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Consequences of being Rhesus D immunized during pregnancy and how to optimize new prevention strategies

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Key words
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Abstract

Objective. To analyze the timing of Rhesus D (RhD) immunization in pregnancy and the consequences for the index pregnancy and for subsequent pregnancies to be able to optimize the design of antenatal screening and prevention programs. *Design.* Retrospective cohort study. *Setting.* Stockholm county, Sweden. *Population.* All RhD immunized pregnant women 1990–2008 before the introduction of routine antenatal anti-D prophylaxis. *Methods.* Data were collected from transfusion medicine registers and databases, medical records, the Swedish Medical Birth Register and the National Perinatal Quality Register and entered into a standardized database before analysis. *Main outcome measures.* The order of pregnancy and trimester when immunization occurred and treatment of hemolytic disease of the fetus and newborn. *Results.* A total of 290 RhD immunized women were included in the study. In 147/290 (51%) of the women, sensitization occurred with their first-born child and in 96/290 (33%) it occurred with their second-born child. Anti-D antibodies developed during the second or third trimester in 212/290 (73%) and in 61/290 (21%) at term or after delivery. In subsequent pregnancies 56% (144/259) of the neonates required treatment for hemolytic disease of the fetus and newborn. *Conclusions.* Based on our study, at least half of the cases could potentially have been avoided by routine antenatal anti-D prophylaxis in the beginning of the third trimester. To optimize the beneficial effects of new prevention programs, we propose providing anti-D prophylaxis in gestational week 28–30 selectively to all RhD-negative women with RhD-positive fetuses.

Abbreviations: HDFN, hemolytic disease of the fetus and newborn; IgG, immunoglobulin G; IUT, intrauterine blood transfusion; RAADP, routine antenatal anti-D prophylaxis; RBC, red blood cell; RhD, Rhesus D.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) due to Rhesus D (RhD) immunization has become an infrequent, but still severe, complication of pregnancy. Once an RhD-negative woman becomes immunized, all sub-

Key Message

Most Rhesus D immunized women become sensitized with their first-born or second-born child. The majority of immunizations occur before delivery and the frequency of hemolytic disease of the fetus and newborn in subsequent pregnancies is high.

sequent pregnancies with an RhD-positive child will be affected, requiring extensive monitoring and timely prenatal or postnatal interventions (1). Prevention of RhD immunization and HDFN has been successful because of the discovery and introduction of anti-D prophylaxis. Postnatal anti-D-prophylaxis was introduced in the late 1960s and the risk of being sensitized decreased from 13% to approximately 1% (2). More recently, routine antenatal anti-D prophylaxis (RAADP) in the third trimester was introduced in several countries, reducing the prevalence of RhD immunization further to 0.2–0.3% (3,4). RAADP has not been introduced in Sweden, and the prevalence of anti-D antibodies in our pregnant population has been reported to be 1% (5).

Prophylaxis programs differ regarding timing of RAADP, dose of anti-D immunoglobulin (IgG) and number of doses (6). Recently routine fetal *RHD* genotyping using cell-free DNA in maternal plasma to selectively provide RAADP to the 60% of RhD-negative women with an *RHD*-positive fetus was introduced in Denmark and the Netherlands (7). This strategy enables a more efficient use of anti-D IgG and will probably be introduced in many other countries. We and others have shown that first-trimester fetal *RHD* screening is highly accurate, enabling selective anti-D prophylaxis to be provided not only in the third trimester but also earlier in pregnancy after miscarriages, terminations of pregnancy and invasive prenatal testing (8,9). A still unresolved issue is when during pregnancy fetal *RHD* screening should be introduced and how to optimize antenatal prophylaxis strategies.

The aim of the present study was to analyze the timing of RhD immunization in pregnancy and the consequences for the index pregnancy and for subsequent pregnancies. Most studies on the epidemiology of RhD immunization are old and updated information is important when designing and introducing new antenatal screening and prevention programs.

Material and methods

This is a retrospective cohort study of all RhD immunized pregnant women in the Stockholm county from 1990 to 2008. During this period, all pregnant women with red blood cell (RBC) antibodies were managed at the Karolinska University Hospital. Women referred to us from outside the region or from other countries were excluded from the analysis.

Post delivery anti-D prophylaxis in nonimmunized women was introduced in Sweden in 1969. In addition, anti-D prophylaxis was administered during pregnancy at interventions posing a risk of fetomaternal hemorrhage and since 1975 after all surgical terminations of pregnancy and spontaneous or induced abortions after 12 weeks of gestation. The dose was 250–300 μg anti-D IgG after 12 weeks of pregnancy and after delivery, before 12 weeks half the dose. RAADP has not been introduced in our country.

Primary outcome variables were timing of sensitization and frequency of HDFN. For the diagnosis of HDFN, antenatal and/or neonatal treatment for hemolytic disease was required, such as intrauterine blood transfusion (IUT), exchange transfusion or phototherapy. Severe HDFN was defined as need for IUTs or exchange transfusion to the newborn. Secondary outcome variables were perinatal outcome including fetal or neonatal mortality, prematurity due to HDFN and number of subsequent children following the pregnancy when sensitization occurred (index pregnancy). In the analysis, the women were divided into three groups depending on parity: women sensitized with their first-born child (group 1), with the second-born child (group 2) and with the child born third or later (range third to seventh child) (group 3).

The antenatal screening program for RhD-negative women in Stockholm has included testing for maternal RBC antibodies in the first trimester and additional testing around weeks 25 and 37 of gestation. In nullipara, antibody testing in the second trimester (i.e. 25 weeks) was not included in the program. Women with RBC antibodies were referred to the Karolinska University Hospital for further management. Women who had previously given birth to an RhD-positive child and had detectable anti-D antibodies in the first trimester in a subsequent pregnancy were considered to have been sensitized at the time of parturition during the previous pregnancy. In women who seroconverted during the ongoing pregnancy we used the date of the first positive antibody test to define in which trimester of pregnancy they were sensitized. In the analysis, prematurity was defined as birth before 37 completed weeks of gestation. Blood group serology and direct agglutination test were performed on cord blood in all neonates born to an RhD-negative mother.

All RhD immunized women in the cohort were identified using the register of the Department of Immunology and Transfusion Medicine. Data on antenatal IUTs and postnatal exchange transfusions were obtained from the same source. Results from RBC antibody screening, identification and titration were obtained from the transfusion medicine laboratory information system. Information regarding obstetric and perinatal outcome, pregnancy interventions and postnatal treatment was retrieved from medical records, local databases at the Department of Obstetrics and Gynecology and the National Perinatal Quality Register for neonatal care (www.pnq.se). All data were entered into a web-based quality register for red cell immunization during pregnancy (www.gravimm.se). In

each case, the date of first positive anti-D antibody titer was confirmed with the laboratory information system at the Department of Immunology and Transfusion Medicine.

To be able to investigate the birth rate in RhD-negative women, all RhD-negative pregnant women were identified from the transfusion medicine laboratory information system and compared with the total number of births in the Swedish Medical Birth Register during the same period in Stockholm county.

All variables were expressed as frequency, unless otherwise indicated. Comparisons between groups were assessed with the Fisher's exact test. We used a nonparametric Mann–Whitney test when calculating birth rate in the RhD-negative population compared with the total population. Statistical significance was set to $p < 0.05$. Missing data were excluded in the analyses and indicated in the tables by the total number of individual data points included for each variable. All statistical analyses were performed using statistical software STATA version 12.1 (Stata Corp, College Station, TX, USA).

The study was approved by the regional ethics committee (approval no. 2010/1996-31/4).

Results

A total of 290 pregnant women with anti-D antibodies during the study period were identified and included in the study. In four cases, it was unclear in which pregnancy the women were immunized and they were added to group 3. Fifty-one percent of the women (147/290) were sensitized with their first-born child and 33% (96/290) with their second-born child. Anti-D antibodies developed during ongoing pregnancy, in the second or third trimester, in 73% (212/290) and at term or after delivery in 21% (61/290). Forty-six percent (132/290) of women became immunized in the third trimester of pregnancy. Thirteen percent (38/290) of women were immunized already in the second trimester of pregnancy and this proportion was 24% in Group 2. Details on the index pregnancy and when the women were immunized are listed in Tables 1 and 2.

Table 1. Order of pregnancy (index pregnancy) in which the women were Rhesus D sensitized ($n = 290$).

	Group 1 First-born child, $n = 147$	Group 2 Second-born child, $n = 96$	Group 3 Third-born child or later, $n = 47$	Total
Number sensitized during ongoing pregnancy	96/147 (65%)	84/96 (88%)	32/47 (68%)	212 (73%)
Anti-D detection in first trimester	6	0	0	6 (2%)
Anti-D detection in second trimester	10 (7%)	23 (24%)	5 (11%)	38 (13%)
Anti-D detection in third trimester	62 (42%)	46 (48%)	24 (51%)	132 (46%)
Unclear if anti-D detected in second or third trimester	24(16%)	15 (16%)	3 (6%)	42 (14%)
Sensitized after delivery	41/147 (28%)	12/96 (12%)	8/47 (17%)	61 (21%)
Unclear when sensitized	4	0	7	11 (4%)

Table 2. Perinatal outcome of index pregnancy and frequency of hemolytic disease of the fetus and neonate in relation to the pregnancy in which the women were immunized.

	Group 1 First-born child, $n = 147$	Group 2 Second-born child, $n = 96$	Group 3 Third-born child or later, $n = 47$	Total	Comparison between groups
Prematurity due to HDFN	7/147 (5%)	4/96 (4%)	2/44 (5%)	13/287 (4%)	$p = 1.00$
DAT positive neonate	65/89 (73%)	65/78 (83%)	25/34 (74%)	155/201 (77%)	Not calculated
IUT	0/144 (0%)	2/95 (2%)	3/45 (7%)	5/284 (2%)	$p = 0.01$
Exchange transfusion	12/127 (9%)	6/75 (8%)	1/35 (3%)	19/237 (8%)	$p = 0.54$
Phototherapy	31/92 (34%)	36/71 (51%)	8/28 (29%)	75/191 (39%)	$p = 0.04$
Intrauterine fetal demise	4/147 (3%)	0/96 (0%)	2/45 (4%)[a]	6/288 (2%)	$p = 0.11$
Neonatal death	1/147[b]	0/96	0/45	1/288 (0.3%)	$p = 1.00$

HDFN, hemolytic disease of the fetus and neonate; DAT, direct agglutination test; IUT, intrauterine blood transfusion.
[a]One intrauterine fetal demise after IUT, the other due to placental abruption.
[b]Neonatal death due to severe asphyxia, unrelated to hemolytic disease.

Phototherapy was needed in one-third of neonates in groups 1 and 3 and in half of the neonates in group 2. This was statistically different ($p = 0.04$). The frequency of IUT in the index pregnancy was higher in group 3 ($p = 0.01$), performed in 3/45 cases compared with 2/95 in group 2 and 0/144 in group 1. The four cases of intrauterine fetal death in the group of women sensitized with their first-born child were unrelated to HDFN. However, it could not be clearly determined if the mother was RhD sensitized before the intrauterine fetal death or if this was the sensitizing event.

Thirty-six of 147 (24%) women who were immunized during ongoing pregnancy with their first-born child had a previous history of at least one first-trimester termination of pregnancy or miscarriage. In eight cases, we could not rule out that this was the sensitizing event. Two of these mothers had detectable anti-D antibodies in the first trimester and two women had seroconverted in week 16 of pregnancy. In four women, it was unclear when exactly anti-D antibodies were detected. We could not exclude that they were sensitized before they became pregnant with their first child. The remaining 28 cases seroconverted during ongoing pregnancy, in the second or third trimester, proven by a previous negative antibody test in first trimester. In addition, two women had anti-D antibodies in the first trimester in their first pregnancy. One had a history of previous blood transfusion and the other was pregnant after in vitro fertilization.

Most women had at least one more child after the index pregnancy: 131/147 (89%) in group 1, 46/96 (48%) in group 2 and 18/47 (38%) in group 3. The perinatal outcome, frequency of HDFN and treatment for HDFN in the first pregnancy following the index pregnancy are presented in Table 3. There was no statistical difference in perinatal outcome between the groups. Table 4 provides results with regard to the cumulative frequency of perinatal outcome of all children born after the index pregnancy. Fifty-six percent (144/259) of the neonates required treatment for HDFN. IUT was performed in 20% and postnatal exchange transfusion in 26% without any statistical difference between the groups. Induced delivery before <37 weeks of gestation was common, but premature delivery before 34+0 weeks due to HDFN only occurred in 12/557 pregnancies (2%). The number of total perinatal deaths confirmed as caused by HDFN or by a complication to treatment for HDFN was 1.6% (9 of 557 pregnancies).

RhD-negative women did not differ significantly in number of children compared with RhD-positive women (mean 1.81 ± 0.96 vs. 1.82 ± 0.97). However, immunized RhD-negative women gave birth to significantly fewer children than RhD-negative women with no antibodies (1.77 ± 0.90 vs. 1.82 ± 0.97, $p = 0.0001$) during the period.

Discussion

In this large cohort of RhD immunized pregnant women during a 19-year period we found that half of the women became sensitized with their first-born child and one-third with their second-born child. The majority developed anti-D antibodies during pregnancy, in the second

Table 3. Perinatal outcome, pregnancy intervention and neonatal treatment for hemolytic disease in the first pregnancy following the index pregnancy ($n = 195$).

	Group 1 Immunized with first-born child $n = 131$	Group 2 Immunized with second-born child $n = 46$	Group 3 Immunized with third born child or later $n = 18$	Total	Comparison between groups
RhD-positive child	99/127 (76%)	36/43 (78%)	13/18 (72%)	148/188 (79%)	Not calculated
HDFN[a]	76/127 (60%)	24/42[b] (57%)	8/18 (44%)	108/187[b] (58%)	$p = 0.49$
Prematurity due to HDFN	41/131 (31%)	9/46[b] (20%)	2/17 (12%)	52/194 (27%)	$p = 0.16$
IUT	28/128 (22%)	6/46[b] (13%)	3/18 (17%)	37/192[b] (19%)	$p = 0.66$
Exchange transfusion	38/116 (33%)	5/39[b] (13%)	3/17 (18%)	46/172[b] (27%)	$p = 0.07$
Phototherapy	67/117 (57%)	20/40[b] (50%)	7/17 (41%)	94/174[b] (54%)	$p = 0.44$
Intrauterine fetal demise	4/131 (3%)[c]	1/46 (2%)[d]	0/18 (0%)	5/195 (3%)	$p = 1.00$
Neonatal death	1/131§	1/46	1/18[e]	3/195 (2%)	$p = 0.14$

RhD, Rhesus D; HDFN, hemolytic disease of the fetus and neonate; IUT, intrauterine blood transfusion.

[a]Frequency of hemolytic disease of the fetus and newborn defined as treated antenatally with intrauterine blood transfusions and/or postnatally with exchange transfusions and/or phototherapy.

[b]One case with severe Kell alloimmunization not included.

[c]All due to HDFN, three of the four as a complication to IUT or cordocentesis.

[d]Intrapartum demise due to extreme prematurity.

[e]Neonatal death as complication to an exchange transfusion.

Table 4. Cumulative frequency of hemolytic disease, intrauterine blood transfusions, preterm birth due to hemolytic disease of the fetus and neonate and neonatal treatment in all pregnancies following the index pregnancy.

	Group 1 Immunized with first-born child	Group 2 Immunized with second-born child	Group 3 Immunized with third born child or later	Total	Comparison between groups
Total no. of children born after index pregnancy	181	62	24	267	
Developed other red cell antibodies in addition to anti-D	50 (34%)	17 (18%)	10 (21%)	77/290[a] (27%)	Not calculated
RhD-positive children	127/176 (72%)	50/59 (85%)	18/24 (75%)	195/259 (75%)	Not calculated
HDFN[b]	98/177 (55%)	34/58[c] (59%)	12/24 (50%)	144/259[c] (56%)	$p = 0.66$
Prematurity due to HDFN	52/181 (29%)	12/62[c] (19%)	4/23 (17%)	68/266[c] (26%)	$p = 0.35$
IUT	40/178 (22%)	9/62[c] (14%)	5/24 (21%)	54/264[c] (20%)	$p = 0.62$
Exchange transfusion	47/163 (29%)	10/54[c] (18%)	5/23 (22%)	62/240[c] (26%)	$p = 0.44$
Phototherapy	88/166 (53%)	30/55[c] (55%)	11/23 (48%)	129/244[c] (53%)	$p = 0.81$
Intrauterine fetal demise	5/180 (3%)[d]	2/62 (3%)[e]	0/24 (0%)	7/266 (3%)	$p = 1.00$
Neonatal death	1/180 (0.6%)[f]	1/62 (2%)[g]	1/24 (4%)[f]	3/266 (1%)	$p = 0.14$

Rh, Rhesus; HDFN, hemolytic disease of the fetus and neonate; IUT, intrauterine blood transfusion.

[a]Presented as cumulative frequency in the total of 290 RhD immunized women.

[b]Frequency of hemolytic disease of the fetus and newborn defined as treated antenatally with intrauterine blood transfusions and/or postnatally with exchange transfusions and/or phototherapy.

[c]One case with severe Kell alloimmunization not included.

[d]All due to HDFN, three of the five as a complication to IUT or cordocentesis.

[e]One due to complication after IUT, one intrapartum demise due to extreme prematurity.

[f]Neonatal death as complication to an exchange transfusion.

[g]Neonatal death unrelated to HDFN. RhD-negative neonate.

or third trimester. Based on our study, at least half of the cases could potentially have been avoided by RAADP at the start of the third trimester.

Our findings confirm the original incidence studies in Canada from the 1960s and 1970s. The Canadian studies showed that, before introduction of postnatal anti-D prophylaxis, 10–20% of RhD-negative primigravidas became immunized during ongoing pregnancy and 8% of these women developed anti-D antibodies before 29 weeks of gestation (10,11). In 16%, anti-D antibodies were present already in gestational week 29–34, indicating that sensitization occurred before the third trimester of pregnancy. Our findings are also in line with the results from a study on the Dutch RhD prevention program, in which the incidence of immunizations occurring between 12 and 30 weeks of pregnancy was 0.24% before introduction of RAADP and 0.25% after (3). In our study as well as in the Canadian studies, there were a few cases of immunization in the first trimester of pregnancy in primigravidae without a history of blood transfusion (11,12). Theoretically, very early sensitization is possible because the RhD antigen is exposed on fetal RBCs from 7 weeks of gestation and very small fetomaternal hemorrhages may happen early (13,14). Based on these data, we suggest that a strategy with first-trimester noninvasive fetal *RHD* genotyping in cell-free DNA in maternal plasma, followed by administration of 250–300 μg anti-D prophylaxis at the start of the second trimester in addition to week 28–30, could potentially be highly efficient in preventing RhD immunization and HDFN. Obviously, the cost-effectiveness of such a strategy needs to be studied. This decision will also depend on the availability and cost of hyperimmunized D plasma and the possible replacement by monoclonal or recombinant anti-D IgG.

Pharmacokinetic studies have shown that anti-D prophylaxis administered in gestational week 28–30 normally lasts for at least 10 weeks, but thereafter many women do not have detectable plasma levels of anti-D IgG (15–18). This means that some pregnant women are unprotected in gestational week 38–42. In our study population some of the women classified as immunized after delivery may have been sensitized at term or post term, but not detected at that time because the last routine antibody screen was carried out at 37 weeks of gestation. With the fetal *RHD* genotype known early in pregnancy, anti-D prophylaxis could be administered routinely in gestational week 38 to provide protection at term and later. A high enough dose at this gestational age will normally protect against sensitization after delivery and a postnatal dose will not be necessary, at least not after an uncomplicated delivery, within 3 weeks of the injection (19–21). Whether a repeat dose at 38 weeks of gestation can safely replace the postnatal administration requires further investigations.

Although immunized primiparous women would be expected to give birth to more children after the index pregnancy than women immunized in later pregnancies, the severity of the disease seems to be similar. The risk for HDFN and need for interventions did not differ between these groups. One could speculate that women being immunized during their first pregnancy should exhibit a certain immunological phenotype with a potentially more severe course. Our results do not support such a speculation, although 34% of the women in this group developed other RBC antibodies than anti-D during their pregnancies, indicating an active immune response. As the consequences in subsequent pregnancies are the same regardless of in which pregnancy sensitization occurs and with the possibility of noninvasive fetal *RHD* determination, we propose that selective anti-D prophylaxis should be offered independently of parity.

In the index pregnancy, the frequency of severe HDFN requiring specialized antenatal or postnatal care was low. The frequency of phototherapy appeared higher in the group of women immunized with their second child. It might reflect that a larger proportion of women in this group had already seroconverted in the second trimester, but this finding has to be interpreted with caution because data were missing in many cases. The difference regarding IUT might reflect a lower threshold for intervention in a multiparous woman compared with a nulliparous woman. In subsequent pregnancies, severe HDFN occurred in approximately 25% of the neonates. In recent studies from both France and the Netherlands, a similar risk of 20–25% of developing severe HDFN (requiring IUT and/or exchange transfusion) was found (3,22). Our analysis showed that HDFN, when it occurs, is still a cause of perinatal mortality, leading to perinatal death directly related to the disease and its treatment in 1.6% of cases (9/557 pregnancies).

Our study showed that immunized women gave birth to fewer children than nonimmunized RhD-negative women. The most likely explanation for this is that some immunized women will refrain from becoming pregnant again. It cannot be ruled out that some of these women have been counselled that they should limit their number of children because the next child will be even more severely affected. The consequence that immunized women will have fewer children needs to be taken into account when performing cost-effective analyses on different prevention strategies. We are not aware of any previous study evaluating the number of children in immunized women and this aspect deserves further investigation.

The strengths of our study include centralized care and registers of all pregnancies with red cell immunization in our region. However, we present a retrospective study retrieving data from medical records and our findings have to be interpreted with caution. For some variables there were a considerable number of missing data because of the retrospective design of the study. Especially in the earlier years of the study period, information was missing from the medical records reviewed. Detection and classification of when immunization occurred depend on screening frequency. We chose to be conservative in the classification of when the women were sensitized, the date of first positive anti-D titer was confirmed with the transfusion medicine register and if there was uncertainty then the latest confirmed date was used. This means that some women could have been sensitized earlier than described in our study. As we were interested in practical implications for new antenatal prevention programs, we used parity instead of pregnancy when defining the three groups of RhD immunized women. Even though the reporting of previous pregnancies is generally good in Sweden, information in medical records about miscarriages and terminations might be less reliable. Because of this, there is a possibility that a miscarriage or termination of pregnancy between children could have caused immunization in a few cases. For nulliparae, however, we analyzed all cases with previous miscarriages or termination of pregnancy and in 5.4% (8/147) we could not rule out that this had been the sensitizing event.

Conclusion

Our study shows that without routine antenatal anti-D prophylaxis, approximately half of RhD immunized women are sensitized during pregnancy or delivery of their first-born child and a third with their second-born child. The majority of immunizations occur before delivery and the frequency of HDFN in subsequent pregnancies is high. At least half of the cases of RhD immunizations could potentially have been avoided by RAADP in the beginning of the third trimester. Noninvasive determination of fetal *RHD* genotype in the first trimester of pregnancy enables more efficient prevention strategies, providing anti-D prophylaxis selectively to women with *RHD*-positive fetuses.

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