


Research Article

Frequency of Alloimmunization Against D Antigen and its Titre among the RhD Negative Pregnant Women at Tertiary Level Hospital in Bangladesh

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Abstract

Background: Rh alloimmunisation in pregnancy and blood transfusion The Rhesus (Rh) blood group system was discovered by Landsteiner and Wiener in 1940. It happens when an RhD-negative mother's blood is exposed to RhD-positive RBCs, causing an immune response and the production of antibodies against RhD-positive cells.

Materials and methods: A cross-sectional study was conducted in the Department of Transfusion Medicine at tertiary level hospital in Dhaka, Bangladesh from August 2023 to July 2024. A total of 51 Participant were enrolled in this study according to inclusion exclusion sampling and a semi-structured questionnaire. The study was carried out over the course of one year, following protocol approval from the Institutional Review Board (IRB).

Results: Nearly one third of the participants, 15 (29.4%), had detectable antibodies, while the remaining 36 (70.6%) participants did not. Almost 1/3, 5 (33.3%), of the participants had an antibody titre ratio of 1:2. 3 (20%) participants had a titre ratio of 1:16. 2 (13.3%) participants each had titres of 1:4 and 1:8, respectively. 1 (6.7%) participant had a titre of 1:32, and another 1 (6.7%) participant had a titre of 1:64. The highest measured titre was 1:256 for 1 (6.7%) participant.

Conclusion: The study on alloimmunization among RhD-negative pregnant women at tertiary level hospital in Bangladesh found that most women were aged 26-30 years and had B- negative blood. While antibody detection was more common in those with B- negative blood and B-positive husbands, the difference was not significant. In our country, the majority of the population has blood group B, so I observed a higher detection of antibodies in individuals with B negative blood group.

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Introduction

The Rhesus (Rh) blood group system is the second most important blood group system in clinical practice; after ABO, it has a critical influence on matters such as transfusion medicine, pregnancy and hemolytic disease of the newborn (HDN) [1]. The Rhesus system was first seen in 1940 when the rh antigen was found in rhesus monkeys (macaca mulatta), a primate which shared this extracellular red cell antigen with man. About 85% of RBC containing individuals are RhD antigen bearing or positive, whereas the remaining 15% lacks the RhD antigens and are said to be Rh-negative [2]. The immunogenicity of the D antigen ensures that Rh-negative individuals will mount a vigorous immune response to

such exposure to Rh-positive blood. These Rh antigens are transmitted as homozygotes or heterozygotes (one gene from each parent). For instance, if the parents are Rh-positive, there is a good chance that the baby will inherit D antigen and be RH positive [3]. If one or both parents are Rh negative, the inheritance pattern of an Rh-negative or Rh-positive offspring may be determined on the basis of those genetic combinations. In the clinical setting, individuals are either classified as Rh-positive or Rh-negative based on whether they carry the D antigen. This difference is particularly relevant in pregnancy. If the mother has Rh- negative blood and her partner has Rh-positive, then instead of receiving an Rh- negative gene from the father, the baby could receive a positive gene. This can cause Rh incompatibility between the mother and fetus, with potential complications. If fetal red cells that express the D antigen are able to penetrate into the maternal circulation (i.e. fetomaternal hemorrhage), the mother's immune system might perceive these cells as foreign and will make antibodies against them. This immune response is known as Rh sensitization [4]. Rh sensitization was first identified as a clinically important condition by Philip Levine in 1941 when he described how RHD negative women who were carrying an RHD positive fetus could have an immune response leading to antibodies that caused the devastating illness, hemolytic disease of the newborn (HDN), including stillbirth or very severe anemia in the fetus [5]. RhD antibodies, predominantly of the IgG type, have the ability to pass through placenta in pregnancy. These antibodies react against Rh positive RBCs in the fetus and cause hemolysis. The FcRn receptor for IgG, expressed on the placenta (placental FcR), is a major determinant of the transplacental transfer of maternal IgG antibodies to the fetus, and in some cases could be associated with overwhelming hemolysis of fetal erythrocytes secondary to Rh immune sensitization causing life-threatening anemia [6]. Rh immunization is most often induced by fetomaternal hemorrhage, which may be associated with miscarriage, abortion, injury, invasive obstetric procedures or delivery. Sensitization is irreversible after it has developed. The mother's immune system has a memory of this, so further pregnancies with an Rh-positive baby carry the risk. During subsequent pregnancies, maternal anti-D IgG antibodies can cross the placenta and bind to fetal RBCs leading to hemolysis. This breakdown in red cell can cause HDN- hemolytic disease of the newborn It may be mild anemia or hydrops fetalis-a form of severe heart failure that can threaten a baby's life [7]. If fetomaternal hemorrhage takes place and maternal sensitization has started, the maternal antibodies are only produced to the paternally derived RhD antigen. Those antibodies then circulate through the placenta and bind to your fetus's red blood cells, marking them for elimination by the immune system. The immune system of the fetus breaks down the coated red blood cells causing HDN. The degree of HDN ranges in severity, from

mild cases with manageable anemia to severe hydrops fetalis or intrauterine demise.

Materials and Methods

It is an observational cross-sectional study. In this study we selected RhD-negative pregnant women after fulfilling the eligibility criteria in the Department of Transfusion Medicine tertiary level hospital in Dhaka, Bangladesh from August, 2023 to July, 2024. In this study a total of 51 participants were included.

Inclusion and Exclusion criteria

RhD-negative women came in Transfusion medicine irrespective of their age, parity, gestational age and administration of Rh Anti-D IgG in previous or present pregnancy. Singleton pregnancy. RhD-positive pregnant women. RhD-negative non-pregnant subject. Fail to give consent.

Data collection procedure

The data collection procedure was initiated by the researcher. First of all the researcher explained the aim and objectives of the study. The written informed consent was taken from all of them. The researcher interviewed every participant face to face asking questions in Bengali. For each participant data concerning age, blood group, number of gravida, para, presence of positive titre, previous rats, history of transfusion, H/O immunization and all other information related to study given by participant was carefully noted. All the information was preserved in the data collection sheet.

Statistical analysis

All the relevant data was compiled on a master chart first and then statistical analysis of the result was obtained by using the Statistical Package for Social Science (SPSS) version 22 was used for statistical analysis (SPSS Inc., Chicago, Illinois, USA).

Ethical Consideration

In this study, random Rh negative women who are directed and non-remunerated were selected as study participants using the inclusion and exclusion criteria. All the participants were properly explained about the study and informed written consent was taken. The study did not involve any additional investigation that might cause financial burden to the participant. Every participant has freedom to quit. This study had to be approved by the "Institutional Review Board" of BSMMU. All the information collected from the participants including the results of the laboratory test was kept confidential under the responsibility of principal investigator. No one except the investigators, regulatory authorities and Institutional Review Board (IRB) were having access to such information. The participant's identity was not disclosed while analyzing or publishing the results.

Quality assurance strategy

After participant selection with proper SOP of the Department of Transfusion Medicine blood is collected. Protective gloves were worn during collection and sterile gauge, syringe, test tube was used very cautiously. Direct supervision by the guide and co- guide was ensured. The investigator was trained properly to conduct the research. Review of the study status was done by guide and co-guide.

Result

Among the 51 Rh-negative pregnant women, 15 (29.4%) were found to have Rh Anti- D antibodies (alloantibody), while 36 (70.6%) had no anti-D antibodies detected. Of the women with Rh Anti-D antibodies, 5 (33.33%) had a titre ratio of 1:2.

Table 1: Distribution of the study population according to age (n = 51)

Age (in years)	Number of population	Percentage
20-25	16	31.4
26-30	20	39.2
>30	15	29.4
Mean ± SD	28.08 ± 4.53	
Range (min-max)	20-36	

Table 1 shows the distributions of the study population by age. It was observed that more than one third (39.2%) of the population belonged to the age 26-30 years. The mean age was 28.08±4.53 years with the range from 20 to 36 years.

Figure 1 shows the distributions of the study population by population blood groups. It was observed that more than one third 18 (35.3%) population had B negative blood group, 17 (33.3%) O negative, 10 (19.6%) A negative and 6(11.8%) had AB negative blood group.

Table 2 shows the distributions of the study population by population husband blood groups. It was observed that one-

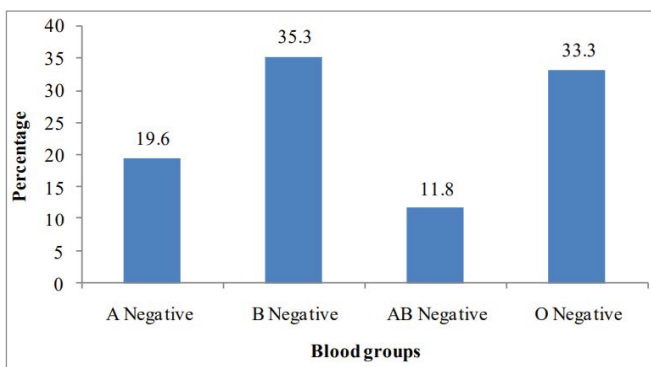


Figure 1: Bar-diagram showing distribution of the study patients by blood groups

third of the 17 (33.3%) population husbands had B positive blood group, 15 (29.4%) A positive, 12 (23.5%) O positive and 7 (13.7%) AB positive blood group.

Table 2: Distribution of the study population according to population husband blood groups (n = 51)

Blood groups	Number of population	Percentage
A Positive	15	29.4
B Positive	17	33.3
AB Positive	7	13.7
O Positive	12	23.5

Table 3: Distribution of the study population according to obstetric history (n=51)

Obstetric history	Number of population	Percentage
Gestational age (weeks)		
<10	6	11.8
Oct-20	15	29.4
20-30	14	27.5
>30	16	31.3
Mean±SD	23.47	±9.49
Range(min-max)	7	-37
Gravida		
First gravida	7	13.7
Second gravida	13	25.5
Third gravida	17	33.3
Fourth gravida	11	21.5
Fifth gravida	1	2
Sixth gravida	1	2
Eighth gravida	1	2
Para		
Nulli Para	20	39.2
Para one	18	35.3
Para two	10	19.6
Para three	2	3.9
Para six	1	2

Table 3 shows the distributions of the study population by obstetric history. It was observed that almost one third 16 (31.3%) population belonged to gestational age >30 (weeks). The mean age was 23.47±9.49 (weeks) with the range from 7 to 37 (weeks). Almost one third 17 (33.3%) of the population had third gravida. More than one third of the 18 (35.3%) population had one para.

Table 4 shows the distributions of the study population by children's living status. It was observed that more than one third of the 22 (43.1%) population had no living child. Almost three fourths of the 36 (70.6%) population had no dead child.

Table 4: Distribution of the study population according to children living status (n = 51)

Children living status	Number of population	Percentage
Number of living child		
No living child	22	43.1
One living child	18	35.3
Two living child	10	19.6
Three living child	1	2
Number of dead child		
No dead child	36	70.6
One dead child	11	21.6
Two dead child	4	7.8

Almost two third (60.7%) of the population had no abortion history, 13 (25.5%) had one abortion, 3 (5.9%) had two abortions, 3 (5.9%) had three abortions and 1 (2.0%) had six abortion histories.

Table 5 shows the distributions of the study population by history of blood transfusion. It was observed that 9 (17.6%) of the population had a history of blood transfusion.

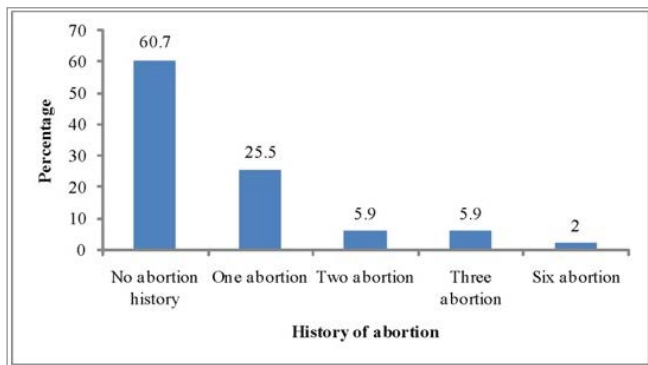


Figure 2: Bar-diagram showing distribution of the study patients by history of abortion

Table 5: Distribution of the study population according to history of blood transfusion (n=51)

History of blood transfusion	Number of population	Percentage
Yes	9	17.6
No	42	82.4

Table 6 shows the distributions of the study population by previous antibody titre. It was observed that eleven (21.6%) population had previous antibody titre detected and 40 (78.4%) were not detected. More than one third 4 (36.3%) population belonged to 1:8 ratio previous antibody titre.

Table 6: Distribution of the study population according to previous antibody titre (n=51)

Previous antibody titre	Number of population	Percentage
Detected	11	21.6
Not detected	40	78.4
Previous antibody titre ratio (n=11)		
01:02	3	27.3
01:04	3	27.3
01:08	4	36.3
01:16	1	9.1

Table 7 shows the distributions of the study population by antibody detection. It was observed that nearly one third 15 (29.4%) of the population had antibodies detected. Almost one third 5 (33.3%) population belonged to 1:2 ratio antibody titre.

Table 7: Distribution of the study population according to antibody detection (n=51)

Antibody detection	Number of population	Percentage
Detected	15	29.4
Not detected	36	70.6
Antibody titre ratio (n=15)		
01:02	5	33.3
01:04	2	13.3
01:08	2	13.3
01:16	3	20
01:32	1	6.7
0.086111111	1	6.7
0.219444444	1	6.7

Table 8 shows the association between gravida and antibody detection. It was observed that one third 5(33.3%) population had third gravida in detected and 12(33.3%) in not detected. One third 5(33.3%) population had one para in detected and 13(36.1%) in not detected. The difference was statistically not significant ($p>0.05$) between two groups.

Table 9 shows the association between history of abortion and antibody detection. It was observed that one third 5(33.3%) population had one abortion in detected and 8(22.2%) in not detected. The difference was statistically not significant ($p>0.05$) between two groups.

Discussion

This was an observational cross-sectional study carried out to find the incidence of alloimmunization against anti RhD and determination of their titre levels in pregnant women who were RhD negative. The eligibility criterion for Rh-negative women was a visit to the Transfusion Medicine department,

Table 8: Association between obstetric history and antibody detection (n=51)

Obstetric history	Detected (n=15)		Not detected (n=36)		P value
	n	%	n	%	
Gravida					
First gravida	1	6.7	6	16.7	0.42
Second gravida	4	26.6	9	25	
Third gravida	5	33.3	12	33.3	
Fourth gravida	3	20	8	22.2	
Fifth gravida	1	6.7	0	0	
Sixth gravida	0	0	1	2.8	
Eighth gravida	1	6.7	0	0	
Para					
Nulli Para	6	40	14	38.9	0.515
Para one	5	33.3	13	36.1	
Para two	2	13.3	8	22.2	
Para three	1	6.7	1	2.8	
Para six	1	6.7	0	0	

Table 9: Association between history of abortion and antibody detection (n=51)

History of abortion	Detected (n=15)		Not detected (n=36)		P value
	n	%	n	%	
No abortion history	8	53.3	23	63.9	0.357 ^{ns}
One abortion	5	33.3	8	22.2	
Two abortion	0	0	3	8.3	
Three abortion	1	6.7	2	5.6	
Six abortion	1	6.7	0	0	

irrespective of age, parity, gestational age and whether or not they had received Rh Anti-D immunoglobulin in previous or current pregnancy. Women with multiple pregnancies were excluded. Discussion The findings of the current study were compared with relevant studies which had been previously conducted. The mean age of the patients was 28.08±4.53 years (range: 20-36 years), and 39.2% of the included patients were between 26 and 30 years of age. This peak age incidence is consistent with other reports that women in their late twenties/early thirties are most likely to attend antenatal care and be tested for alloimmunization [8]. The reason why a higher rate was in this age group could be related to the more reproductive self-memberships and increased attention on prenatal health. This population feature emphasizes the need for focused interventions in maternal care to avoid alloimmunization [2]. In this study, a marked difference in blood group frequencies were noted between RhD negative pregnant women and their Rh positive husbands. Blood

groups common in the females were B Negative (35.3%) > O Negative (33.3%), A Negative (19.6%) and AB Negative (11.8%). In contrast, the husbands were mostly Rh positive with B Positive (33.3%) as the commonest followed by A Positive (29.4%), O Positive (23.5%) and AB Positive (11.8%). This discrepancy indicates the possible risk of alloimmunization because of Rh incompatibility, especially among B Negative and O Negative women with Rh Positive husbands. Published studies such as Shah et al. [8] and Arthur & Stowell [9] observed that the balance of these blood groups among Rh-negative women have not been different from other population data, so highlighting for an universal use of comprehensive Rh immunoprophylaxis to prevent the risk and associated complications with alloimmunization.”. The commonest blood group in the ABO series of our country is type B. In the present study, 49.1% of the studied subjects had the B negative blood group. This study demonstrates a high proportion of nulliparous RhD-negative women (39.2%) in the population studied and less prevalence of parity. In other studies, e.g., Maier et al. [10], the people factor s, for example number of previous pregnancies have been shown to affect alloimmunization rates, supporting the need for careful monitoring of multiparous women. Even with new advances in Rh prophylaxis, a small number of studies have indicated ongoing risk of sensitization even when receiving prophylactic anti-D. Rentta et al. [2], which reported a similar balance of parity in Rh-negative pregnancies. They may reflect different sensitization behaviours, where the probability of alloimmunisation is increased in multiparous women because fetal blood exposure was enhanced during their previous pregnancies. The study therefore emphasizes the importance of taking measures for active alloimmunization prevention, specifically in areas like Bangladesh where Rh-incompatibility still poses as a significant burden [11]. The history of blood transfusion was noted in 17.6% patients of the study. This is important because prior transfusions can cause alloimmunization and raise the risk of complications in future pregnancies. According to Arthur and Stowell [9], red blood cell transfusions are frequently administered as a therapeutic intervention, however, they carry an alloimmunization risk profile particularly in Rh-negative individuals.

In this study, 29.4% of the patients had present antibody titres. 34.3%, 1:2 titre; and 20.0% for titres of 1:16, then titres of both 1:4 and 1:8 were equally distributed at the percentage frequency of 13.3% accounted for sera with titres of either, then values steadily declined as follows; (6.7%) each for representing titres at maximum dilutions recorded as; (6.7%). This distribution demonstrates the variation in Rh-specific antibody response among Rh-negative pregnant women and is a reflection of their alloimmunization status. The existence of high antibodies titres, a titre greater than or equal to 1:32 for example, might indicate that there is

significant alloimmunization and potential danger to HDFN [12,13]. The relationship between blood group and antibody detection was investigated; over one half of the Rh- negative pregnant women infected with C.D. had B negative blood, while their husbands and a majority of them had B positive. The aforementioned trends were statistically insignificant, however ($p>0.05$). This is consistent with the results of Maier et al. [10] found, that particular blood group combinations have a higher risk of alloimmunization, particularly when including B negative and positive pairs. For ABO, if both partners were of group B the protection against RhD alloimmunization was approximately 55%. If either the fetus is AB positive or the father is AB positive and mother RhD negative, then there is a greater chance of RhD alloimmunization [14]. In our country, it is the most common blood group and I saw more antibodies detection in B negative. However, Rentta et al. [2] and they highlighted that even if some blood groups increase the risk of alloimmunization, in this study there was no meaningful evidence which show otherwise; as a matter of fact previous pregnancies or transfusions might be the relevant factors. Such findings further emphasize the necessity of more extensive studies on blood group effects in alloimmunization and antibody detection [15,16].

Conclusion

The study on the frequency of alloimmunization due to red cell Anti-D antibodies among Rh-negative pregnant women at tertiary level hospital in Dhaka, Bangladesh. The majority of the women were aged between 26-30 years, with a significant proportion having a B-negative blood group. While antibody detection was more common in women with B-negative blood and in those with husbands having B- positive blood. If the fetus is AB positive or the father is AB positive and the mother is RhD negative, there is a higher likelihood of RhD alloimmunization. In our country, the majority of the population has blood group B, so I observed a higher detection of antibodies in individuals with B negative blood group. However, a statistically significant difference was observed in women with a history of blood transfusion, highlighting the importance of these factors in alloimmunization. The findings emphasize the need for improved Rh immunoprophylaxis and monitoring, especially in women with prior transfusions or previous sensitization.

Limitations

Adequate medical history was not documented. The immediate impact was observed, but long-term effects were not assessed. Some associated factors could have been addressed but were not. The reliability and validity of the findings were not confirmed. The study was limited by a small sample size.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee.

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