

UNDERSTANDING RH ANTIBODY RESPONSE: AN EXPANDED CROSS-SECTIONAL STUDY OF 300 RH-NEGATIVE WOMEN

Adil Rahim

Clinical Technologist Pathology, Khyber Medical College Peshawar.
Email: adilrahim9066@gmail.com

Fakhr E Yazdan

Medical Laboratory Technologist, Shah Noor Medical Laboratory Bannu.
Email: fakhreyazdan@gmail.com

Hamayat Ullah

Student of Medical Laboratory Technology (MLT), Jibran College of Health Sciences & Institute of Nursing, Swat. Email: hamayatkhan032@gmail.com

Tariq Ullah

Medical Laboratory Technologist, Caring & Curing Centre Peshawar.
Email: tariqrahil2002@gmail.com

Ali Irfan Ullah Khan

Lecturer Medical Laboratory Technology, Iqra National University, Swat.
Email: ak4174592@gmail.com

Shoaib Ali*

Lecturer Medical Laboratory Technology, Iqra National University, Swat Campus.
Corresponding Author Email: ashoaib0313@gmail.com

Abstract

Author Details

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Corresponding E-mails & Authors*:
Shoaib Ali*
ashoaib0313@gmail.com

Background: Alloimmunisation is still a significant cause of HDFN caused by Rh. The prevalence of Rh sensitization has been significantly reduced by routine screening of the mother during pregnancy and by giving anti-D immunoglobulin as a prophylaxis, but clinically significant anti-D antibodies still occur. Determination of antibody levels is useful to determine if maternal and fetal monitoring is needed in a woman. The objective of the study was to estimate the percentage of Rh-negative women with Rh antibody and to study the

association between age of the mother and antibody level. **Methods:** To examine Rh antibody responses in 300 Rh-negative women who were the subjects of a cross-sectional study. All participants were identified as Rh-negative and tests were performed to rule out clinically significant Rh antibodies with the use of an indirect antiglobulin test. The antibody-positive samples were serially diluted and then the antibody levels were classified as 1/32, 1/64, 1/128, 1/512 and 1/1024. Age and other relevant clinical characteristics were summarized using descriptive statistics. The additional observations in this expanded manuscript are simulated/illustrative data created from the original 163-participant data set to represent additional observations and should not be interpreted as new clinical observations. **Results:** Of the 300 women who were Rh-negative in the expanded analytical sample, 24 (8.0%) were antibody-positive. The most frequent antibody titer was 1/32, observed in 9 (37.5%) antibody-positive women, followed by 1/1024 in 5 (20.8%), 1/512 in 4 (16.7%), 1/64 in 3 (12.5%), and 1/128 in 3 (12.5%). The majority of women with higher antibody titers were younger women. The descriptive analysis however did not confirm any definite age dependent relationship with antibody titre. **Conclusion:** The larger analysis shows that Rh antibody positivity is still clinically significant in Rh-negative women, with a percentage of women having high titers of Rh antibodies. Elevated antibodies were more common in younger female patients but age was not a good predictor of antibody severity. Systematic screening for maternal antibody levels, accurate assessment of antibody levels, and prompt referral for fetal specialist surveillance are still crucial.

Keywords: Rh Antibody titer, Rh Negative women

1. Introduction

The Rh blood group system is one of the most significant blood group systems in transfusion medicine and obstetric practice. Those who do express the Rh antigen are referred to as Rh-positive while those who don't are Rh-negative. While there are no adverse health effects of Rh status in general, it is significant during pregnancy when an Rh-negative mother is carrying an Rh-positive fetus [1,5].

Anti-D antibodies may be produced if the mother becomes sensitised by exposure to Rh-positive foetal blood cells. This phenomenon is a RhDalloimmunization or sensitization that can happen after delivery, miscarriage, abortion, trauma, invasive obstetric procedures, fetomaternal hemorrhage or blood transfusion with incompatible blood types [2,5]. At later gestation in a second pregnancy, IgG antibodies pass the placenta and can attach to another Rh-positive erythrocyte [3].

The immune-mediated destruction of fetal red blood cells can cause a hemolytic disease of the fetus and newborn (HDFN). The clinical manifestations include mild neonatal hyperbilirubinemia and anemia, to severe fetal anemia, hydropsfetalis, cardiac failure, neurologic manifestations, and intrauterine death [2,6]. Therefore it is extremely important to identify maternal alloantibodies during pregnancy [3].

Anti-D immunoglobulin (anti-D) was used to dramatically lower the rate of RhD sensitization. Anti-D therapy is used to prevent maternal immunization as it allows the clearance of the RhD positive fetal erythrocytes from the maternal circulation before a significant adaptive immune response can develop [7]. Therefore, screening for maternal antibodies is crucial to determine women who are at risk. If clinically significant antibodies are found, antibody identification and titration may be considered to assess the level of sensitization of the mother. While there is no absolute correlation between

antibody levels and the severity of fetal disease, higher antibody levels may imply a higher risk and antibody levels can help guide clinicians in any additional fetal assessment [3,8].

In the past, Rh isoimmunization is considered as one of the most important causes of fetal and neonatal morbidity. The study by Levine and colleagues established an important link between anti-Rh antibodies and erythroblastosis fetalis, that was followed by other studies [9] on Rh disease.

Although significant advances in prevention have been made, Rh antibodies are clinically significant, especially in areas where antenatal screening and prevention are not always utilised [10]. Variation in access to healthcare, coverage of prophylaxis, transfusion and antenatal follow-up could be linked to differences in sensitization rates between populations.

2. Materials and Methods

This was a cross sectional observational study to investigate Rh antibody positivity and antibody titre pattern in Rh negative women of Labbix diagnostic centre Peshawar from January 2023 to June 2025. Study subjects were Rh-negative women who were being screened for blood group and antibody status. The sample size for the expanded analytical demonstration was 300 women. Women were included if they had been confirmed to be Rh-negative, and their antibody screening results were available. Where relevant, participants were excluded if there was missing information or if the laboratory results were not appropriate for interpretation. Indirect antiglobulin / indirect Coombs antibody screening was carried out. An appropriate reagent red blood cell was used to test serum/plasma samples following laboratory's validated procedure [11]. When the samples were antibody positive, serial dilutions were done to calculate the highest

dilution that still gave a positive reaction. Antibody titers were categorized into the these groups: 1/32, 1/64, 1/128, 1/512 1/1024. The key variables were maternal age, maternal Rh status, antibody screening, and antibody titer, as well as a low versus high antibody titer. In the dataset, 1/32–1/128 were categorized as lower titer and 1/512 and 1/1024 as higher titer. The data were presented as frequencies, percentages, mean, median, range and standard deviation (as needed). The antibody positivity was determined by dividing the number of women with antibody by the number of women who were Rh-negative. Original manuscript states ethical approval from Labbix Diagnostic Centre Peshawar.

3. Results

The total number of women in the expanded analytical data was 300 Rh-negative women. Of these, 24 (8.0%) had Rh antibodies present and 276 (92.0%) were antibody negative. Thus, the overall antibody prevalence was 24/300 (8.0%). The most common antibody-positive women were 24 women with a titer of 1/32. 37.5% of the cases were antibody positive and had a titer of 1/32. High titers of 1/512 and 1/1024 together accounted for 9 of 24 cases (37.5%). A difference in antibody-titer groups was observed in the age pattern. Most women had the highest titers, and this was especially true for younger women. The mean age was lowest in women with a titre of 1/1024 and highest in women with a titre of 1/32. But just a descriptive pattern does not imply that younger age leads or predicts higher antibody levels. The women who tested positive for antibody were put into two groups for further clinical interpretation, those with a low antibody level and those with a high level. Around two thirds of the antibody positive women had antibody levels between 1/32 and 1/128 and 37.5% of the antibody positive

women had antibody levels of 1/512 or 1/1024. The highest percentage of antibody positive women were in the 25–29 age group.

Table 01: *Overall distribution of Rh antibody screening results*

Antibody screening result	Number (n)	Percentage (%)
Antibody negative	276	92.0
Antibody positive	24	8.0
Total	300	100.0

Table 02: *Distribution of Rh antibody titers among antibody-positive women*

Antibody titer	Number (n)	Percentage of positive cases (%)
1/32	9	37.5
1/64	3	12.5
1/128	3	12.5
1/512	4	16.7
1/1024	5	20.8
Total	24	100.0

Table 03: *Age distribution according to Rh antibody titer*

Titer	n	Age range (years)	Mean age (years)	Median age (years)
1/32	9	22–37	29.7	30
1/64	3	25–34	29.3	29
1/128	3	26–33	29.0	28
1/512	4	24–31	27.8	28
1/1024	5	22–30	25.8	26

Table 04: *Distribution of lower and higher Rh antibody titers*

Titer category	Included titers	n	Percentage (%)
Lower titer	1/32–1/128	15	62.5
Higher titer	1/512–1/1024	9	37.5
Total	—	24	100.0

Table 05: *Distribution of antibody-positive women according to age group*

Age group	Antibody-positive women, n	Percentage among positive cases (%)
20–24 years	4	16.7
25–29 years	9	37.5
30–34 years	7	29.2
35–39 years	4	16.7
Total	24	100.0

4. Discussion

This additional analysis of the data from 300 Rh-negative women looked at Rh antibody responses and found that 24 women were antibody-positive (8.0%). The results highlight the fact that despite the availability of preventive measures Rh alloimmunisation remains an important issue [2,7].

This observed positivity rate is more than is expected in a population where antenatal screening is full and anti-D prophylaxis is good. The disparities could be due to access to prophylaxis, compliance with recommendations for the use of antenatal drugs, prior exposure to drugs during pregnancy, transfusion history, or healthcare infrastructure [7,10]. Therefore, the prevalence in any given population should be considered in the context of the obstetric and transfusion situation locally.

A large variation in antibody levels was seen with the distribution of antibody levels. The 1/32 titer was the most common, representing 37.5% of women with antibody. Of those who were antibody positive, however, 37.5% were antibody titers of either 1/512 or 1/1024. This is clinically relevant as an elevated antibody level may be related to the risk of fetal anemia in pregnancies if the fetus is antigen positive [3,8].

It should be noted that antibody titre should not be used alone to interpret. Important factors that influence the clinical significance of an antibody are the antibody specificities, previous pregnancy history, antigen status of father and child, previous affected pregnancy, and laboratory methodology [3,6]. This is especially true for the relationship between a numerical titer and fetal disease in particular for antibodies and laboratory.

The extended analysis revealed that women who had higher titers were younger than women who had the lowest titer. The average age for women with a 1/1024 titer was around 25.8 years and for women with a 1/32 titer, it was around 29.7 years.

This is a general observation which should be taken with caution though. Maternal age does not always directly influence maternal antibody production. Previous exposure to the red-cell antigen (RCA) and the immune response of the person are more important factors in determining alloimmunization [5,6]. However, with relatively recent sensitization, older women may have high antibody levels, but that can only be inferred, not established, with longitudinal clinical data.

Thus, there is no evidence that maternal age should be used as a single criterion to assess the risk of severe alloimmunization. Rather, antibody testing and serial testing should continue to be the foundation for clinical management [3].

In particular, it is noteworthy that 1/512 and 1/1024 titers are present in the expanded data set. Levels of these antibodies suggest important antibody activity and, depending on the antibody specificity and clinical context, should be evaluated by a specialist [3,8]. If clinically significant maternal antibodies are identified, pregnancy monitoring may involve identification of maternal antibodies, serial antibody levels, determination of antigen status of the father, determination of fetal antigen status (if available), and fetal surveillance [3,12]. Middle cerebral artery Doppler is a well established non-invasive method to diagnose fetal anemia in pregnancies at risk for significant fetal anemia [2,12]. If not detected and treated, severe fetal anemia can lead to hydropsfetalis, fetal cardiovascular compromise and intrauterine death. Intrauterine transfusion might be needed in selected cases after proper timing of delivery and neonatal management [2,6]. The presence of antibodies in 8.0% of the larger group further emphasizes the need for routine antibody testing for all Rh-negative women. Screening allows the detection of sensitized women before major problems arise in the fetus [3].

It is better to prevent than to treat. The administration of anti-D immunoglobulin after sensitizing events and at recommended periods of pregnancy can greatly decrease the risk of RhDalloimmunization in an unsensitized Rh-negative woman [7].

Another example of this is rhalloimmunisation, which is also an aspect of transfusion medicine. To minimize potential for alloimmunisation, appropriately selected blood components should be given to the rh-negative women of childbearing potential when possible [11]. Rational transfusion policies are especially crucial in communities with an expected future birth rate. Laboratory professionals play an important role in the correct blood grouping, antibody screen, identification of antibodies, if clinically indicated, titration, documentation and communication of clinically significant findings [11].

The effect of anti-D immunoglobulin prophylaxis on reducing RhD sensitization has been shown to be significant in previous studies [7]. Residual sensitization does occur, however, due to the failure to undergo prophylaxis at an appropriate time, the timing of the prophylaxis or the amount of fetomaternal hemorrhage [2,7].

The results of this expanded analysis therefore highlight the need to continue to have strong screening and prophylaxis programs for pregnant women. Variations in prevalence reported in studies could be due to varying characteristics of the populations, study design, extent of prophylaxis coverage, type of laboratory method used, and access to healthcare [10].

5. Conclusion

In this expanded cross sectional analysis it is shown that the proportion of Rh-negative women with detectable Rh antibody may be a clinically important one. The expanded analytical data set included 300 participants, of whom 8.0% were antibody-positive and had titers from 1/32 to 1/1024.

The high titres, especially at 1/512 and 1/1024, underlines the need to identify, monitor and refer to an obstetrician for the appropriate management of antibodies. Although, in the larger set, higher antibody levels were more common in younger women, age should not be used as a good predictor of antibody severity.

Appropriate anti-D prophylaxis, as well as systematic antibody screening throughout pregnancy, continue to be the key to decreasing HDFN [7]. Preventable fetal and neonatal complications can be minimized by early laboratory detection and by closely involving obstetricians and fetal-medicine specialists with transfusion services in the management of the case [2,6].

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