was performed at a later date. An 11-cell panel was used for antibody identification(11 cell ID-DiaPanel ,DiaMed GmbH, 1785 Cressier FR, Switzerland of Bio-Rad Laboratories Pvt. Ltd.).

A review was conducted regarding medical history,obstetric history (including any stillbirths,abortions,medical termination of pregnancy (MTP) and cases of HDFN among siblings) and any past blood transfusions of all the participants.

Comparison of categorical data between antibody screen positive and negative individuals was done using Chi-square test or Fisher's Exact test as appropriate. Demographic and clinical variables were presented as frequency (%). All statistical analysis was carried out at 5% level of significance and a p-value <0.05 was considered significant. The data collected were entered and analysed with appropriate SPSS IBM version 21.

III. Result

Their mean age was 28.9+ 5.3 years,(range 18-41 years);majority of the cases were from the age group 26-35 years (52.5%) followed by age < 25 years (33.5%) and >35 years (14%). Most of the cases were from the group Meitei (84.5%) followed by Meitei Pangal (13.5%) (Table 1). Blood group B was the most common blood group found among the cases followed by blood group A and the least common was blood group AB (Table 2). There were 195 (97.5%) Rh-D positive women while 5 (2.5%) were Rh-D negative (Table 3).

Alloantibodies were absent in majority of the cases (99%) and only 2 cases were found positive for alloantibodies (1%),giving the overall prevalence of red cell alloimmunisation of 1% (Table 4). The

alloantibody anti-E was found only in one case and it belongs to Rh blood group system while the alloantibody anti-Lea was also found only in one case. It belong to the Lewis blood group (Table 5).Alloantibody was positive in 0.5% (1/195) of D antigen positive cases and in 20% (1/5) of D antigen negative cases.It was found to be statistically significant (Table 6)

Out of the 171 women with normal obstetric history alloantibody was detected only in one case (0.58%). Also alloantibody was detected in only one case out of the 29 women with bad obstetric history (3.45%) (Table 7).

One alloantibody each was detected in women belonging to gravida 3 and gravida 4 out of the 73 gravid 3 women and 11 gravid 4 women (1.4% and 9.1%) respectively. The relation of alloantibodies to gravida was found to be statistically significant (Table 8).

Table 1: Distribution of the cases according to their ethnic group

Ethnic group	Frequency	Percentage
Meitei	169	84.5
Meitei Pangal	27	13.5
Tribal	2	1.0
Others	2	1.0
Total	200	100.0

Table 2: Distribution of ABO blood group among the cases

ABO blood group	Number of cases	Percentage
A	63	31.5
B	68	34.0
O	53	26.5
AB	16	8.0
Total	200	100.0

Table 3: Distribution of Rhesus D antigen among the case

Rhesus D antigen	Number	Percentage
Positive	195	97.5
Negative	52	2.5
Total	200	100.0

Table 4: Distribution of the cases according to the presence of alloantibodies

Alloantibodies	Frequency	Percentage
Absent	198	99.0
Present	2	1.0
Total	200	100.0

Table 5: Specificities and frequency of alloantibodies detected

Blood Group System	Alloantibodies	Frequency
Rh	Anti-E	1
Lewis	Anti-Le[a]	1

Table 6: Relation between D antigen with red cell alloimmunisation

D antigen	Alloimmunised	Non-alloimmunised	Fisher exact test
Positive	1	194	Value =18.7
Negative	1	4	

Table 7: Incidence of alloantibodies detected among normal and bad obstetric history

Obstetric history	Total cases	Alloantibodies detected	Percentage of positivity of alloantibodies
Normal	171	1	0.58
Bad	29	1	3.45

Table 8: Alloantibodies detected in relation to gravida status

Gravida	Number of cases	Alloantibodies	Percentage of positivity of alloantibodies	Chi-square test
2	103	0	0	Value = 8.9
3	73	1	1.4	p-0.04
4	11	1	9.1	
5	10	0	02	
6	2	0	0	
7	1	0	0	
Total	200	2		

IV. Discussion

The present study was conducted to find out the prevalence of red cell alloimmunisation in multigravida attending or admitted at the Department of Obstetrics and Gynaecology, RIMS, Imphal. It also studied the factors contributing to red cell alloimmunisation like age, gravida, blood group and ethnicity. The knowledge of the prevalence of maternal alloimmunisation is useful in diagnosis and management of cases of haemolytic disease of foetus and newborn (HDFN).

HDFN is a condition caused by maternal antibodies to foetal red cell antigens which cross the placenta and cause haemolysis in foetus. The sensitizing event causing alloimmunization is frequently a previous pregnancy or a transfusion, where the mother was exposed to the relevant antigen. HDFN due to alloimmunisation shows wide spectrum of severity, some may have only mild jaundice on first day of life, but rapid fall of haemoglobin than other newborn infants. In others jaundice develops more rapidly, unless treated by exchange transfusion may lead to kernicterus and permanent brain damage. With a still more severe haemolytic process, profound anaemia develops and the infant may die in utero at anytime from about seventh week of gestation onwards[4].

In the present study, maximum cases were from the age group 26-35 years (52.5%).The mean age of the study population was 28.9 + 5.3 years, minimum age being 18 years and maximum age 41 years. Suresh B et al[5] also reported the similar finding where the mean age of their study were 23.8 + 3.3 years (range 18-39 years).

In the present study, the frequency of A, B, O and AB phenotypes of the patients were found to be 31.5%, 34%, 26.5% and 8% respectively. There is wide variation in the frequency of ABO not only in population of various parts of India but also in different countries of the world. In a study conducted by PahujaS et al[3] in New Delhi, the most common blood group phenotype was B group (37.18%) which is similar to the present study. In contrast to a study by Suresh B et al[5], O group was the most common phenotype (41.9%) followed by B group (31.8%).

The prevalence of red cell alloimmunisation in the multigravida in the present study population was 1%.The result was consistent with those reported in previous studies such as Suresh B et al[5] 1.1%, PahujaS et al[3] 1.3%, Varghese J et al[6] 1.5%, Devi SA et al[7]1.4%, Howard et al[8] 1%.In contrast, Gottvall et al[9] found an alloimmunisation rate of 0.4% in all pregnancies with clinically significant alloimmunisation in 0.16% of pregnancies. The alloimmunisation rate recorded by Vrijer B et al[10] was 2.71% which is higher compared to the present study.

In the present study, 5(2.5%) women were RhD negative. Incidence of RhD negative phenotype reported from other studies was comparatively higher. The frequency of RhD negative is slightly higher in other parts of India i.e. 6.4% in a study by Varghese J et al[6] in Vellore, by PahujaS et al[3] in Delhi, by Suresh B et al[5] in Tirupati. Karim F et al[11] found 13.6% women of their study population in Southern Pakistan to be RhD negative which is much higher compared to the present study. Naje AA et al[12] also reported higher rate of RhD negative (5.9%) in the study conducted in Saudi Arabia.

The red cell alloimmunisation rate in the D antigen negative group was 20 % (1/5) in the present study. There was a significant correlation between alloimmunisation and D antigen negative group of women. In the literature, there is a wide variation in alloimmunisation rates among Rh-negative women. Lurie et al[13] found a low alloimmunisation rate of only 0.9% among D antigen negative group women in their study conducted in Israel. The alloimmunisation rate among D antigen negative group women as reported by Naje AA et al[12]was 7.1%, by PahujaS et al[3] was 10.4%, by Suresh B et al[5] was 12.8%.The allosensitization observed in RhD negative women in the present study was 20% which is higher compared to other studies. This may be attributed to the better access of health care services in other places and moreover the low incidence rate of RhD negative women in the present study which was only 2.5%.

The red cell alloimmunisation rate in the D antigen positive women is 0.5% in the present study. Suresh B et al[5] and Lurie S et al[13] also reported the alloimmunisation rate of 0.3% and 0.2% respectively in the RhD positive women in their study population. PahujaS et al[3] found a low alloimmunisation rate among the RhD positive women (0.1%).This is much lower as compared to the present study, which may be due to the higher prevalence of RhD positive women in the present study than in the study by PahujaS et al[3] (97.5% vs 89%).

In the present study, red cell alloantibodies detected in two (1%) patients were identified as anti-E and anti-Le[a]. No anti-D was identified in the present study despite 2.5% of the study population being RhD negative. This finding was in accordance to the previous study conducted by Jeremiah ZA et al[14] where it was found that no anti-D was identified despite 8.6% of the study population being RhD negative.

Red cell alloantibodies identified in the present study, anti-E and anti-Le[a] were from gravida 3(1.4%) and gravida 4(9.1%) respectively. The gravida status of women showed a statistically significant correlation with the rate of alloimmunisation in the present study. The result was consistent with those studies reported by Suria AA et al[2], PahujaS etal[3], Suresh B et al[5].Each pregnancy increases a woman's risk of fetomaternal circulation leak, and therefore increases the chance of alloimmunisation.

The incidence for alloimmunisation shown by the present study was 0.58% (1/171) in women with normal obstetric history and 3.45% (1/29) in those with bad obstetric history. In the present study, the gravida 3 had a history of abortion and she was positive for anti-E.The other patient, who was a gravida 4 was a Rh negative mother with a previous normal obstetric history.PahujaS et al[3] also reported a high incidence of alloimmunisation in women with bad obstetric history (5.5%).But the incidence found in the present study was more compared to the study conducted by Suresh B et al[5] 1.92%, Suria AA et al[2] 2.86%.

Regarding the ethnicity, the majority (84.5%) of cases in the present study were Meiteis followed by MeiteisPangal (13.5%).The rate of alloimmunisation among Meiteis in the present study is 0.6% and that of Meitei Pangal is 3.7%.Lee et al[15] reported the prevalence of clinically significant antibodies among Chinese pregnant women to be 0.27%.Different ethnic backgrounds may be a confounding factor that influences the prevalence of alloantibody production in pregnant women. Nevertheless, due to the small sample size, a bigger study is needed to improve the power of study and to differentiate between ethnic groups in this part of the country.

In developing countries like India, antenatal screening is generally targeted solely at detection of anti-D in Rh negative mothers and routine antenatal antibody screening is done for RhD negative mother only as reported by Franco A et al[16].However,Pahuja S et al[3],Suresh B et al[5],Varghese J et al[6] reported the development of alloantibodies in RhD positive women also. In the present study,0.5% of alloantibodies were observed in RhD positive women. The present study also included both Rh positive (97.5%) and Rh negative (2.5%) women. Hence, antibody screening of both Rh positive and negative women is necessary.

V. Conclusion

The prevalence of red cell alloimmunisation in multigravida in the present study was 1%. The alloantibody identified was anti-E and anti-Le[a]. They were found in women of gravida 3 and 4 and it was statistically significant. Red cell alloimmunisation was significantly associated with ethnicity (more in Meitei ethnic group). Alloimmunisation was found in 0.5% of the Rhesus D positive women and in 20% of the Rhesus D negative women, which was statistically significant. But, it was not statistically significant with age and ABO blood group.In conclusion, red cell alloimmunisation is prevalent in around 1% of the multigravida women and significantly associated with gravida and ethnicity.

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