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Occurrence of Rhesus c, D, e Antigens amongst Blood Donors and Recipients in Awka and Asaba, Nigeria.

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Abstract

The Rhesus blood group antigen D, C, c, E and e are highly immunogenic and are of importance in blood transfusion services. The study determined the occurrence of Rhesus blood antigens D, c and e amongst the participants. One hundred and forty-two participants were recruited randomly for the study at the Regina Caeli Specialist Hospital, Awka and Federal medical Centre Asaba. They were aged between 18 years and 34 years. Monoclonal anti-D, Anti-c and Anti-e reagents (50ul) were added differently into corresponding test tubes containing 50ul of 3% washed red blood cells in phosphate buffered saline. The presence of agglutination reaction in any tube indicates the presence of the corresponding Rhesus antigen while negative agglutination reactions is indicative of absence of the particular Rhesus antigen in the blood. The finding showed that 78(55%) of the participants were Rhesus D+/c+/e+ blood group, 53(37%) were Rhesus D+/c-/e- blood group, 5(3.5%) were Rhesus D-/c-/e- blood group, 4(2.8%) were Rhesus D+/e+/c- blood group, 1(0.7%) were D+/c+/e- blood group and 1(0.7%) were D-/c-/e+ Rhesus blood group. The study has shown that some participants had different profiles of the Rhesus antigens D, c and e. Since these three Rhesus antigens are known to cause haemolytic transfusion reactions it is important to screen blood and blood products for Rhesus c and e in addition to screening for Rhesus D.

Keywords: Rhesus antigens, Blood donors, Blood recipients, Rhesus blood group.

Introduction

Rhesus blood cell antigens are considered as important blood group system. About 49 different Rhesus blood cell antigens have been identified (Dean, 2005; Flegel, 2007) amongst which Rhesus D, C, c, E and e have been considered amongst the Rhesus antigens that are highly immunogenic with significant blood transfusion importance (Dean, 2005; Hackney *et al.,* 2004). In Nigeria amongst these Rhesus antigens, blood or blood products are mandatorily screened for Rhesus D antigen but not others. Extensive reports exist in Nigeria about Rhesus D blood group, but scanty reports exist about other Rhesus antigens such as C, c, E and e. Available reports on prevalence of Rhesus antigens such variations from one community to another in Nigeria (Akinnuga *et al.,* 2011, Anifowoshe *et al.,* 2017, Adedoyin et al., 2018; Etura *et al.,* 2020; Serekara *et al.,* 2021) and elsewhere in the world (Garraty *et al.,* 2004; George and Simon, 2014; Jue *et al*., 2017). These Rhesus antigens have been implicated in haemolytic transfusion reactions (Dean, 2005). This study tried to identify the pattern of distribution of Rhesus D, c and e amongst the participants in Awka and Asaba, Nigeria.

Methods

One hundred and forty-two individuals (female= 104; Male= 38) participated in the study. The participants were recruited randomly amongst the blood donors and patients waiting for blood transfusion at Regina Caeli Specialist Hospital Awka and Federal Medical Centre Asaba. The participants were aged 18 to 34 years. Three (3) mls of Blood sample was collected from each of

the participant and dispensed into EDTA anticoagulant tube of which 1ml was washed in 4mls of phosphate buffered saline by centrifugation at 1000g for 20 seconds. This process was repeated thrice and thereafter 3% suspension of red cells in PBS was prepared for each participant and labelled accordingly. The tube technique for blood grouping was used to determine the Rh D, c and e blood group status of the participants. The procedure was as described by the manufacturer of the anti-D, Anti-c and Anti-e reagents (Lorne Laboratories LTD, Great Britain). Fifty microlitres (50ul) of monoclonal Anti-D, Anti-c and Anti-e reagents were added to 50ul of 3% red cell suspension of participants in a properly labelled test tube. The reaction mixture was mixed thoroughly and centrifuged at 1000g for 20 seconds. After which the red cells were gently re-suspended and viewed macroscopically for agglutination. The test tubes with positive agglutination test were recorded while those with negative agglutination test were incubated for 15minutes at room temperature (20^{o}C) and the reaction mixture centrifuged at 1000g for 20second and viewed for agglutination again. No evidence of agglutination hereafter is recorded as negative for the antigen in respect. Agglutination of red cells constitute a positive test result which indicates the presence of Rhesus antigen-D, or Rhesus antigen-c or Rhesus antigen-e on the red cells. While red cells that did not agglutinate with anti-D, Anti-c or Anti-e were regarded as lacking the expression of the antigen(s). Negative control was also used as quality control measure. The frequency of occurrence of agglutination or lack of agglutination was expressed in percentages.

Ethical clearance and informed consent.
The Ethics Committees of both Federal medical Centre Asaba (Ref.FMCASB/A&I VOL.XII/305) and Regina Caeli Specialist Hospital Awka (R/C/S/H/Ethics.Cmte/2022/012) approved the study design. The participants after being dully informed of the purpose of the study gave informed consent to participate in the study. There was no form of inducement for the participants.

Results
The prevalence of Rhesus antigens on the same blood cells of the individuals are shown in Table 1. Rhesus D+/c+/e+ antigens among the participants was 78(55%); Rhesus D+/c-/e- antigens among the participants was 53(37%); Rhesus D+/c+/e- antigens among the participants was 1(0.7%); Rhesus D+/e+/c- antigens among the participants was 4(2.8%); Rhesus D-/c-/e- antigens among the participants was 5(3.5%) while Rhesus D-/c-/e+ antigens among the participants was 1 (0.7%). Table 1 shows the expression of Rhesus D, c and e antigens amongst the participants.

Table 1: Expression of Rhesus D, c and e antigens amongst the participants

Rhesus Status	frequency (%)
D+/c+/e+	78(55%)
D+/c+/e-	1(0.7%)
D+/e+/c-	4(2.8%)
D+/c-/e-	53(37%)
D-/c-/e-	5(3.5%)
D-/c+/e-	0(0.0%)
D-/c-/e+	1(0.7%)

Discussion

Reports have shown variations in prevalence of Rhesus D antigens in Nigeria along ethic faults (Akinnuga *et al.,* 2011; Anifowoshe *et al.,* 2017; Adedoyin *et al.,* 2018; Etura *et al*., 2020; Serekara *et al*., 2021). Such variations have also been reported amongst population in China (Jue *et al*., 2017) and America (Garraty *et al*., 2004). Although these studies cited their reports based on prevalence of Rhesus D antigen alone. However, in this study, the prevalence of Rhesus antigens c/D/e was considered.

Rhesus antigens c/D/e are part of Rhesus blood group system that are expressed on the red cells. The ratio of expression of Rhesus c/D/e among the participants in this study was 2.7:4.6:2.8. The finding of this study revealed that 55% of the population of the participants expressed Rhesus c+/D+/e+ antigens on their red blood cells while 37% of the participants expressed Rhesus c-/D+/e- antigens on their red blood cells. These were the major expression of Rhesus antigens encountered in this study.

Interestingly, 2.8% of the participants expressed Rhesus c-/D+/e+ antigens on their red blood cells while 0.7% of the participants expressed rhesus c+/D+/e- antigens on their red blood cells. Similarly, 3.5% of the participants did not express Rhesus c-/D-/e- antigens on their red blood cells. However, Rhesus c-/D-/e+ antigen was also encountered in about 0.7% of the participants. This tripartite Rhesus antigen screening adopted in this study has shown that these three Rhesus antigens exist among the Nigerian population although we did not determine their genotypes. Therefore, blood/blood product screening should incorporate Rhesus c and e screening alongside Rhesus D screening in Nigeria. Any mix-match of blood or blood product involving Rh c, D, or e antigen can induce haemolytic transfusion reactions and haemolytic disease of the New-born in pregnant mothers (Dean, 2005).

Earlier studies among Nigerians have shown prevalence of cDe as 46.2% in Calabar Cross River State (Etura *et al.,* 2020), 60.8% in Port Harcourt among major ethnic groups (Jeremiah and Buseri, 2003), 73.6% amongst Ibibio, Efik and Ibo ethnic groups in Calabar (Jeremiah and Odumody, 2005) and 53.3% in Benin (Adedoyin *et al*., 2018). In this study the occurrence of c+/D+/e+ was 55% amongst participants drawn from Awka in Anambra State and Asaba in Delta State, both in Nigeria. Similarly, the prevalence of c-/D-/e- was 0.8% in Calabar Cross River State (Etura *et al*., 2020), 3.0% in Port Harcourt among major ethnic groups (Jeremiah and Buseri, 2003), 5.3% amongst Ibibio, Efik and Ibo ethnic groups in Calabar (Jeremiah and Odumody, 2005) but was 3.5% amongst participants in this study. In this study it is also important to note the high percentage occurrence of c-/D-/e- (37%) amongst the participants in this study.

The cause of disparity in findings of the present study and other studies in Nigeria need to be identified. One of such possible ways of identifying the cause is to intensify studies on complete Rhesus phenotype and genotype in Nigeria. Currently the research activities on complete profile of Rhesus red cell antigens is not robust.

Fortunately, the ratio of female to male in this study was 7:3. This showed that more females present with conditions that need blood transfusion. Transfusion reaction in females of childbearing age should be avoided entirely because of the consequences. Apart from the Haemolytic disease of the newborn, death in-utero and haemolytic reaction, the emotional challenges and trauma the woman may experience may not be quantifiable. Such emotional challenge may result in withdrawal syndrome or depression.

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Competing interests

There are no competing interests by any of the authors.

Authors' contribution

Conception and design: This was carried out by Onyenekwe C. Chinedum, Osakue O. Nosakhare, Ehiaghe F. Alfred, and Onyenekwe N. Ogochukwu.

Acquisition of data: This was carried out by Onyenekwe C. Chinedum, Ezugwu U.

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Analysis and Data interpretation: This was carried out by Onyenekwe C. Chinedum, Osakue O. Nosakhare, Ehiaghe F. Alfred, Onyenekwe N. Ogochukwu.

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Final approval of the version to be published: This was carried out by Onyenekwe C. Chinedum, Ezugwu U. Modestus, Osakue O. Nosakhare, Obu S. Ifeoma, Ehiaghe F. Alfred, Osakue N. Eric, Akaahan Achiaku, Edet S. Eno and Ehiaghe I. Joy, Onyenekwe N. Ogochukwu.

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