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Frequency of Rhesus D-Responder and Non-Responder among the D-Negative Mother in Bangabandhu Sheikh Mujib Medical University, Bangladesh

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Abstract
Objective: *To determine the prevalence of anti-D antibody formation among the Rhesus D negative women in response to D antigen exposure either by previous transfusion, delivery or immunization by Ig-D.*
Methodology: *Cross sectional prospective study. This study had done in the Transfusion Medicine Department, BSMMU from 1st January 2007 to 31st January 2015.*
Subject: *All 783 Rhesus negative mothers were sent for detection of Rh D antibody as a part of their antenatal check up.*
Result: *Higher prevalence of O group found in this study. More alloantibody detected in the age group 25-29 years and other ABO group shows the relationship of alloantibody formation with age and parity. Husband blood group was more or less competent with the mother's blood group. Abortion plays a more important role in development of antibody than menstrual regulation (MR). History of receiving Rhesus immunoglobulin (RhIg) is less detected than the non-immunization cases. It shows the importance of giving RhIg in a Rh negative mother.*
Conclusion*: Routine antenatal anti-D immunoglobulin prophylaxis (RAADP) is recommended for all non-sensitized D- negative mother.*

Keywords: *D-Responder, D-negative, Hemolytic, fetomaternal, Hemolytic Disease of Fetus and Newborn (HDFN) or Hemolytic Disease of Newborn (HDN).*

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I. Introduction

The terms Rh positive and Rh negative refer to the presence or absence of the red cell antigen D. The first human example of the antibody against the antigen later called D was reported in 1939 by Levine and Stetson; the antibody was found in the serum of a woman whose fetus had hemolytic disease of the fetus (HDFN) and who experienced a hemolytic reaction after transfusion of her husband's blood. With the exception of the A and B antigens, Rh D is the most important RBC antigen in transfusion practice. (3) The Rh D antigen has greater immunogenicity than virtually all other RBC antigens. Expression of Rh D antigen varies quantitatively and qualitatively among individuals. (5) Persons whose red cells lack the D antigen do not regularly have anti-D. Formation of anti-D results from exposure, through transfusion or pregnancy, to red cells possessing the D antigen. (4) The D antigen has greater immunogenicity than other red cell antigens. The Rh antigen are highly immunogenic among those the D antigen is the most potent, less than 0.1 mL of Rh D

positive RBCs can stimulate antibody production in an Rh –D negative person. [(6)] It is estimated that 30% to 85% of D-ve persons who receive a D+ve transfusion will develop anti-D. Rh antibodies are almost always IgG and do not bind complement thus they lead to extra vascular rather than intravascular RBC destruction.

Haemolytic disease of the newborn (HDN) is a condition in which the lifespan of the infant's red cells is shortened by the action of specific antibodies derived from the mother by placental transfer. [(2)] The disease begins in intrauterine life and is therefore correctly described as hemolytic disease of the fetus and newborn, but the simple term HDN has been used for a long time and can be taken to include haemolytic disease of the fetus (HDFN).

Pregnancy causes immunization when fetal red cells, possessing a paternal antigen foreign to the mother, enter the maternal circulation as a result of fetomaternal hemorrhage (FMH). FMH occurs in the vast majority of pregnancies, usually during the third trimester and during delivery. Delivery is the most common immunizing event, but fetal red cells can also enter the mother`s circulation after amniocentesis, rupture of an ectopic pregnancy, and blunt trauma to the abdomen. The antigen that most frequency induces immunization is D, but in theory, any red cell antigen present on fetal cells and absent from the mother can stimulate antibody production. The probability of immunization to D correlates with the volume of D-positive red cells entering the D-negative mother`s circulation. [(1)] Trans-placental hemorrhage of fetal RBCs into maternal circulation occurs up to 7% of women during gestation as determined by the acid elution method for fetal hemoglobin.

The overall incidence of D sensitization in untreated D-negative mothers of D-positive infants is about 16%; 1.5 to 2% become sensitized at the time of their first delivery, an additional 7% become sensitized within 6 months of the delivery, and the final 7% become sensitized during the second affected pregnancy.[(1)] The sensitization during the second affected pregnancy probably reflects primary immunization during the first D-positive pregnancy and delivery that happened without production of detectable levels of antibody. The small numbers of D-positive fetal red cells entering the maternal circulation early during the second affected pregnancy constitute a secondary stimulus sufficient to elicit overt production of IgG anti-D. In susceptible woman not immunized after two D-positive pregnancies, later pregnancies may be affected but with diminished frequency. Once immunization has occurred, successive D-positive pregnancies often manifest HDFN of increasing severity, particularly between the first and second affected pregnancies. After the second affected pregnancy, the history is predictive of outcome, although, in rare instances, some women have a stable or diminishing pattern of clinical disease in subsequent pregnancies.

Rh immunization of untreated D-negative women occurs less frequently after delivery of an ABO-incompatible D-positive infant than when the fetal cells are ABO-compatible with the mother. ABO incompatibility between mother and fetus has a substantial but not absolute protective effect against maternal immunization by virtue of the increased rate of red cell destruction by anti-A or anti-B. Statistically mother & infant are ABO incompatible is one in every five pregnancies. The rate of immunization is decreased from 16% to between 1.5% and 2%.[(1)]

The risk of immunization is only about 9% for an Rh negative mother after an Rh positive pregnancy if RhIg is not administered.

In general, a titre of 16 to 32 is considered significant antibody titer cannot predict the severity of HDN because it depends on sub class of IgG whether the antibody is IgG1 or IgG3.

ABO incompatibility between mother and fetus has a substantial but not absolute practice effect against maternal immunization by the increased rate of red cell destruction by anti-A or anti-B. The rate of immunization is decreased from 16% to between 1.5% to 2% (1) chap23.page 531.

II. Materials And Method:

This study was done in Transfusion Medicine Department, BSMMU from 1st January 2007 to January 2015. Total 802 blood records of Rh D negative mother with Rh D positive husband were reviewed. 121 cases with detected antibody were analyzed.

The 3 ml of venous blood was collected in a dry sterile plain test tube serum is separated by centrifuge method and test done by enzyme (Bromelin), Albumin (20% bovine) and ICT method at 37^0C for detection of antibody and double dilution method for titer. The higher dilution where fetal cell show agglutination microscopically taken as titer value which is indicated as ratio value. The red cell used was blood group O D-positive. (5% cell suspension)

Data collected by a structured questionnaire form filled by the respondent herself who came for detection of Rh D antibody as a part of their antenatal check up from 3 months onwards. Any discrepancies observed during test were repeated by close supervision.

III. Result:

During the study period total 802 data of D negative patients were interpreted. Excluding the repeat cases, 783 negative mothers' data were analyzed, out of them 121 cases detected irregular antibody ranging

from 1:2 to 1: 1024. In this study, higher prevalence of O group and more alloantibody were detected in the age group 25-29, husband's blood group was more or less competent with mothers' blood group.
All the data presented in table from.

Table 1: Percentage of ABO Blood Group

	A group	B Group	O Group	AB group
783 patient	194(24.8%)	266(34%)	272(34.8%)	51(6.5%)
121 detected patient	31(25.6%)	41(33.9%)	41(33.9%)	8(6.6%)
783 husband	129(16.5%)	283(36.2%)	232(29.7%)	74(9.5%)
121 husbands of detected cases	24(19.8%)	35(28.9%)	53(43.8%)	9(7.4%)

Table 2: ABO Blood group compatibility of couple

Blood Group of Patient	Blood Group of Husband							
	A+ve		B+ve		O+ve		AB+ve	
	In 783	In 121	In 783	In 121	In 783	In 121	In 783	In 121
A-	49	15	76	8	51	7	18	1
B-	78	3	95	19	72	16	20	3
O-	57	3	95	17	92	**26**	29	**5**
AB-	8	3	17	1	19	4	7	0
Total	192	24	283	35	234	53	74	9

Table 3: Child of 121 detected cases

Have single living child	**80**
More than one living child but no dead child	13
No living or dead child	8
History of dead child	57
4 or more dead child but no living child	4
Child received exchange transfusion	10

Table 4: Immunization history

History of	Abortion	MR	RhIg
783 patient	79(10.1%)	91(11.7%)	171 (21.8%)
121 Antibody detected	11(9.1%)	14(11.6%)	22(18.2%)

Among total 783 patients, had the history of abortion 79 (10.1%), MR 91 (11.7%), receiving RhIg 171 (21.8%), and out of them antibody was detected in 121 patients, and who had abortion 11 (9.1%), MR 14 (11.6%), RhIg 22(18.2%).

Table 5: Age distribution

	15-19	20-24	25-29	30-34	35-39	40+
783 Cases	43	213	283	174	63	7
121 detected	8	22	44	33	10	4

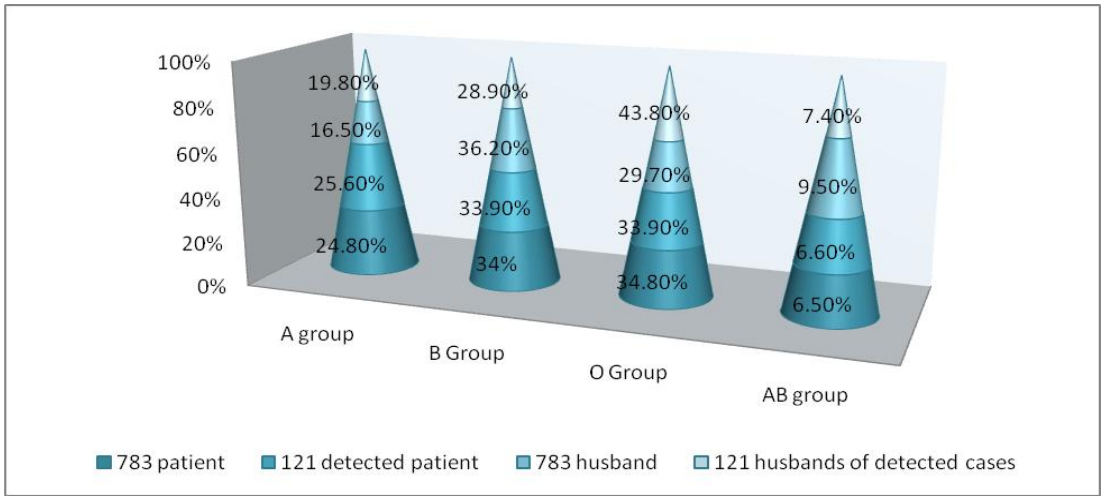

Figure 1: The Bar diagram showing the percentage of different ABO blood group among patients and their husband.

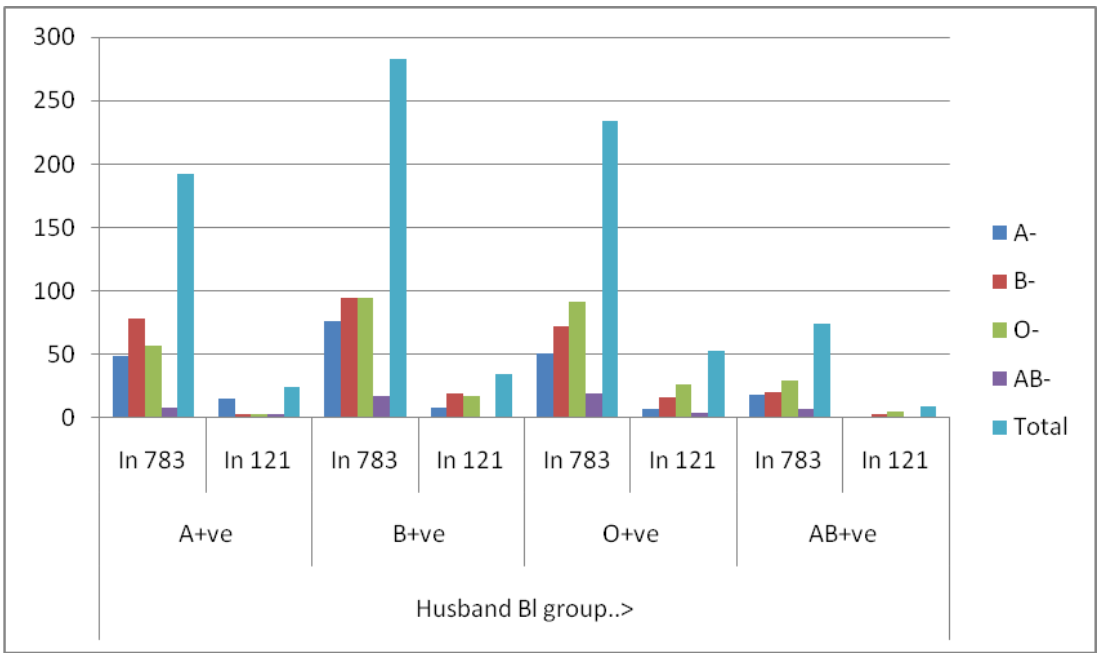

Figure2: The diagram showing ABO blood group compatibility of couple.

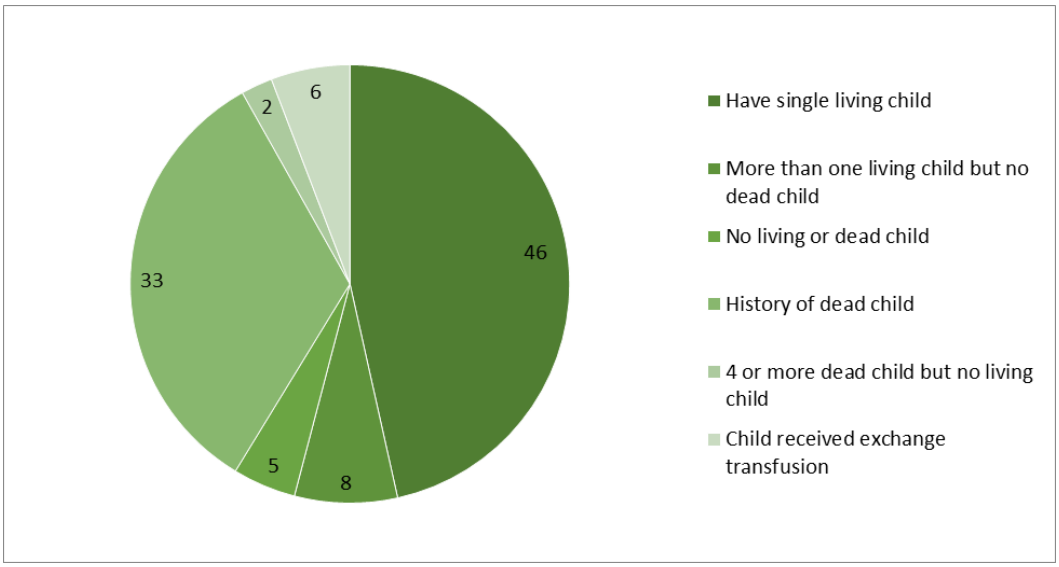

Figure3: This Pie diagram illustrating the number of child in 121 antibody detected cases.

IV. Discussion:

It shows higher number of O blood group in participants. It shows awareness of physician who want to investigate about alloantibody as O blood group may possess IgG. Relatively less A blood group of husbands of 65 detected cases justify established knowledge.

It is also observed that husband's blood group was more or less competing with the mother's blood group those who produce antibody.

More participants as well as more frequency alloantibody detected cases in (25-29) age group shows the relationship of alloantibody formation with advancement of age and parity.

M.R and abortion in early gestation is the risk factor for primary immunization by transplacental hemorrhage the most dangerous period is during parturition, especially if the labour is complicated. This two are risk factors for antibody production due to increase amount of trans placental hemorrhage. More rate of abortion in detected cases interprets that abortion plays a more important role in development of antibody than MR.

A good number of patient 171 (21.8%) received RhIg. The use of RhIg should be more specially at the time of abortion or M.R. which might be a cause of low prevalence of antibody formation. H/O receiving RhIg

is less in detected cases and higher percentage of non immunization after administration of RhIg shows the importance of giving in a Rh-negative mother.
Comments of present or detected in data are applicable for ICT positive cases only in 1:1 titre.
Relatively less number of A group husband of 121 antibody detected mother shows that group incompatibility protects the mother more in case of A group.

V. Conclusion:

Self-responding questionnaire was used, so cross validation was not possible in this study. More important data were missing in some cases. So routine antenatal anti-D immunoglobulin is recommended prophylaxis (RAADP) is recommended for all non-sensitized D- negative mother.

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