# Evaluation of Postnatal Prophylactic Program for Rhesus Isoimmunization

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## ABSTRACT:

**BACKGROUND:**

Since the introduction of routine postpartum prophylaxis in the 1960s, the crude incidence of Rhesus isoimmunization has been declined all over the world. Iraq and due to many years of sanctions and wars had many occasions where there was a limited supply of this valuable injection.

**OBJECTIVE**

To evaluate the effectiveness of our preventive postnatal prophylactic protocol for Rhesus isoimmunization.

**METHODS:**

A Cohort observational study conducted at a private clinic and AL-Elwyia Maternity Teaching Hospital between the start of April 2010 till the end of June 2011. A total of 500 women were enrolled in the study; who were Rhesus D-ve pregnant women, married to Rhesus D+ve husbands, and had a Rhesus D+ve neonate and received the usual postpartum prophylactic dose after the previous deliveries when needed. Maternal plasma level of IgG-D concentration was determined by performing Indirect Coomb's test to the mother on admission and neonatal blood group and plasma level of IgG-D concentration was determined by performing direct Coomb's test to the neonate.

All the previous and current relevant obstetrical and gynecological events were included in the study and analyzed. Data analysis was done using SPSS which included percentages according to cross tabulation of background of sample groups and Chi- square test for the associations.

**RESULTS:**

The prevalence rate of positive Indirect Coomb's test in the study sample was (10.4%) (95% confidence interval ranging between 7.9 to 13.5%), and it was strongly related to gravidity, were gravidity group (G5+) increased the rate of positive Indirect Coomb's test to (25.4%), which is significantly higher than that of primigravida(G1). A positive past history of early pregnancy loss significantly increased the rate of having a positive Indirect Coomb's test by (29.9%).

**CONCLUSION:**

There is an urgent need in our country to improve our current postnatal prophylactic program based on the high sensitization rate which is so far from the global rate.


***KEY WORDS*:** rhesus isoimunization, anti D immunoglobulin, postnatal prophylactic.

## INTRODUCTION:

The Rhesus blood group from its discovery 60 years ago has become second in importance only to the ABO blood group in the field of transfusion medicine. It has remained of primary importance in obstetrics, being the main cause of hemolytic disease of the fetus and newborn (HDFN). [(1)]

The development of anti-D antibodies usually occurs as a result of fetomaternal haemorrhage (FMH) in a Rhesus D (RhD)-negative woman with an RhD-positive fetus. [(2)] This process called sensitization or alloimmunisation, which can happen at any time during pregnancy, but is most common in the third trimester and during childbirth and it can follow events in pregnancy known to be associated with FMH, such as medical interventions (chorionic villus sampling, amniocentesis or external cephalic version), terminations, late miscarriages, antepartum haemorrhage and abdominal trauma. Once sensitization has occurred it is irreversible.[(3)]

On January 31st, 1964 the first postpartum prophylaxis was given.[(4,5)] The vaccine was finally approved in England and the United States in 1968, and the FDA approved the drug under the name RhoGAM, with a fixed dose of (300 µg) and given within three days postpartum. Time magazine picked it as one of the top ten medical achievements of 1960s. [(5, 6)]

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Prior to the development of anti-D immune globulin, approximately 16 % of Rh(D)-negative women became alloimmunized after two deliveries of Rh(D)-positive ABO compatible infants. [7] This rate fell to 2 % with routine postpartum administration of a single dose of anti-D immune globulin and was further reduced to as low as 0.1 percent with the addition of routine antenatal administration in the third trimester, [8] which use to be given at 28th and 34th weeks of gestation according to the Royal College & NICE guidelines. [2,3] However, Rh(D) alloimmunization has not been eliminated and reasons for continued emergence of sensitized pregnancies include both failure to administer anti-D immune globulin in accordance with the published guidelines and sensitization in early gestation before routine third trimester antenatal anti-D prophylaxis. [9] In addition to that with more severe FMH, one (300µg) dose of RhD IgG may not be sufficient when FMH exceeding (30 ml). Thus, at least (1%), and perhaps more, of susceptible mothers would have been given insufficient immunoglobulin if not tested. Because of the above observations, the American Association of Blood Banks recommends that all RhD-ve women should be tested at delivery with the Kleihauer-Betke test and the dosage of anti-D IgG is calculated from the estimated volume of the FMH. One (300µg) ampoule (which is the only dose available in Iraq) is given for each (30 ml) of fetal whole blood. [10]

Iraq is one of the countries who used to give Anti-D IgG postpartum when it is available, where sometimes it is not; due to the subsequent wars and sanction, and during the last few years it was used to be given occasionally during pregnancy to RhD-ve unimmunized women after any sensitizing event which is the case in some of the teaching hospitals usually.

For that reason; this research was conducted to determine efficacy of our current postnatal preventive protocol of Rhesus D isoimmunization.

## PATIENTS AND METHODS:

A Cohort observational study was conducted in a private clinic and AL-Elwyia Maternity Teaching Hospital. It was approved by the Ethical and Scientific Committee of Alkindy College of Medicine and the Administrative board of the hospital and informed consent by the participants was ensured. It conducted between the 1st of April 2010 and 30th of June 2011. A total of 500 RhD-ve pregnant women were enrolled in the study who fulfill the inclusion criteria; who were RhD-ve woman married to RhD+ve husband and their pregnancies were ended with early pregnancy loss, or delivery of RhD+ve neonate. They were previously received the routine postpartum immunization after the previous deliveries (when needed), while women who received antenatal Anti-D immunoglobulin or on medications which can cause positive Indirect Coomb's test; as Methyldopa, were excluded as it can lead to drug-induced hemolysis. A questionnaire format was designed for the current study including detailed history of current pregnancy, previous pregnancies, complications and outcomes. With special concern to any sensitizing events in current or previous pregnancies, fait of previous pregnancies, Anti-D immunoglobulin administration; antenatal or postnatal**.** Maternal plasma level of IgG-D concentration was determined by performing Indirect Coomb's test to the mother on admission and neonatal blood group and plasma level of IgG-D concentration was determined by performing direct Coomb's test to the neonate.

An expert statistical advice was sought for Statistical analyses were computer assisted using SPSS version 13 (Statistical Package for Social Sciences). Frequency distribution for selected variables was done first. The following tests were used as needed; including t-test, ANOVA test, Chi-square test, the relative risk (RR) and P value less than the 0.05 level of significance was considered statistically significant. The 95% confidence interval of RR gives an idea about the expected range of the parameter in the target population with 95% confidence.

## RESULTS:

The study sample consists of (500) RhD-ve pregnant women (400 ended with delivery and 100 with early pregnancy loss) and some of the study sample characteristics were shown in table 1.

**Table 1: Frequency distribution of the study sample by selected variables.**

| Variables | N | % |
|---|---|---|
| Gravidity | | |
| Primigravida | 118 | 23.6 |
| G2-G4 | 264 | 52.8 |
| G5+ | 118 | 23.6 |
| Total | 500 | 100 |
| Past history of miscarriage | | |
| Negative | 356 | 71.2 |
| Positive | 144 | 28.8 |
| Total | 500 | 100 |
| Gestational age (weeks) for labor-categories | | |
| <37 | 45 | 11.3 |
| 37-38 | 214 | 53.5 |
| 39-40 | 133 | 33.3 |
| 41-42 | 8 | 2 |
| Total | 400 | 100 |
| Vaginal bleeding in this pregnancy | | |
| Negative | 399 | 79.8 |
| Positive | 101 | 20.2 |
| Total | 500 | 100 |
| History of previous postnatal Anti-D (excluding primigravida) | | |
| Negative | 81 | 21.2 |
| Positive | 301 | 78.8 |
| Total | 382 | 100 |
| Outcome of current pregnancy | | |
| Vaginal delivery | 350 | 70 |
| CS | 50 | 10 |
| Miscarriage | 90 | 18 |
| Ectopic pregnancy | 10 | 2 |
| Total | 500 | 100 |

As shown in (table 2) and (figure1); the prevalence rate of a positive Direct Coomb's test in a sample of (395) infants born alive was (3.3%) [The 95% confidence interval for this estimate was (1.8 – 5.7%) which represents the expected range of positivity rate in a reference population]. The prevalence rate of a positive Indirect Coomb's test among the total sample of pregnant women examined was (10.4%) (Its 95% confidence interval ranging between 7.9 to 13.5%), and as shown also in (figure 1).

**Table 2: The prevalence of positive Direct and Indirect Coomb's tests in the total study sample.**

| Study group | N | % | 95% confidence interval |
|---|---|---|---|
| Positive Direct Coomb's test (n=395) | 13 | 3.3 | (1.8 – 5.7) |
| Positive Indirect Coomb's test (n=500) | 52 | 10.4 | (7.9 – 13.5) |

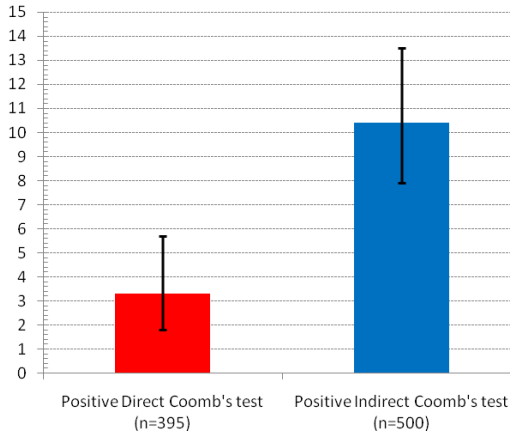


**Figure 1: Error bar chart showing the prevalence (and its 95% confidence interval) of positive Direct and Indirect Coomb's test in the total study sample.**

While gravidity, parity and history of miscarriage, all have significant effects on having a positive Indirect Coomb's Test, as shown in (table 3).

**Table 3: The difference in median gravidity, parity and miscarriage by results of Indirect Coomb's test.**

| Variables | Indirect Coomb's test | | P(Mann-Whitney) |
|---|---|---|---|
| | Negative | Positive | |
| Gravidity | | | <0.001 |
| Range | (1 - 13) | (1 - 13) | |
| Median | 3 | 5 | |
| Inter-quartile range | (1 - 4) | (3 - 7) | |
| N | 448 | 52 | |
| | | | |
| Parity | | | 0.001 |
| Range | (0 - 10) | (0 - 8) | |
| Median | 1 | 3 | |
| Inter-quartile range | (0 - 3) | (1 - 5) | |
| N | 448 | 52 | |
| | | | |
| History of miscarriage | | | <0.001 |
| Range | (0 - 4) | (0 - 5) | |
| Median | 0 | 2 | |
| Inter-quartile range | (0 - 0) | (1 - 2) | |

Risk factors for a positive Indirect Coomb's test were shown in (table 4); as the blood group of the mother and the fetus has no significant effects on the Indirect Coomb's test results.

**Table 4: Maternal & fetal ABO blood group for mother and positivity rate of Indirect Coomb's test.**

| Variables | Total N | Positive Indirect Coomb's test | | P |
|---|---|---|---|---|
| | | Negative | % | |
| ABO blood group-Mother | | | | 0.45[NS] |
| A | 137 | 10 | 7.3 | |
| B | 141 | 16 | 11.3 | |
| AB | 39 | 6 | 15.4 | |
| O | 183 | 20 | 10.9 | |
| | | | | |
| ABO blood group-Neonate | | | | 0.64[NS] |
| A | 125 | 15 | 12 | |
| B | 110 | 11 | 10 | |
| AB | 31 | 2 | 6.5 | |
| O | 129 | 10 | 7.8 | |

While (table 5) & (figure 2) shows that the ABO blood group incompatibility between the mother and the newborn had no important, or statistically significant, association with the risk of having a positive Indirect Coomb's test, the history of previous postnatal Anti-D immunoglobulin (excluding primigravida) also had no important or statistically significant association with the risk of having a positive Indirect Coomb's test, however this variable is of limited relevance in the present study, since there is no documented history for previous pregnancies regarding the actual need for the anti-D immunoglobulin injection and its availability. A positive history of vaginal bleeding increased the risk of having a positive Indirect Coomb's test by (1.3 times), the risk estimate (calculated relative risk RR) was however not significant statistically. Delivery by C/S did not significantly increase the risk of having a positive test.

Primigravida was associated with a (0.8%) positive Indirect Coomb's test, the second group (gravida 2 to 4) (G2-4) increased the rate of positive test to 8%, and it is significantly higher than that of primigravida. The highest gravidity group (G5+) increased the rate of a positive Indirect Coomb's test to (25.4%), which is significantly higher than that of primigravida. A positive past history of miscarriage significantly increased the risk of having a positive Indirect Coomb's test by (29.9%).

**Table 5: The risk of having a positive Indirect Coomb's test by selected factors.**

| Variables | Total N | Positive Indirect Coomb's test | | P | RR | 95% CI for RR |
|---|---|---|---|---|---|---|
| | | N | % | | | |
| ABO incompatibility between mother and neonate | | | | | | |
| Negative | 225 | 18 | 8 | | Ref | |
| Positive | 170 | 20 | 11.8 | 0.21[NS] | 1.47 | (0.8 - 2.69) |
| | | | | | | |
| Vaginal bleeding in this pregnancy | | | | | | |
| Negative | 399 | 39 | 9.8 | | Ref | |
| Positive | 101 | 13 | 12.9 | 0.36[NS] | 1.3 | (0.7 - 2.4) |
| | | | | | | |
| History of previous intake of Anti-D (excluding primigravida) | | | | | | |
| Negative | 81 | 13 | 16 | | Ref | |
| Positive | 301 | 38 | 12.6 | 0.42[NS] | 0.8 | (0.4 - 1.4) |
| | | | | | | |
| Gravidity | | | | | | |
| Primigravida | 118 | 1 | 0.8 | | Ref | |
| G2-G4 | 264 | 21 | 8 | 0.006 | 9.4 | (1.3 - 68.9) |
| G5+ | 118 | 30 | 25.4 | <0.001 | 30 | (4.2 - 216.4) |
| | | | | | | |
| Past history of miscarriage | | | | | | |
| Negative | 356 | 9 | 2.5 | | Ref | |
| Positive | 144 | 43 | 29.9 | <0.001 | 11.8 | (5.9 - 23.6) |
| | | | | | | |
| Early preg. loss as an outcome for the current pregnancy | | | | | | |
| Negative | 400 | 41 | 10.3 | | Ref | |
| Positive | 100 | 11 | 11 | 0.83[NS] | 1.07 | (0.57 - 2.01) |
| | | | | | | |
| Mode of delivery | | | | | | |
| Vaginal delivery | 350 | 37 | 10.6 | | Ref | |
| C/S | 50 | 4 | 8 | 0.57[NS] | 0.76 | (0.28 - 2.03) |

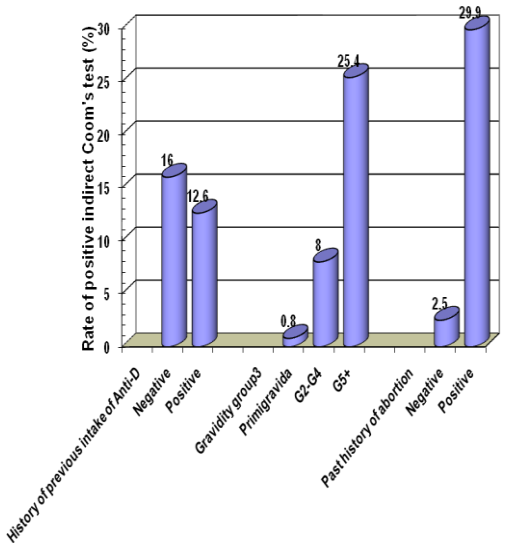


**Figure 2: Bar chart showing the rate of positive Indirect Coomb's test by selected explanatory variables (risk factors).**

## DISCUSSION:

Anti-D immunoglobulin injection availability in Iraq is fluctuating and had undergone long history of shortage. The only dose available in our country is 300 µg (1500 IU), which costs the health system around 56.650 thousands ID ($ 47.2) for each. It is a blood product, prepared from paid donors' blood.

Since the introduction of routine postpartum prophylaxis in the 1960s, the crude incidence of D isoimmunization had fallen and as example in the U.S. and Canada it has fallen from 9.1-10.3 cases to 1.3 cases/1,000 total births.[11,15]

The problem of isoimmunization, which can lead to perinatal and neonatal morbidity and mortality, is preventable and the prevention of its occurrence by giving Anti-D immunoprophylaxis according to a local protocol is the Golden standard and by now given Anti-D for RhD-ve women after delivery of a RhD+ve baby had an evidence level (Ia) and a grade of recommendation (A).[2]

It has been shown that a RhD-ve woman who is not immunized and who didn't receive antenatal prophylaxis has, in every pregnancy, a (16%) risk of becoming immunized when carrying a RhD+ve fetus. [16] However, with the introduction of the preventive protocol, the global success rate of post-natal RhD immunoprophylaxis has reached (98.4 – 99%). [16,17]One of the studies done in Manitoba had reported a positive Indirect Coomb's test at 1.62% in 2768 controls who received the postnatal prophylaxis only without the antenatal prophylaxis. [18] Also a meta-analysis by the Cochrane Collaboration in 2000 which included only two studies, for a total of more than 4500 women treated with Anti-D prophylaxis; comparing different dose of antenatal prophylaxis showed that the risk of alloimmunization of RhD-ve pregnant women was about (1%) for those use only the postnatal prophylaxis. [16] Comparing that with the prevalence rate of sensitization in our study which was 10.4% (95% confidence interval ranging between 7.9 to 13.5%) that is much higher than the global rate.

The only previous reported research in Iraq about RhD-isoimmunization was carried out in Al-Ramadi, from 1993 to 1997. Of (487) RhD-ve mothers who were followed during the study period; (172) were primigravida, (1.7%) of them were RhD-isoimmunized, and the frequency of isoimmunization increased with the increasing number of pregnancies (4.9% for 2$^{nd}$ pregnancies to 45.4% for 5$^{th}$ pregnancies). Comparison of this study with other earlier studies showed that the incidence of RhD-isoimmunization was considerably greater in Iraq than other countries. It also showed a significant rise in the percentage of RhD-isoimmunization among RhD-ve mothers in Al-Ramadi during mid-1990s. [19] Comparing these results with our results that is in accomplished in early 20I1; it showed a decrement in sensitization rate to certain extent, as in primigravidae where the sensitization was (0.8%), while gravidity 2-4 increased the risk of sensitization to (8%) and the

fifth pregnancy increased the risk to (25.4%). While a study accomplished at 2003 in United Kingdom showed much lowered incidence of a positive Indirect Coomb's test among multigravidae who received the postnatal prophylaxis alone, which was (5.5%) only. [20]

Although the general awareness about the problem in our country had been increased and the pregnant women by the time of labour usually their blood group would be tested and they use to receive the postnatal prophylactic dose within 72 hours of delivery if it available and especially if their deliveries were at hospital but this injection sometime not available at the public hospitals and the cost of which at the private pharmacist is around 100 ID ($ 83.3) where not all the families can offer.

Iraq; is one of the countries who still not use the routine antenatal prophylactic Anti D. Comparing a study done in Yorkshire, regarding Primigravida showed that of the (2000) un-sensitized Primigravida who didn't receive the antenatal Anti-D prophylaxis only 0.9% become sensitized. [20] In another study on the use of low-dose Anti D given at 28th and 34th wk gestation; showed that 0.77% of those who received the low-dose Anti-D became sensitized postnatally, [21] while in our study the sensitization rate in primigravida was 0.8% which is nearly comparable to those who received the antenatal dose in those studies. This is supporting the idea that there is no urgent need to use a routine antenatal proghylaxis at the time being and for that the 12.6% sensitization rate in those who previously received postnatal prophylaxis could be attributed to suboptimum dose of postnatal anti D intake due to the larger fetomatenal haemorrhage rather than the non administration of antenatal prophylaxis.

In our study a positive past history of early pregnancy loss significantly increased the risk of having a positive Indirect Coomb's test to (29.9%) and a positive history of vaginal bleeding in the current pregnancy increases the risk of having a positive Indirect Coomb's test, but the association was not significant statistically and needs further evidence to be established. Otherwise no obvious association could be elicited between the risk of having Indirect Coomb's test and ABO incompatibility between the mother and the newborn and the mode of delivery. This may justify selective administration of antenatal Anti-D after some sensitizing events.

Finally, attainment of the Millennium Development Goals demands a reliable and comparable method for estimating resource needs. Achieving the Millennium Development Goals may also require reform of health-care systems to allow the poor to access care. One of the parameters included in the List of health interventions coated in WHO normative approach in the preventive intervention is the Postpartum administration of anti-D immunoglobulin to rhesus-negative women with a rhesus positive foetus.[22]

## CONCLUSION:

Strengthening of our own postnatal protocol with the Indirect Coomb's tests should be the usual trend in our hospitals to test the eligibility for providing the Anti-D immunoglobulin injection for rhesus negative women. Kleihauer test needs to be done routinely in our hospitals, since it is a simple and of low cost, for the RhD-ve mother after delivery of a RhD+ve neonate to determine the need for an extra-dose of Anti-D immunoglobulin injection and so to increase the effectiveness of postnatal prophylactic protocol. Selective administration of antenatal Anti-D immunoprophylaxis when there is a sensitizing event in pregnancy especially early pregnancy loss or when there is a history of bleeding during current pregnancy. This should be done in conjunction with efforts to scale-up maternal and newborn health services.

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