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FREQUENCY OF Rh ANTIGEN C AND c AMONG PREGNANT WOMEN IN SUB-URBAN AREA IN EASTERN NIGERIA

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ABSTRACT

Antigen C and c which are found in approximately 80% of the united states population is considered one of the clinically significant Rh antigen whose antibody has been associated with Haemolytic disease of New born hence the need to carry out this study in my locality. The study was aimed to investigate the frequency of antigens C and c in pregnant women in a hospital in Enugu, Eastern Nigeria. A total of 150 blood samples were collected from pregnant Women and commercially prepared anti- C and anti c were used to determine the presence of antigen C and c using tube agglutination method. The frequency of both antigens was determined using tube method. Eighty-five (85) samples were positive to antigen C, representing 56% while sixty-five (65) were negative representing 43.3%. Also, Ninety-one (91) samples were positive to antigen c representing 60.7% while fifty-nine (59) 0f the samples were not negative. This concludes that both antigens have a high frequency in the subjects tested and for that reason; Alternate hypothesis is accepted which says that antigen C and c are frequent in pregnant women in Enugu Nigeria.

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***Keywords**: frequency, Rh antigen C and c, pregnant women*

INTRODUCTION

The Rh blood group system is a human blood group system. It is the second most important blood group system, after the ABO blood group system (Eze *et al.,* 2021; Okoroiwu *et al.,* 2015). The Rh blood group is one of the most complex blood groups known in humans. From its discovery 60 years ago where it was named (in error) after the Rhesus monkey, it has become second in importance only to the ABO blood group in the field of transfusion medicine. It is highly polymorphic and immunogenic; second to the ABO group, it is the most clinically significant in transfusion medicine (Chávez *et al.,* 1991). It is comprised of at least 45 independent antigens, the most important of which are D, C, c, E, and e. These antigens are encoded by the RHD and RHCE genes, located together on chromosome 1 (Westhoff, 2004, Obeagu *et al.,* 2013, Okorie *et al.,* 2020). The Rh system is one of the most immunogenic blood groups in humans and is well-known for its role in hemolytic disease of the newborn (HDN), in which mothers who are Rh negative are sensitized by the corresponding antigen. The D antigen being the most immunogenic has been associated with severe HDN. Several researches have also associated other Rh antigen with HDN (though usually mild). Sensitization is usually during the first Rh-positive pregnancy or exposure to Rh-positive blood. A severe immune response to the Rh antigen during subsequent Rh-positive pregnancies will ensure leading to a hemolytic reaction that may be life threatening to the fetus. The alloantibodies produced are primarily IgG and react optimally at warm temperatures, with obvious clinical significance. It has remained of

primary importance in obstetrics, being the main cause of hemolytic disease of the newborn (HDN) (Bowma and Chown, 1979)

The c-antigen (little c) which is found in approximately 80% of the United States population, is considered the most clinically significant Rh antigen after D and is associated with severe HDN. Anti-c antibodies arise through previous exposure, such as fetomaternal hemorrhage or transfusion, and can produce acute and delayed hemolytic reactions. As with the D antigen, pregnant women and girls are usually sensitized to the c-antigen during an initial pregnancy, and complications occur with repeat exposure during subsequent pregnancies. Pregnancies complicated by anti-c are not extremely common; however, one may gather an idea of their incidence from the retrospective review by Hackney and colleagues (102 cases over a 34-year period at one United States institution and dozens of other cases at various institutions worldwide) (Avent and Reid, 2000). Similar to the other Rh antibodies, anti-c is also primarily of the IgG type. IgM anti-c, however, has been reported, as well as other Rh IgM antibodies (Janeway *et al.*, 2001). As IgM antibodies are the first immunoglobulins to be produced during any humoral immune response followed by IgG, it is not unexpected that the process of sensitization and sero conversion after c-antigen exposure (and exposure to other Rh antigens) involves the same course of anti-c IgM production before anti-c IgG is formed. It is possible that the IgM component of the antibody does not remain very long in that phase and is difficult to capture, or it is also possible (and more likely) that the existence of the IgM component is known and therefore unlikely to garner much interest in demonstrating it even when observed (Delaflor *et al.*, 2005). The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen

will produce anti-D if they encounter the D antigen on transfused RBCs (causing a hemolytic transfusion reaction, HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in pregnant women (Avent and Reid, 2000).

The study was done to determine frequency of antigen C and c among pregnant women in a Sub -urban Area in Eastern Nigeria.

MATERIALS AND METHODS

STUDYAREA

The study was carried out in a sub-urban community in Eastern Nigeria.

RESEARCH DESIGN

This research is a cross sectional study designed to determine the presence of Antigen C and antigen c in pregnant women attending antenatal in a hospital in south Eastern Nigeria.

SAMPLE SIZE CALCULATION

The incidence rate of Antigen C and c found in pregnant women is 3.4%cases. Making the prevalence rate a total of 0.034%, using the formula below, my sample size was calculated using Leslie kish formular

$N = Z2 \text{ X } p(1 – p)/ d2$

Where

N = Minimum sample size

d = desired level of significance (0.05)

Z = confidence interval (1.96)

P = prevalence rate or proportion of occurrence =0.388 %

Therefore

N = 3.8416 X 0.034 (1 – 0.34)/ 0.0025

N = 150 samples

STUDY POPULATION

The study was carried in 71 adult pregnant females between the age ranges of 25 to 45 years who are attending antenatal clinic in a hospital in eastern Nigeria.

ETHICAL APPROVAL/ CONSIDERATION

Ethical approval was gotten from the hospital management. The study was carried out according to the Good Clinical Practice Guidelines of the modified Helsinki declaration.

INCLUSION AND ECXLUSION CRITERIA

Inclusion: Pregnant women who are within the age range of 25 to 45

Exclusion: Non pregnant women

SAMPLECOLLECTION AND PREPARATION

Blood sample was collected from each patient and dispensed into an EDTA specimen container and taken to the laboratory as soon as possible following collection for analysis of Antigen C and c. If a sample was delayed in testing, it was stored at 2-8 degrees Celsius. Samples displaying

gross haemolysis or microbial contamination should not be used for testing. It is preferable to wash all blood samples with PBS or isotonic before been tested.

METHODOLOGY

Method: Tube method

Procedures

1. 2-3 percent of red cells suspension was prepared in an isotonic solution and placed in a labeled test tube containing 1 volume of Lorne anti- Rh reagent.

2. Then I added 1 volume of the red cell suspension to the Lorne reagent. This was mixed and thoroughly and spun for 20 seconds at 1000 revolution.

3. The red cells were re-suspended and red macroscopically to check for the presence of agglutinations.

4. Tubes which showed negative or questionable results were incubated for 15 minutes at room temperature after which cells were re-suspended and read microscopically.

RESULTS

Prevalence of Antigen C

Table 1: Prevalence of Antigen C

C Antigen	frequency (%)
Negative/Non agglutination	65 (43.3)
Positive/Agglutination	85 (56.7)
Total	150 (100.0)

Most participants were positive for antigen C (n=85, 56.7%).

Table 2: Prevalence of Antigen c

c Antigen	Frequency (%)
Negative/Non-agglutination	59 (39.3)
Positive/Agglutination	91 (60.7)
Total	150 (100.0)

Most of the participants were positive for antigen c (n=91, 60.7%).

Relationship between Antigen C and Antigen c

There was a significant association (X^2=50.126, p <0.0001) and a significant correlation (r=0.578, p < 0.0001) between antigen C and antigen c (Table 3. From Table 3, 26 (17.3%) of the participant were positive for both antigens, while 0 (0.0%) were negative for both antigens. Participants were more positive for antigen c (n=65, 43.3%) than antigen C (n=59, 39.3%). Since none 0 (0%) were not reactive for either antigen C or Antigen c.

Table 3: Relationship between Antigen C and Antigen c

	c Antigen		**Total**	**X^2**	**r**
C Antigen	Negative/ Non-agglutination n (%)	Positive/ agglutination n (%)	**n (%)**	**(p-value)**	**(p-value)**
Negative/Non-agglutination	0 (0)	65 (43.3)	65 (43.3)	50.126 (0.000*)	0.578 (0.000*)
Positive/Agglutination	59 (39.3)	26 (17.3)	85 (56.7)		
Total	59 (39.3)	91 (60.7)	150 (100.0)		

Fisher's Exact Test*

DISCUSSION

Few countries in sub-Saharan Africa have systemic testing for antigen C and c in the donor and recipient, thereby exposing transfused patients to the risk of developing antibodies that can cause HTR and HDN.

From the study carried out, of the 150 samples collected from pregnant women. Eighty-five 85 (56%) samples were Rh C positive while sixty-five 65 (42.6%) samples were negative. The prevalence of antigen c in pregnant women were Fifty-nine 59 (39.3%) negative and Ninety-one 91 (60.7%) positive. This high prevalence in the number of positive participants to anti C and c is in agreement to Mitchel (1999) who reported that antigen C and c are implicated in hemolytic disease of the new born, and that antigen c has higher prevalence than C because c appears in 80% of population while C appears in 70% of population. Prevalence of Rh c antigen among 200 pregnant women attending antenatal clinic in Usmanu Danfodiyo university teaching hospital Sokoto was determined and the frequency of Rh c was 92%. In another study carried out on

pregnant women in Port Hacourt, Rivers State, frequency of Rh c and C was 84.0% and 24.3% respectively. Among 651 blood donors tested in Abidjan for antigen C and c, the antigen frequency was 99.85 and 21.97. Among Saudi voluntary blood donors, prevalence of c was 86%. From a study carried out in north India and south Gujarat, frequency of Rh C and c were 85.1% and 62.3% respectively.

The result of this present study is in line with studies carried out in different region stated above. High frequency of the Rh C and c antigens among the pregnant women means there are few women with antibodies against the antigen thus making HDN due to rhesus C and c rare. In table 3 there was a significant association ($p<0.01$) and a significant correlation ($p<0.01$) between antigen C and c when both were compared. 26 (16.7) of the participants reacted positive to both antigens, but participants in the reacted more to antigen c. This significant difference in relationship could be as a results that both antigen cause hemolytic disease of the new born (Hammerrning, 2005).

CONCLUSION

The result concluded that both Antigen C and c are highly prevalence in pregnant women in sub urban area of Eastern Nigeria. There is a low frequency of anti C and c among the pregnant women in Eastern part of Nigeria.

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