

## Research Article

# Prevalence of rhesus negativity among pregnant women

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### ABSTRACT

**Background:** Hemolytic disease of the newborn (HDN), secondary to Rhesus D (Rh D) iso - immunization, contributes significantly to perinatal morbidity and mortality. The objective of the study was to determine the frequency of Rh factor in this region, which would not only help in blood transfusion services but also eliminate the risk of Erythroblastosis fetalis in the neonates.

**Methods:** A two year retrospective study of rhesus negative pregnant women was carried out at Tertiary care center, Modern Government Maternity Hospital, Petlaburz, Hyderabad, from January 2014 to December 2015.

**Results:** The prevalence rate of Rh D negative women for antenatal care, were 895 (4.29%). Out of that 304 (33.96%) of the Rh D negative women were of blood group B followed by blood group O of 292(32.62%), blood group A of 218 (24.35%) and blood group AB of 81 (9.05%), respectively.

**Conclusions:** The prevalence of Rh D negative women is low when compared to Rh D positive women. There is a need for adequate counselling of pregnant women on the importance of Rh D negative factor during the antenatal period in order to prevent hemolytic disease of the newborn.

**Keywords:** Rhesus negative, Pregnant women, Perinatal morbidity, Hemolytic disease of the newborn, Counselling

### INTRODUCTION

The AB and Rhesus factor (Rh D antigen) are recognized as the major blood group antigens present in the red blood cells. In 1900, the A, B and O types were determined by Karl Landsteiner. Rhesus blood group system was the fourth system to be discovered by Landsteiner and Alexander S. Wiener in 1937.<sup>1,2</sup> The Rhesus system is named after Rhesus monkeys which were used in the experiments that led to the discovery of the system. The presence of the Rh factor, a protein on the red cell surface, constitutes Rh+positive (Rh) person, whereas the absence of Rh factor indicates a negative (Rh) person. Dr. Philip Levine made a connection between the Rh factor and the incidence of Erythroblastosis fetalis resulting from the Rh factor. Wiener realized adverse reactions from transfusions were also resulting from the Rh factor.<sup>2,3</sup>

Determination of blood type in ABO Rh D negative pregnant women allows reasonable precautions which limit the risk to fetus. Erythroblastosis is a very serious medical condition for about 4000 babies a year. In 15% of cases of babies die before birth. Those who survive may suffer from jaundice, which leads to the deaf-muteness, speech disturbances, cerebral palsy and mental retardation.<sup>4,5</sup> The frequency of Rh D iso-immunization in the general population continues to be a point of significance for the clinician, as this significantly contributes to morbidity and mortality in obstetric practice.<sup>6</sup> There is a need for further studies in Rh D negative pregnant women because several factors affect the development of allo-immunization and its prognosis.<sup>7</sup>

Rh antigens are lipoprotein molecules, which are sparsely located at the erythrocyte surface. About 50 of them can be identified, which indicates the specific complexity of the Rh antigen. D antigen is the most immunogenic and therefore the most important antigen. It causes the

formation of antibodies 50 times more often than the C and E antigens. Rh D negative people do not have the D antigen in the Caucasian population, 85% of people are Rh positive and 15% Rh negative. The frequency of Rh negative women is more common for Caucasian women (15%) than African American (5%) and is less common in Asian women.<sup>5,8-10</sup>

Antibodies from Rh system are almost always immune, predominantly in the IgG class, passing due to its size through the umbilical cord and cause hemolytic disease of the new born. Under Hemolytic disease of the new born (HDN) in the strict sense is considered disease whose basis is accelerated immune destruction of fetal/child erythrocytes that are bound to IgG antibodies of maternal origin. These antibodies are directed against antigens of father's origin, which are present in the fetal/children's erythrocytes and that the mother's immune system recognizes them as foreign antigens. This happens if the fetal red blood cells enter mother's circulation.<sup>10,11</sup> Sensitization occurs during childbirth when the mother's bloodstream penetrates certain amount of Rh positive child erythrocytes. Erythrocytes, as foreign substance to the mother, encourages her body to begin to produce Rh antibodies. Therefore, the second and other pregnancy can have complications related to maternal Rh antibodies to Rh positive fetal red blood cells.<sup>4,5,8-10</sup> Their appearance in the circulation is also possible after amniocentesis, spontaneous or induced abortion, cardiocentesis, chorionic villus sampling, ruptured ectopic pregnancy and a blunt trauma to the abdomen.<sup>11</sup>

## METHODS

Data regarding the blood group details of women visiting for antenatal care at Modern Government Maternity Hospital (MGMH), from January 2014 to December 2015, for delivery were collected from records. Blood group details of the patients visited for antenatal care during each month of the year were noted down. Number of candidates belonging to A, B, AB and O groups were consolidated. Rh factor details of the patients were also consolidated. A total number of 20863 subjects were found during the study period. They belonged to both rural and urban areas, mostly lower middle class. Their age group ranged from 18 to 40 years.

The ABO and Rh D factors are part of the routine investigations during the antenatal booking of women attending to Modern Government Maternity Hospital. The previous obstetric history, transfusion history, and obstetric findings were noted. Other information including age, religion, tribe, occupation, and social and family history on the booked Rh D negative pregnant women were obtained from their case files. The Rh D blood group systems of the husbands of women booked for antenatal care is not routinely carried out in this hospital unless specifically requested by the managing clinicians. Ethical review and clearance was obtained from the hospital and ethics committee. Department

protocol was via informed written consent prior to data collection.

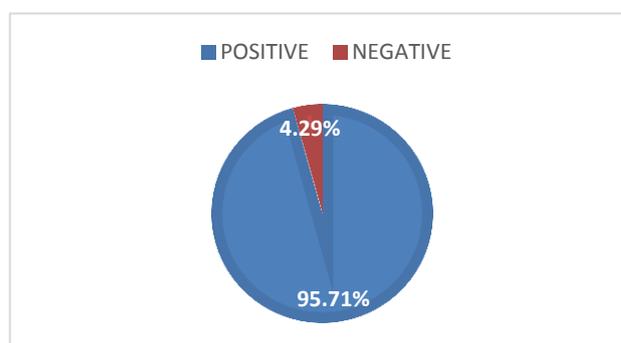
After aseptic washing with 70% ethyl alcohol, blood samples were collected on grease free clean slide from left ring finger tip with the help of a sterile lancet. Blood groups were determined in a single slide to minimize any errors.

The determination of ABO blood group and Rh (D) blood group was done according to the principle of slide method.<sup>12</sup> A drop of blood from each volunteer was placed on a glass slide in three places. A drop of each of the antisera A, B and D was added and mixed with each blood sample, with the aid of glass rods. Monoclonal blood grouping antibodies, in vitro diagnostic reagent of tulip diagnostics private limited are used. Then, the mixture was rocked gently for 60 seconds to observe for agglutination. The results of agglutination were recorded immediately after mixing. The agglutination in blood drop A was considered as group A, and agglutination in blood drop B as group B. The agglutination in both drops was considered as group AB, and if both blood drops were not agglutinated, it was considered as group O. The agglutination in rhesus blood drop was considered as rhesus positive and non-agglutination as rhesus negative. All statistical analyses were done by Microsoft Office Excel 2007. The result was calculated as frequency of each blood group expressed as percentage.

## RESULTS

Women who were visiting for antenatal care at Modern Government Maternity Hospital are included in this study for the period of two years from January 2014 to December 2015.

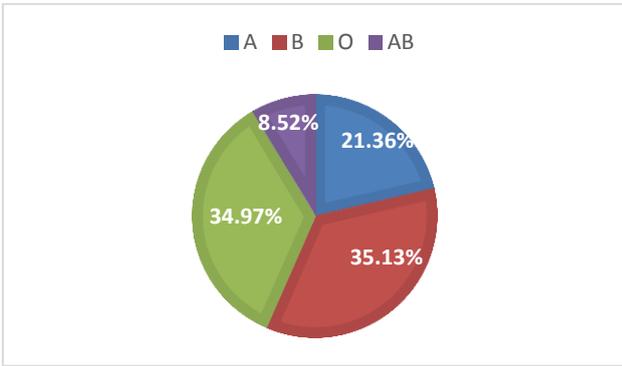
Figure 1 shows the Rhesus blood group distribution among the pregnant women. The frequency distribution of ABO blood group among the participants is shown in Figures 2.



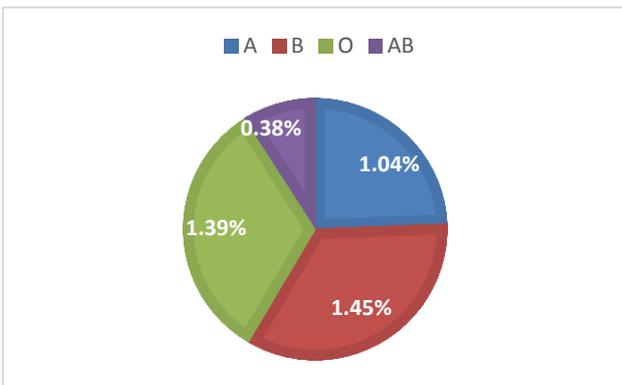
**Figure 1: Rhesus blood group distribution among the pregnant women at modern government maternity hospital, Hyderabad.**

The percentages of the ABO blood group and Rhesus blood group varies significantly. The Rhesus negative

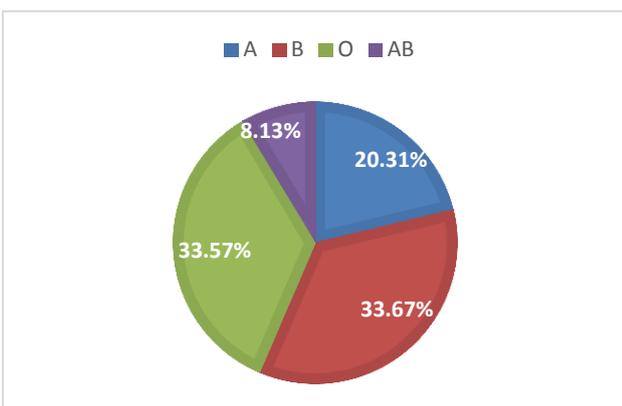
blood group distribution is shown in Figure 3 which states that 1.04% are of group A, 1.45% are group B and 1.39% O, 0.38% are group AB.



**Figure 2: Abo blood group distribution among the pregnant women at modern government maternity hospital, Hyderabad.**



**Figure 3: Rhesus-negative blood group distribution among the pregnant women at modern government maternity hospital, Hyderabad.**



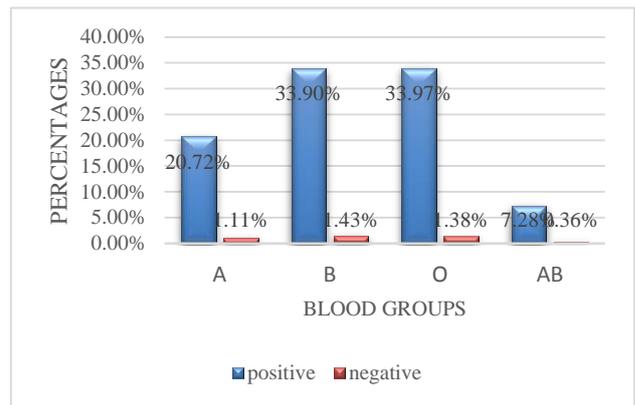
**Figure 4: Rhesus-positive blood group distribution among the pregnant women at modern government maternity hospital, Hyderabad.**

In the rhesus-positive blood group distribution, as shown in Figure 4, blood group A has percentage frequency of

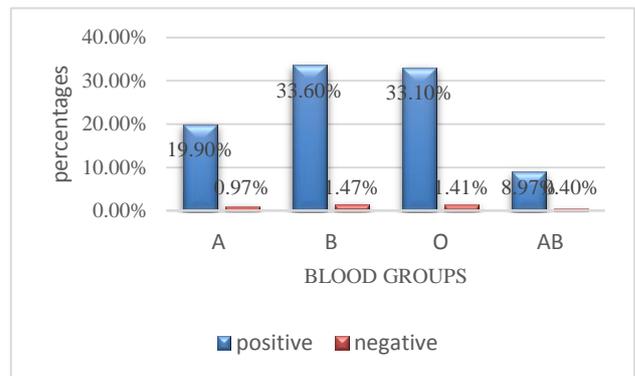
20.31%; blood group B 33.67%; blood group AB 8.13% and blood group O 33.57%.

Blood group B had the highest frequency followed by blood groups O and then A. Blood group AB had the least.

The Rhesus-positive and Rhesus-negative vary among the ABO blood group. Rhesus positive has the highest frequency (95.71%) while Rhesus negative has the lowest frequency (4.29%). Distribution of ABO blood group among the pregnant women based on rhesus blood group in the year 2014 and 2015 are shown in Figure 5 and 6.



**Figure 5: Distribution of ABO blood group among the pregnant women based on rhesus blood group in the year 2014 at modern government maternity hospital, Hyderabad.**



**Figure 6: Distribution of ABO blood group among the pregnant women based on rhesus blood group in the year 2015 at modern government maternity hospital, Hyderabad.**

**Table 1: Distribution of rhesus blood group in pregnant women at modern government maternity hospital, Hyderabad.**

Rhesus blood group	Frequency	Percentage
Rh d positive	19968	95.71%
Rh d negative	895	4.29%
Total	20863	100%

**Table 2: Distribution of rhesus negative blood group among pregnant women in modern government maternity hospital, Hyderabad.**

Rhesus negative blood group	Frequency	Percentage
A rh d negative	218	24.35%
B rh d negative	304	33.96%
O rh d negative	292	32.62%
Ab rh d negative	81	9.05%
Total	895	100%

## DISCUSSION

The knowledge of the blood groups and Rhesus factor is important in evolution, related to diseases and environment, essential in blood transfusion, organ transplantation, forensic pathology, anthropology and training ancestral relation of human, and also helps to prevent complications due to Rhesus incompatibility.<sup>13,14,15</sup> The prevalence of Rh D negative women in this study is 4.29%. This is similar to previous studies done at Ibadan and Abraka in Nigeria.<sup>7,16</sup> This rate shows a low frequency of Rh D negative blood group system in this environment. This finding is similar to that previously reported amongst African subjects, West Indians, and blacks in Great Britain.<sup>17,18</sup> The results are, however, different from those reported from the Eastern highlands of Papua Guinea where the entire population was reported to be 100% Rh D positive.<sup>19</sup> The Rh D negative blood system is of great clinical significance, especially in medical emergencies where appropriate group compatible blood may not be available. In pregnancy, Rh D negative women whose husbands are Rh D positive need adequate counselling on the etiology of Rh D iso-immunization during the antenatal period to prevent hemolytic disease of the newborn.<sup>7,17,20</sup> Rh D positive women were more commonly seen than Rh D negative women.<sup>2,18</sup>

Before the introduction of immune prophylaxis the immunization incidence of Rh D negative multiparas with Rh D positive child to Rh D antigen was approximately 18%. Introducing postnatal Rh D immunization of pregnant women reduced the incidence of immunization to less than 1%. The introduction of combined antenatal (at 28<sup>th</sup> week of gestation) and postnatal Rh D immunization of pregnant women the incidence of immunization is reduced to less than 0.1%.<sup>9,23</sup> With or without antenatal immune prophylaxis all Rh D negative pregnant women who gave birth to Rh D positive fetus and not immunized with the D antigen, should receive 100-300 mg (500-1500 IU) Rh Ig IM within the first 72 hours after delivery. Rh Ig dose should be adjusted to the size of fetus-maternal haemorrhage. Significant fetal-maternal haemorrhage, which usually occurs during the traumatic delivery (including caesarean section), the manual removal of the placenta, intrauterine fetal death, stillborn, abdominal trauma in the third trimester of pregnancy, simultaneous delivery of two or

more children and/or unexplained fetal hydrops requires Rh D immunoprophylaxis in doses higher than the standard. Prevention of HDN is successful when hyperimmune Rh D gammaglobulin is used in 28/29 and 32/34 week of pregnancy.<sup>5,9,23</sup>

Financial inability to purchase the anti-D immunoglobulin had been identified to be a major reason why women don't receive immune prophylaxis, in the condition when the women are unaware about the free supply of anti-D in Government maternity institutions in India. A vial of anti-D immunoglobulin of 1,500 IU or 250µg costs between 2500-7000 INR (75-94 USD). The reasons given by the women for not receiving immune prophylaxis, apart from financial inability, showed the poor knowledge of the women about Rhesus isoimmunization, and there is need to improve their knowledge via the antenatal health talk.<sup>21</sup>

Anti-D immunoglobulin is given only as a prophylaxis and is useless once sensitization has occurred. There is need for proper public education about this preventable disease and its free supply in India. Obstetricians, Hematologists, and Neonatologists also need to put in place a proper protocol for the management of Rh D negative pregnant women to prevent Rh D iso-immunization and to properly care for affected children.<sup>2,22</sup>

However, large-scale studies on pregnant women need to be done in order to collect sufficient evidence to be able to formulate guidelines regarding testing and interventional modalities for alloimmunisation in pregnancy.

## CONCLUSION

In order to reduce the occurrence of alloimmunization of the mother to erythrocyte antigens of the newborn that can lead to major complications in subsequent pregnancies of Rh D negative mothers and HDN, constant monitoring in order to prevent them is necessary. Prevention is essential because once immunized mother will remain immunized for life. There is need for proper public education about this preventable disease. Study of blood grouping not only generates a simple database but also create a great social awareness about self-blood grouping and safe blood transfusion among the population of a country. The identification of the Rh D antigen and its description is a cornerstone of modern immunohematology.

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