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Role of antenatal antibody screening in multiparous women: experience of a single tertiary care center

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Abstract

Introduction: Alloimmunization occurs when an incompatible antigen introduced in an immune-competent host evokes an immune response. Since IgM antibodies are complement-binding, these can cause immediate and severe intravascular hemolysis after transfusion. We carried out this study to determine the prevalence and trends of Rh D negativity among pregnant females and to analyze antenatal Rh alloimmunization by fetomaternal hemorrhage.

Material and methods: Institutional review board approved study, carried out on 6,100 multiparous pregnant females, irrespective of their period of gestation and obstetric history. Demographics for age, sex, obstetric history, blood group, history of having received anti-D immunoprophylaxis (in the current pregnancy) and history of previous blood transfusions were recorded.

Results: The most common phenotype was ‘B’ & Rh Positive. 5734 females were D antigen-positive (94 %) and 366 D antigen-negative (6.0%) (Table 1). 69

antibodies were detected in 63 patients, thus the prevalence of alloimmunisation was 1.03% (63/6,100). Among the 366 females in the D antigen-negative group, 47 developed antibodies, so the prevalence of alloimmunisation in this group was 12.84 %.

Conclusion: Antenatal screening in all pregnant females is essential since Rh D^+ positive females are just as likely as D^- females to form alloantibodies. A close follow up throughout pregnancy is required to detect irregular antibodies and will be helpful to limit fetal hydrops as well as decrease in perinatal morbidity and mortality rates.

Keywords : Alloimmunisation, Antigen, Antibody, Anti-D,Immunoprophylaxis.

Introduction

Alloimmunization occurs when an incompatible antigen introduced in an immune-competent host evokes an immune response. The immune response to carbohydrate antigens is usually thymus independent. Individuals lacking a particular carbohydrate blood group antigen on their red cells can have 'naturally occurring' IgM antibodies, which are most probably stimulated by cross-reacting antigens present in the environment, such as on gut bacteria. The most important carbohydrate antigens for blood transfusion practice are the A- and B-antigens. Normal individuals who lack either the A or B antigen make IgM B- or IgM A antibodies respectively. Since IgM antibodies are complement-binding, these can cause immediate and severe intravascular hemolysis after transfusion of incompatible red cells leading to serious or fatal complications.

The most important and frequent irregular red blood cell alloantibodies in daily transfusion practice, in terms of frequency of occurrence, are directed towards the RH (anti-D, -C, -E, -c and -e), KEL (anti-K), FY (anti-Fya and -Fyb), JK (anti-Jka and -Jkb)and the MNS (anti-M, -S and -s) blood group systems. Of these, the D-antigen is the most immunogenic, resulting in more than 80% of immunocompetent D negative persons becoming alloimmunized after a transfusion of D-positive erythrocytes [1,2]. We carried out this study to determine the prevalence and trends of Rh D negativity among pregnant females and to analyze antenatal Rh alloimmunization by fetomaternal hemorrhage.

Material and method

This was an Institutional review board approved study conducted from June '12 to November'13. Study was carried out on 6,100 multiparous pregnant females attending antenatal outpatient department of obstetrics & gynecology were included in this study irrespective of their period of gestation and obstetric history. Demographics for age, sex, obstetric history, blood group, history of having received anti-D immunoprophylaxis (in the current pregnancy) and history of previous blood transfusions were recorded.

Sample collection

4 ml of whole blood was collected in K2-EDTA and in red (plain) vacutainer for determination of blood group.

ABO typing (Cell & Serum grouping): ABO and Rh(D) typing was done on fully automated blood grouping & matching system using electromagnetic technology from EDTA vacutainer. Blood grouping of samples found screening positive were duly confirmed by standard tube technique Commercial antisera were used for forward grouping (cell grouping) and in-house prepared pooled A,B,O cells were used for reverse grouping (Serum grouping).

Screening for irregular antibody: screening for irregular antibodies were done by the electromagnetic technology on a fully automated blood grouping & matching system from Plain blood samples. We

additionally confirmed antibody screening on semi automated platform by Indirect Coombs Test (ICT) method using a commercial 3-cell antibody screening panel (ID Diacell I, II, III ; DiaMed ID microtyping system). A commercially available 3- cell antigen panel(ID Diacell I, II, III ; DiaMed ID microtyping system) was used for the antibody screening. Patients serum was reacted with panel cells in AHG gel cards. The cards were incubated at 37°C for 15 min followed by 10 minutes of controlled centrifugation. If antibody screening with 3- cell antigen panel was positive, an extended 11 cell panel was used for antibody identification.

Antigens present on

Diacell I :- D, C, e, C^w, k, Kp^b, Fy^b, JK^a, JK^b, Fy^b, Le^a, P, N, S, s, Lu^b and Xg^a.

Diacell II :- D, E, c, k, Kp^b, Fy^b, JK^a, Le^b, M, S, Lu^a, Lu^b and Xg^a.

Diacell III :- c, e, K, k, Kp^b, Fy^a, JK^b, P_1, M, N, s, Lu^b and Xg^a.

Antibody identification: antibody identification was performed for samples positive with Diacell I/II/III or all using a commercially available 11-cell antibody identification panel (ID Diacell I, II, III……XI ; DiaMed ID microtyping system), This system consists of 11 different group O red Cells, each having variable antigens of Rh, Kell, Duffy, Kidd, Lewis, P, MNS, Lutheran and Xg blood group system(D, C, E, c, e, C^w, K, k, Kp^a, Kp^b, Js^a, Js^b, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P_1, M, N, S, s, Lu^a, Lu^b, Xg^a).

Results

Blood group distribution among multigravida females: A total of 6,100 multigravida females were screened for the presence of alloantibody. With regards to the major blood group systems (ABO and Rh), the most common phenotype was 'B' & Rh Positive respectively, 5734 females were D antigen-positive (94 %) and 366 D antigen-negative (6.0%) (Table 1). 69 antibodies were detected in 63 patients, thus the prevalence of alloimmunisation was 1.03% (63/6,100).

Association of D antigen with alloimmunization: Among the 366 females in the D antigen-negative group, 47 developed antibodies, so the prevalence of alloimmunisation in this group was 12.84 % (Table 3).Six patients had two types of antibodies; hence 53 types of antibodies were detected in these 366 patients. In the D antigen-negative group, out of 53 antibodies 45 (84.9%) were anti-D (alone or in combination with anti C), 6 (11.32 %) were anti-C (in combination with anti-D) and 1 (1.88 %) was anti-S and 1 (1.88) was anti – Fy^a

Frequency of antibodies in relation to Adverse obstetric history: Of all 69 antibodies detected in this study, 16 were found in D antigen-positive females, giving an overall prevalence of alloimmunisation in the D antigen-positive group of 0.28 % (16/5734). There was 6 cases of anti-E 4 cases of anti-c, 3 cases of anti-S, 2 cases of anti-M and 1 case of anti-C^w (Table 4).

Frequency of alloantibodies according to blood group systems: Within the whole study group (n=6100), anti-D was the most common antibody, accounting for 65.21 % of all the antibodies formed (either alone or in combination). Multiple antibodies (dual) were present in 8.69 % (6/69) patients. The most common combination in our study was anti-C and anti-D (shown to be two different antibodies by selective adsorption studies). Antibodies belonging to the Rh system accounted for 89.85 % of overall alloimmunization in our study group, belonging to the MNS is 8.69%, and belonging to Duffy group is 1.44 % (Table 5).

Adverse Obstetric history and alloimmunization: In our study, alloantibodies were found in 4.39 % (48/1091) of antenatal females with an adverse obstetric history and in 0.49 % (15/3,068) of antenatal females without an adverse obstetric history ($p<0.001$) (Table 6).

An adverse obstetric history (any history of stillbirth, abortion or medical termination of pregnancy) was present in 82 % of patients with anti-D (32/39) and in 66.7% of patients with combined anti-C and anti-D (4/6). A history of blood transfusions was present in 7.93 % (5/63) females with alloantibodies in 1.03% (63/6,100) of all antenatal females.

Out of a total of 45 D antigen-negative females with anti-D, the husband's blood group could be confirmed in only 30 cases and was found to be D antigen-positive in all. Among the non-anti-D group, the husbands of four females had the corresponding positive antigen on their red cells.

Antibody formation in relation to gravida status: The data relating to antibody formation and the number of pregnancies are presented in Table 7.

Discussion

Antenatal services in India are fragmented and not uniform and there is a limited amount of published data on alloimmunisation rates among pregnant females in India. Although guidelines for screening have been laid down by the Drug Controller General, India[3], screening for alloantibodies is being done primarily for Rh D-negative females or patients presenting with an adverse obstetric history. In this study we found an overall alloimmunisation rate in pregnant females of 1.03 %.Koelewijnet al.[4], in their study to assess the efficacy of a universal antibody screening programme for pregnant females, found a total alloimmunisation rate of 1.2%. They detected alloantibodies other than anti-D of more than one specificity in 14% of index pregnancies, with anti-C and anti-E being most common.

Al-Ibrahim et al.[5] found a 2.0% alloimmunisation rate while Howard et al.[6]detected clinically significant antibodies among 1.0% of all pregnant females. In contrast, Gottvallet al.[7] found an alloimmunisation rate of 0.4% in all pregnancies with clinically significant alloimmunisation in 0.16% of pregnancies. The alloimmunisation rate recorded by De Vrijeret al.[8] among 2392 females was 2.71%.

In our study, the alloimmunisation rate in the D antigen-negative group was 12.84 %. In the literature, there is a wide variation in alloimmunisation rates among Rh-negative females. Lurie et al.[9]found a low alloimmunisation rate of only 0.9% in Israel whereas Al-Ibrahim et al.[5]found a higher rate of 7.1% in Saudi Arabia. Salolaet al.[10] recorded an alloimmunisation rate of 2.98% in Rh-negative females. The rate of alloimmunisation in Rh-negative females in our study is much higher than that in western studies. This can be attributed to the lack of implementation of standardised and universal anti-D immunoprophylaxis in India. Anti-D does therefore continue to the main culprit responsible for alloimmunisation in our country, accounting for 65.21% of all alloantibodies in our study. Our results are in concordance with the results of several other studies. Gottvallet al.[7] found that anti-D was the cause of alloimmunisation in 60% of cases (Table VII). Lenkiewiczet al.[11] and Howard et al.[6] found that anti-D was responsible for 45.5% and 41%, respectively, of cases of significant immunisation. In these studies, anti-D was the leading offender despite immunoprophylaxis.

The alloimmunisation rate within the D-positive group in our study was 0.28%. This is in accordance with the

findings of several other studies, such as those by Lurie et al.[9] and Adenijiiet al.[12], who reported alloimmunisation rates among D-positive females of 0.2% and 0.15%, respectively.

In our study, we found a statistically significant correlation between frequency of Rh-alloimmunisation and adverse obstetric history ($p<0.001$, odds ratio=15.32,) which means that the odds of an antibody-positive females having an adverse obstetric history were more than 10 times higher than females who were antibody negative. The gravida status of females also showed a statistically significant, positive correlation with alloantibody formation. There are limited published data, particularly from India and South East Asia, on such correlations.

It is difficult to compare the results of different studies because of the heterogeneity of populations involved, varied screening protocols, variation in the definition of clinically significant antibodies and difference in the techniques used for antibody identification.

Despite prophylactic use of Rh immunoglobulins, anti-D is still a common antibody identified as the major cause of alloimmunisation. Koelewijnet al.[4] found that the prevalence of alloantibodies other than anti-D is 0.38%. They emphasised that HDFN caused by antibodies other than anti-D occurred in 7–8 cases per 100,000 pregnancies. Without a universal antibody screening programme for red cell alloantibodies in the first trimester of pregnancy, there would be approximately two foetal deaths due to severe intrauterine anaemia in 100,000 pregnancies (in which intrauterine transfusion could have been beneficial).

Lurie et al.[9] have suggested that antibody screening is not warranted from a cost-clinical benefit perspective. Lee et al.[13] supported the view that routine antenatal antibody screening for Chinese females may not be worthwhile. Moreover, they found different specificities of antibodies compared to those reported for western countries, with anti-Mi being the most frequently encountered antibody. However, long-term extensive studies have not been done to assess the severity of problem of alloimmunisation in pregnancy, the clinical significance of these non-D antibodies and their impact on outcome and interventional modalities in the Indian population.

Based on the fact that anti-D accounted for 78.4% of all alloantibodies, we need to focus more on anti-D immunoprophylaxis. In our study, there was a glaring, statistically significant difference between alloimmunisation rates in Rh D-negative versus Rh D-positive group (12.84% versus 0.28%; $p<0.001$). Moreover, follow-up and treatment facilities for antibodies other than anti-D are not available in most of centres across India. However, large-scale studies on pregnant females 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.

HDFN is a condition caused by maternal antibodies to foetal red cell antigens, which cross the placenta and cause haemolysis. The antibodies can be natural or immune. In the latter case, the sensitizing event is frequently a previous pregnancy or a transfusion, where the mother was exposed to the relevant antigen.

In developing countries, antenatal screening is generally targeted solely at detection of anti-D. Moreover, the applicability of western guidelines, and the utility of antibody screening panels developed within western populations are not well established. The issue of whether routine antibody screening in Rh positive females is warranted, especially in developing countries has also been debated . The presence of

alloimmunization in 1.03 % per cent females in our study and the general profile of clinically significant antibodies correlated with other studies from India . It is possible that some antibodies in our study were missed by the absence of routine third trimester screening. Studies have shown that first trimester screening alone can miss a significant fraction of clinically significant antibodies . In addition, our study included only hospital attendees, and is not representative of the prevalence of anti D among a large number of Indian females who do not have access to obstetric care. Given the low occurrence of allosensitization among Rh(D) positive females, a routine screening programme may not be feasible, as perhaps one out of approximately 1250 Rh(D) positive females would have clinically significant antibodies. However, we suggest that where facilities for management of an

allosensitized pregnancy are accessible, the option of screening should be extended to Rh(D) positive females.

Our study found non-D antibodies to constitute a significant proportion of clinically relevant antibodies. In a Croatian study, clinically significant non-D antibodies produced HDFN in approximately 55 per cent of alloimmunized pregnancies, and severe HDFN, defined by perinantal transfusion requirement or death, in approximately 25 per cent . Prevalent screening methods using random O positive pooled cells or cells phenotyped for Rh alone thus ignore a significant component of sensitization. Non-Rh antibodies contributed to 75 per cent of the clinically significant antibodies in Rh(D) positive females, implying the need in this group to use screening panels that are not restricted to Rh but incorporate a wider range of clinically significant antigens.

The antibody identification panel used in our study was not framed to identify anti-Mi, which was reported to be the most frequent irregular antibody in a study from China . Whether this or/and some other population specific antigen can account for the large proportion of unidentified antibodies in our study needs further evaluation. Antibodies that have been reported to cause HDFN in the Indian population include anti-c, anti Jk , anti E and anti M . However, there are possibly others which remain unreported, or unidentified owing to limitations in facilities for their identification. ‘

Conclusion

Our study highlights the importance of antenatal screening in all pregnant females since Rh D^+ positive females are just as likely as D^- females to form alloantibodies. A close follow up throughout pregnancy is required to detect irregular antibodies. The prevention and treatment of Rh D alloimmunization leading to fetal hydrops is a true success story in obstetrics. Discovery of the Rh D antigen and implementation of anti-D IgG prophylaxis to prevent sensitization in 99% of potential cases now allows for good outcomes in Rh D-negative females. Furthermore, in patients who have become sensitized, close monitoring of antibody titers, the use of MCA-PSV or delta OD_{450} to recognize hemolysis or anemia, and treatment with IUT have led to a dramatic decrease in perinatal morbidity and mortality rates.

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Legend Tables and Figures

Table 1: Blood group distribution among multigravida females

Blood group	No. of Females	%
A	1498	24.56
B	2234	36.62
O	1754	28.75
AB	614	10.07
Total	6100	100.00

Table 2		
Blood group	No. of Females	%
D antigen Positive	5734	94
D antigen Negative	366	6

Table 3: D antigen with alloimmunisation

	Antibodies not detected	Antibodies detected
In D antigen Positive females	5716	16(0.28)
In D antigen Negative females	319	47(12.84)

Table 4: Distribution of alloantibody detected.

Antibodies (n=63)	No. of patients with alloantibodies	D antigen-positive females	D antigen-negative females	Females with Adverse obstetric history
Anti-D	39	-	39	32
Anti-C and anti-D	6	-	6	4
Anti –E	6	6	0	6
Anti-M	2	2	0	2
Anti-c	4	4	-	3
Anti-S	4	3	1	Nil
Anti – Fy^a	1	-	1	Nil
Anti- C^w	1	1	-	1

Table 5: Frequency of alloantibody according to blood group systems.

Antibody type	Subtype	Number	Percentage of total	Total
Rh	Anti-D	45	65.21%	89.85%
	Anti-C	6	08.69%	
	Anti-c	4	05.79%	
	Anti-E	6	08.69%	
	Anti-C^w	1	01.44%	
MNS	Anti-M	2	02.89%	8.69%
	Anti-S	4	05.79%	
Duffy	Anti – Fy^a	1	01.44	01.44%
Total		69 (in 63 patients)		

Table 6: Association of adverse obstetric history with alloimmunisation.

	Antibodies detected	Antibodies not detected
Adverse obstetric history present (n=1091)	48 (4.39%)	1043
Adverse obstetric history absent (n=5009)	15 (0.49 %)	4994

P<0.001, odd's ratio=15.32 (95% confidence interval=5.75–21.64)

Table 7: Antibody formation in relation to gravida status.

Gravida status	II	III	IV	V	Total
Total cases	4084	1740	218	58	6100
Antibody positive	28	19	8	8	63
% of antibody to total cases of respected gravida	0.69	1.09	3.67	13.79	

p<0.001 (by χ^2 test=18.29, degrees of freedom=3)

Figure 1: Frequency of ABO type of Blood group in percentage (n=6100)

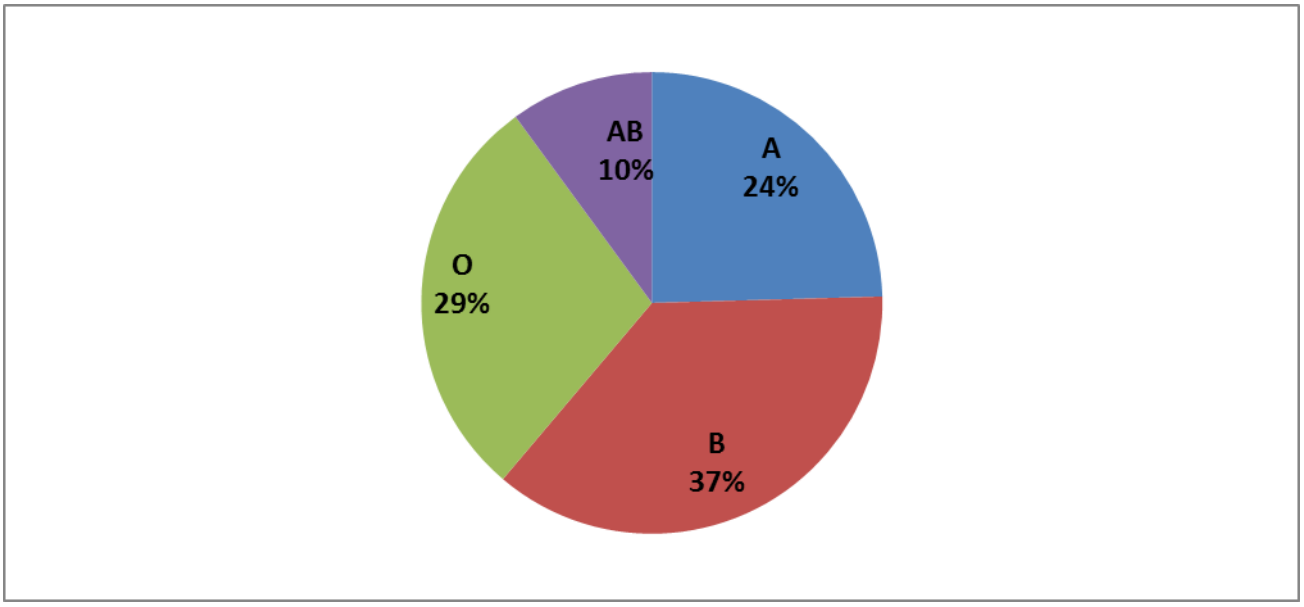

Figure 2: Percentage of antibodies detected

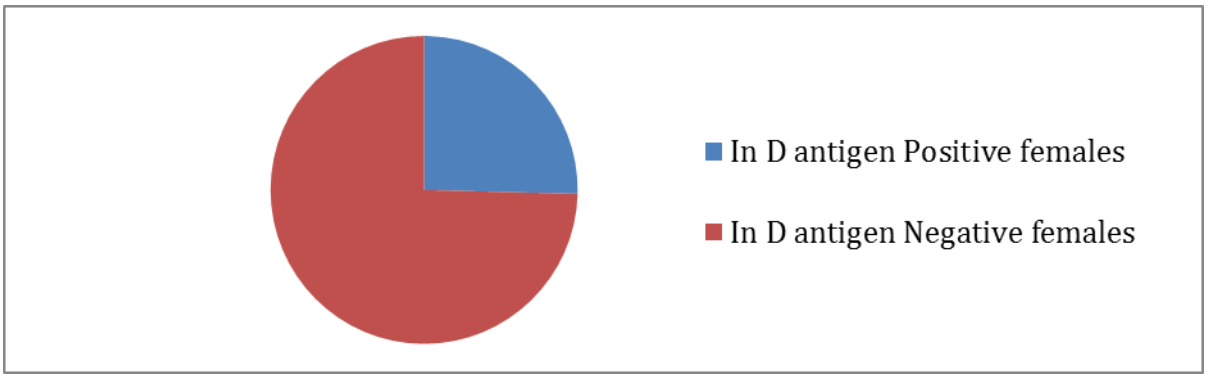

Figure 3: Type of antibodies detected in the study population

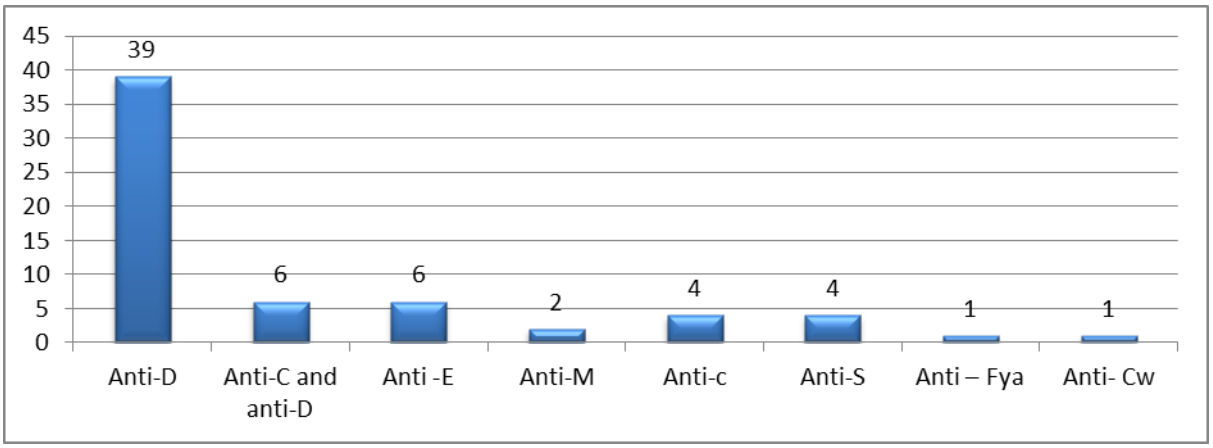

Figure 4: Frequency of alloantibodies according to blood group systems

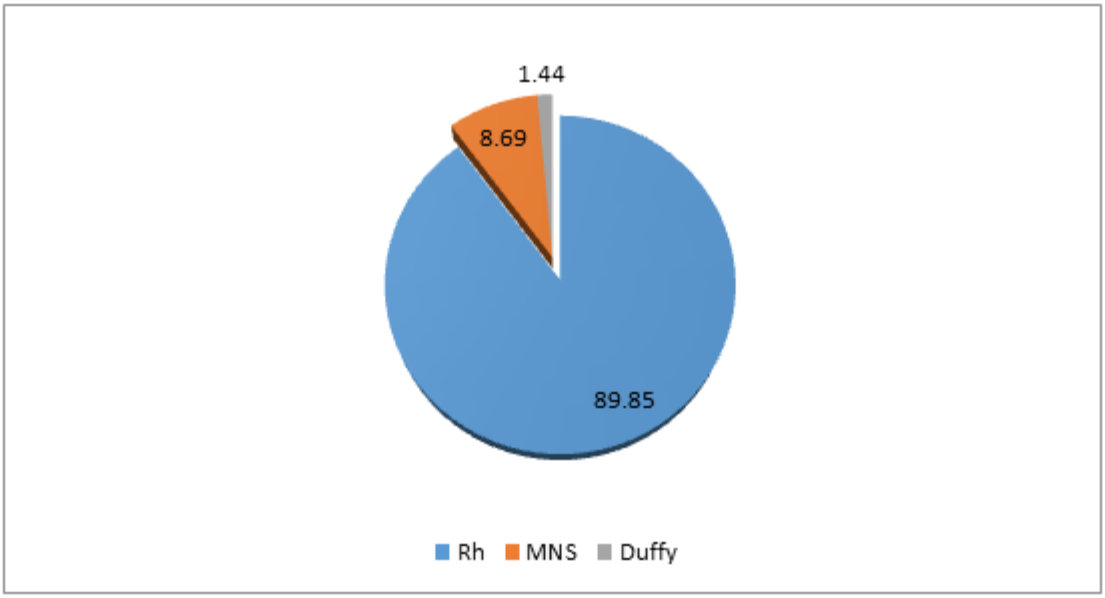

Figure 5: Percentage of antibody to total cases according to gravida