Original Article

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Obstetrical and Perinatal Outcome in Rhesus Antigen Negative Pregnancy

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Abstract

Introduction: Incidence of Rh negativity in India is 5-10%. Rh negative pregnancy poses a risk only when there is incompatible mating, leading to antigen-antibody reaction, and hemolysis. However, it can be prevented by adequate measures.

Aims and Objectives: To assess obstetrical and perinatal outcome in terms of perinatal morbidity and mortality, to correlate maternal outcome with increased gravidity and impact of anti-D immunoglobulin (Ig) on the outcome of Rh negative pregnancy.

Materials and Methods: The prospective observational study was conducted in Rh negative pregnant women with incompatible mating attending the Department of Obstetrics and Gynecology, Rajendra Institute of Medical Sciences over a period of March 2013 to October 2014. Maternal outcome was assessed on the basis of mode of delivery and risk factors in present pregnancy. Neonatal outcome was assessed for weight, gestational age of birth, APGAR score, anemia, distal convoluted tubules positively, hyperbilirubinemia, need for phototherapy, and exchange transfusion.

Results: In the present study, 16% of patients in the study group were indirect coomb's test (ICT) positive, 84% were ICT negative. Isoimmunization was found to be significantly associated with increasing gravidity, *P* value being <0.05. Isoimmunization was found to be significantly less in mothers with a history of anti-D administration in previous pregnancies *P* value being <0.05. Of 150 births, 94% were live birth, 3.3% neonatal death, and 2.7% intrauterine death. Neonatal anemia (hemoglobin <18 g%) and hyperbilirubinemia (cord blood bilirubin ≥3.5 mg%) were significantly associated with isoimmunized mother, *P* value being <0.05. Association of isoimmunization with need of phototherapy ($P < 0.5$) and with exchange transfusion was highly significant ($P < 0.01$). In the present study, 48 out of 51 booked patients received routine antenatal anti-D prophylaxis in contrast to only 8 out of 99 unbooked patients. 3 of booked patients refused it because of the cost factor. 94 patients received post-natal prophylaxis with 300 mg anti-D. 61 patients did not receive because 24 were isoimmunized and 24 babies had Rh negative blood group. 10 patients refused to take anti-D, 6 because of the cost factor, and 4 as they adopted for permanent sterilization.

Conclusions: Though Rh hemolytic disease of the newborn forms common and preventable cause of maternal and perinatal morbidity, conditions are still encountered in India. Technically simple investigations and anti-D IgG when required decreases the burden of disease.

Key words: Erythroblastosis fetalis, Heterozygous, Hyperbilirubinemia, Isoimmunization, Perinatal, Rhesus, Utero

INTRODUCTION

Rhesus antigen negative pregnancy is one of the major causes of perinatal mortality and morbidity. "Rhesus antigen" was named after monkey Macacus rhesus with whom about 85% human being shares this red cell antigen.[1] The incidence of Rh negative blood group is highest among Basques that is 34%. It is 13% among Caucasians, 7% among African-American, and 1% among Americans, Chinese, and other Asiatic peoples.[2] The incidence of Rh negative blood group in India varies between 3% and 5.7%.[3-5] In general, 60% of Rh positive men are heterozygous, and 40% are homozygous.[6] Rh negative pregnancy poses a risk to mother and baby only when there is incompatible mating, i.e., husband blood group is

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Rh positive. If genotype of the husband is homozygous, all the babies will be affected by hemolytic disease and if heterozygous 50% of babies will be affected.

Before the discovery of Rh antigen by Landsteiner and Weiner in 1940, little was known about etiology of erythroblastosis fetalis, a condition in which fetus becomes edematous and dies in utero due to severe anemia and high output cardiac failure.[7] Levine *et al.* in 1944 showed that in 90% cases of erythroblastosis fetalis, the mother was Rh negative.[8] With discovery of Rh immune type of antibody by Race and Weiner (1944) which can cross placental barrier, it became evident that it is responsible for erythroblastosis fetalis.[9]

Rh disease accounts for 97% of hemolytic disease of the newborn (HDN), remaining 3% is caused isoimmunization against other fetal antigenic groups such as Kell, non-D Rh, Duffy, Kidd, and MNS.[10] HDN is preventable disease when measures to prevent fetomaternal hemorrhage in Rh negative pregnancy and antenatal and post-natal immunoprophylaxis with anti-D immunoglobulin (Ig) are practiced correctly. HDN due to Rh isoimmunization is yet a significant health problem in India. The incidence of Rh sensitization during pregnancy is 1-9%[5] and perinatal loss due to Rh alloimmunization has been reported to be between 1% and 2.5%.[13] Risk of isoimmunization decreased 1.5% by post-natal anti-D prophylaxis and to 0.18% by additional routine antenatal anti-D prophylaxis (RAADP).[14]

The main cause of sensitization in present day practice is a lack of awareness in many places in India, particularly in rural areas where the mother is not routinely tested for their ABO Rh blood group, also in small rural areas where facilities for laboratory testing for Rh isoimmunization are non-existent. Finally, the benefits of protecting non-immune Rh negative mothers from isoimmunizaton with the use of prophylactic IM anti-D Ig are either unknown or ignored because of cost consideration.

While the prevention of Rh alloimmunization is the responsibility of all health care workers, the management of alloimmunized pregnancies requires specialized care. From a public health viewpoint, emphasis must be placed on prevention; hence, we have some areas to focus on. There must be increased awareness among doctors and patients about antenatal prophylaxis at 28 weeks or after any sensitizing event. The present study is done to show risk factors associated with isoimmunization, perinatal outcome, and impact of anti-D Ig on the outcome of Rh negative pregnancy.

MATERIALS AND METHODS

This was an observational study conducted in all Rh negative pregnant women with incompatible mating who presented in outdoor and emergency Department of Obstetrics and Gynecology, RIMS, Ranchi over a period of March 2013 to October 2014. Ethical approval from ethical committee was taken. Informed consent was taken from all the patients regarding the necessity of follow-up and compliance.

All pregnant women with Rh negative blood group irrespective to their age, parity, booking status, gestational age, and administration of Rh anti-D Ig in previous or present pregnancy were included in the study. Rh negative pregnant women with compatible mating were excluded. Those women who refused to comply with the follow-up visits were excluded from the study.

A proper history of patients was taken, and all the antenatal records were reviewed. Thorough general and obstetrical examination was done and all the routine antenatal investigations along with indirect coombs test were sent. The maternal chart was reviewed for parity index, gestational age, blood group, and history of anti-D administration in previous pregnancies. Neonatal outcome was assessed for weight, APGAR score, anemia, distal convoluted tubules (DCT) positively, hyperbilirubinemia, need for phototherapy and exchange transfusion.

RESULTS

In the present study, 44% belonged to age group 20-25 years followed by 38% in 26-30 years age group. This scenario was most probably due to early marriage and early childbearing among the Indian population. 42% mothers were primigravida, 24% were the second gravida, 14.7% were the third gravid, and only 9.3% were the fourth gravida. Only 4.1% of the populations were gravida 4 onward; this is most probably due to the adoption of family planning services. 62% of mothers presented to us at 37-40 weeks, 18% at 40-42 weeks, and 20% before 37 weeks. Most of them were in labor at the time of presentation. Only 34% of the patients were booked. Most of the unbooked patients presented to the hospital in labor. This is most probably due to illiteracy, lack of health facilities in rural areas, and loopholes in the implementation of the health program.

Among them, 36% patients were O negative, 30% B negative, 28% A negative, and only 4% AB negative. Husband's blood group in most, i.e., 39.3% was not

known. This is because all of these cases were unbooked and husband was not available at the time of presentation. Of those whose blood group was known 18.6% were A positive, 16.6% were B positive, 18.6% were O positive, and only 6.6% were AB positive. Husband's blood group was known in all of the booked cases.

Only 34% patients have received anti-D in previous pregnancies. 54% patients did not receive anti-D, this was either due to lack of awareness, home delivery or due to cost factor. Labor outcome shows that 37.3% delivered normally, 31.3% by emergency lower segment caesarean section (LSCS), and 16.7% by elective LSCS. Labor was induced in 12.7% patients, and 2% required instrumental delivery. The outcome of 141 (94%) pregnancy was live birth. 4 (2.7%) patients had intrauterine death (IUD), and 5 (3.3%) had early neonatal death. Out of 4 cases of IUD, one had features of hydrops fetalis. There are large number of Rh antigens due to its complex genetic makeup. Two Rh genes RhD and RhCE located on short arm of chromosome number 1 encodes for RhD antigen and E,e,C,c respectively.[11,12]

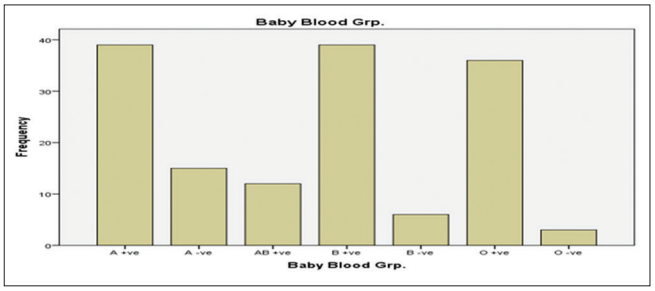
39 (26%) of babies were A positive, 39 (26%) were B positive followed by 36 (24%) being O positive. Only 12 (8%) of babies were AB positive. 24 (16%) babies born were Rh negative, these babies were free from complications (Graph 1).

Out of 150 patients, 24 (16%) were indirect coomb's test (ICT) positive. Out of 24 ICT positive cases, only 1 was booked. Titer of booked case was 1:16 and was followed weekly but rising trend was not observed. Titer of 6 out of 24 was >1:32, one of these presented in active labor and delivered a dead baby with features of hydrops fetalis. One had IUD, 4 babies required exchange transfusion and phototherapy.

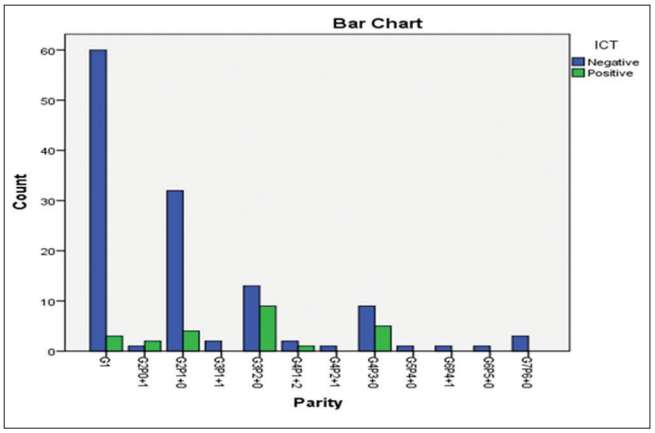
In the present study, 12 out of 24, i.e., (50%) isoimmunized patients belonged to 20-25 age groups. This is most probably because most of the patient in my study group belonged to this age group. It was also observed that isoimmunization is significantly associated with increasing gravidity, *P* value being <0.05. 9 out of 24 ICT positive cases were the third gravida. Association with gravida 4 onward is found to be comparatively less because of less number of patients in that age group, may be due to the adoption of family planning methods (Graph 2).

Isoimmunization was significantly associated with the unbooked status of the patient, *P* value being <0.01. 23 out of 24 ICT positive patients were unbooked as compared to 76 out of 126 ICT negative patients. It was more among unbooked patients. This was probably because most of the unbooked patients were illiterate belonging to remote areas; they were deprived of antenatal checkup and anti-D administration in previous pregnancies (Graph 3).

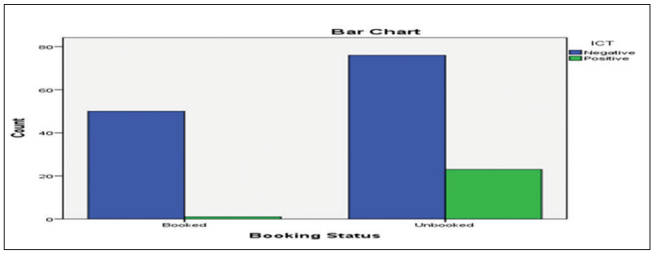
Isoimmunization was found to be significantly associated with mothers who did not have an anti-D administration in previous pregnancies, *P* value being <0.05. There was anti-D prophylaxis is a failure in 3 cases; all of these had a history of increased feto-maternal hemorrhage (FMH) in previous pregnancies. 1 of them had a history of mentally retarded offender program, 1 had a history of twin pregnancy, and other had a history of antepartum hemorrhage in the previous pregnancy (Graph 4).



Graph 1: Distribution of blood groups among newborns



Graph 2: Association of isoimmunization with parity



Graph 3: Association of isoimmunization with booking status of patients

In the present study, the neonatal outcome in terms of mortality at the time of birth was not significantly affected by isoimmunization, *P* value being >0.05. However, an adverse outcome like IUD was found more frequently in ICT positive cases. Neonatal death in ICT positive cases was due to severe HDN. Among ICT negative cases, death was due to other cause (Graph 5).

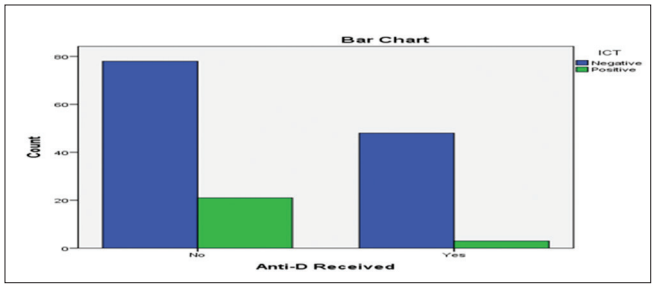
However, 5 min APGAR is <5 in 10 out of 24 ICT positive babies as compared to 5 out of 126 ICT negative babies. Thus, 5 min APGAR score was significantly low among isoimmunized babies, *P* value being <0.01. Furthermore, 6 out of 24 ICT positive babies and 9 out of 126 ICT negative babies were having birth weight <2.5 kg. Thus, low birth weight was also significantly associated with babies of isoimmunized mothers, *P* value being <0.01.

Hemoglobin (Hb) <12% was found in 3 out 24 ICT babies as compared to 1out of 126 ICT negative babies. Hb between 12% and 18% was found in 12 out of 24 ICT positive babies as compares to 14 out of 126 ICT negative babies. Thus, neonatal anemia was significantly associated in babies of isoimmunized mothers, *P* value being <0.01 (Graph 6).

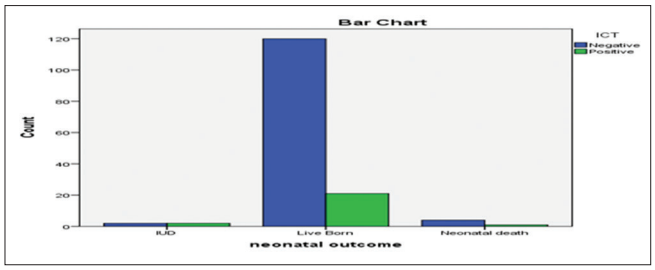
Furthermore, cord blood hyperbilirubinemia was found in 10 out of 24 ICT positive babies as compared to 1 out of 126 ICT negative babies and was found to be significantly associated with isoimmunization, *P* value being <0.05. All these babies developed pathological jaundice (Graph 7).

19 out of 24 babies of isoimmunized mother needed phototherapy as compared to 11 out of 125 non-immunized mothers. Thus, need for phototherapy was significantly associated with isoimmunization, *P* value being <0.05 (Graph 8).

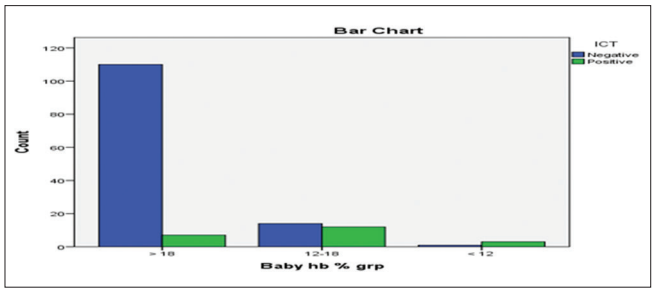
Four babies of isoimmunized mother needed exchange transfusion as compared to 1 out of 126 non-immunized mothers. Thus, need for exchange transfusion was significantly associated with isoimmunized mother, *P* value being <0.01 (Graph 9).



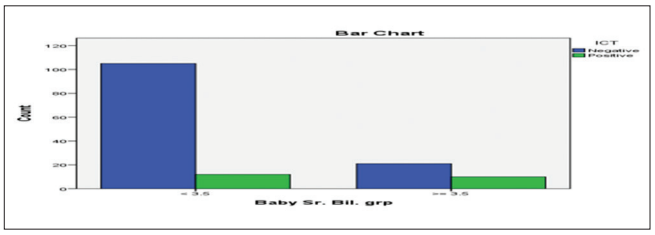
Graph 4: Association of isoimmunization with anti-D administration in previous pregnancy



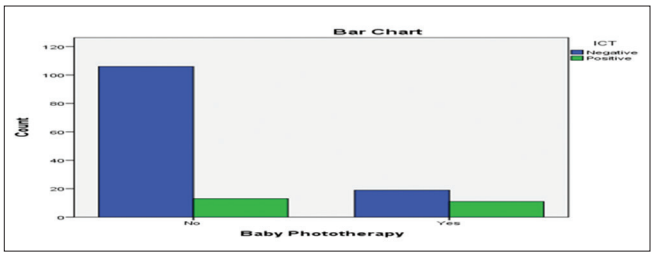
Graph 5: Neonatal outcome in isoimmunized and non-immunized pregnancy



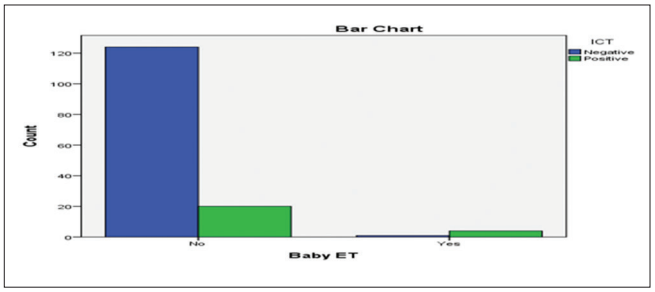
Graph 6: Cord blood hemoglobin in isoimmunized and non-immunized pregnancy



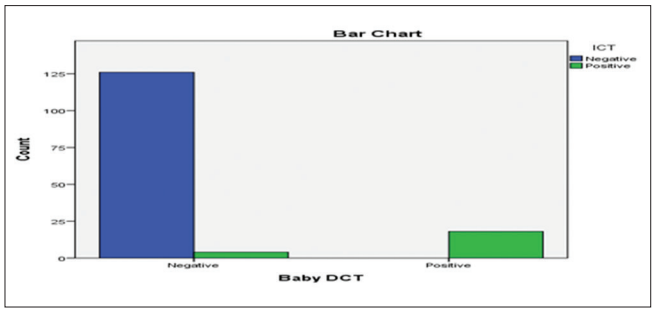
Graph 7: Cord blood bilirubin in isoimmunized and non-immunized pregnancy



Graph 8: Need of phototherapy in isoimmunized and non-immunized pregnancy



Graph 9: Need of exchange transfusion in isoimmunized and non-immunized pregnancy



Graph 10: Distal convoluted tubules positivity in isoimmunized and non-immunized pregnancy

DCT positivity was significantly associated with babies of isoimmunized mother, *P* value being <0.01 (Graph 10).

Only 3 out of 51 booked patients in contrast to 91 out of 99 unbooked patients had not received anti-D prophylaxis in previous pregnancies. In the present pregnancy, 79 (52.6%) patients received post-natal anti-D prophylaxis. 61 patients did not receive because 24 were isoimmunized and 24 babies had Rh negative blood group. 6 patients refused to take anti-D due to the cost factor, and 4 were not prescribed as they adopted permanent sterilization.

DISCUSSION

Rhesus isoimmunization causing erythroblastosis fetalis is a distressing obstetric problem which is still seen in large numbers in India. It is the single most common yet preventable cause of HDN and also an important cause of neonatal hyperbilirubinemia.

Since post-natal anti-D prophylaxis was introduced to prevent sensitization of women lacking Rh antigens, the incidence of Rh hemolytic disease has markedly reduced. A further reduction will probably be achieved by proper and widespread antenatal anti-D prophylaxis. However, despite of these measures the incidence is unlikely to decline to zero. Factors contributing to the grave sequelae resulting from mismanagement of pregnancy in Rh negative women are: No prenatal care (home deliveries), non-availability of Rh testing in many health centers especially in peripheries; inadequate or no anti-D prophylaxis antenatally (after abortion including medical termination, ectopic pregnancy, threatened abortion, ante partum hemorrhage) or even post-nataly many a times. The conventional treatment measures are appropriate hydration, phototherapy, and exchange transfusion. The number of affected cases is still at a stage where the management must be undertaken at a regional level, where the appropriate obstetrics, pediatric, and blood transfusion skill should be made available. Joshep from CMC Vellore used decision analysis technique in the year 2000 and found that there was gross underutilization of anti-D prophylaxis in India.[15] Deka *et al.* observed that failure to administer post-natal anti-D prophylaxis was responsible for Rh D alloimmunization in more than 50% cases followed by failure to administer anti-D after medical termination of pregnancy (MTP) (10%).[14]

In India, Federation of Obstetric and Gynaecological Societies of India guidelines suggest that all patients going for MTP should have documentary proof of blood group of both partners. It recommends routine antenatal prophylaxis by 100 mcg anti-D at 28 and 34 weeks or a single dose of 300 mcg at 28 weeks followed by post-natal prophylaxis by 300 mcg as soon as possible if the baby in Rh positive and DCT is negative. It also recommends 100 mcg anti-D after the sensitizing event of the first trimester. This post-partum anti-D dose is sufficient enough to neutralize 30 ml of fetal blood and is given without quantitative test for FMH. These test, i.e. Kleihauer test, flow cytometry, and rosette test is not readily available in India, also cost benefit of such testing has not been determined.[16]

The present study was undertaken to show the burden of HDN due to Rh incompatibility which is a preventable condition and to illuminates various causes for the persistence of this.

CONCLUSION

Over the 20th century, Rh alloimmunization was clinically recognized, its pathophysiology was understood, its treatment was established, and preventive measures were created to eliminate it. Unfortunately, the incidence of this disease is decreasing at a very slow place in India, in part because of lack of medical information and in part because of the high cost of medication used to prevent it. As shown in the present study, there is a risk of perinatal mortality of 12.5% among Rh negative isoimmunized mothers. Increased morbidity in term of congenital anemia and jaundice poses a great burden to medical professionals leading to increased NICU admissions, phototherapy and need for exchange transfusion. Anemia in newborn adversely affects the growth and development of the baby and increases the risk of neonatal sepsis. About 1/3rd of newborn of Rh negative mothers need treatment for hyperbilirubinemia. This risk is 3.8 times higher among multigravida.

All pregnant women at their first antenatal visit should have documentary proof of blood group; otherwise, a new test should be done. Rh negative mother should be counseled about the importance of Rh immunoprophylaxis and HDN. Routine antenatal prophylaxis with 300 mcg at or around 28th weeks or 100 mcg at 28th and 34th weeks followed by 300 mcg within 72 h of delivery is recommended. 100 mcg

of anti-D injection must be given after any sensitizing event in the first trimester. There should be increased awareness among doctors for RAADP and prophylaxis after MTP, abortion, ectopic pregnancy, etc., which is still lacking. In our country, the high cost of anti-D Ig and lack of its supply by government facilities amounts for significant risk of isoimmunization. While the public health system should guarantee the constant and adequate supply of Ig to all women who need it, physicians are responsible for its correct prescription to ensure the prevention of Rh disease. Family planning should also be encouraged for immunized women since the severely of hemolytic disease increases with consecutive pregnancies.

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