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The Distribution of ABO and Rhesus Antigens and the Presence of Irregular Antibodies to Rhesus Antigens in Individuals Resident in the Centre Region of Cameroon.

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Research Article

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ABSTRACT

The Rhesus blood group is the second most clinically significant blood group system after the ABO. Like the ABO blood group system, the distribution of the Rhesus antigens varies from one population to another. A cross sectional study was designed to determine the distribution of the ABO and Rhesus antigens, as well as irregular antibodies against Rhesus antigens in the study area. The ABO and Rhesus blood groups were determined by the use of monoclonal antibodies and irregular antibodies were determined by detection of anti-human globulin on washed red blood cells. Of the 126 participants, O (44.44%) was the most prevalent ABO blood group followed by A (31.7%), B (17.46%) and AB (6.34%). 118 (93.65%) of the 126 participants were Rhesus-D positive and only 8 (6.35%) were Rhesus-D negative. The distribution of the other Rhesus antigens in decreasing order of prevalence were c (99.21%), e (95.24%), E (31.75%) and C (14.28%). ccDee (57.15%) was the most prevalent Rhesus phenotype while CCdee (0.79%) and ccD (0.79%) were the least prevalent. Of the 50 cross-match samples that were tested for irregular antibodies, 18 (36%) had irregular antibodies. The prevalence of irregular antibodies was 12 (24%) anti-E, 9 (18%) anti-C and 2 (4%) anti-e.These findings are similar to that of many studies performed elsewhere but yet also differ considerably with many other studies performed in other areas. This emphasizes the need for studies like this to be performed on different populations in different areas.

INTRODUCTION

The Rhesus blood group system is one of the most important blood group systems in blood transfusion, second only to the ABO blood group system [1]. The Rhesus blood group system is also one of the most polymorphic and immunogenic of all the blood group systems [2]. Like the ABO blood group system, it is an inherited trait with different significance and ethnic distribution in all countries. The first discovery that the blood groups differed in one population to another was made in the early 20th century [3]. Subsequent studies in other areas have supported this. In a study in Nigeria, the authors reported a frequency of 96.7% for Rhesus D positive cases, with 67% of cases having DD alleles, 30% with Dd meanwhile only 3.3% of cases were Rhesus D negative (dd) and the frequency of D and d alleles were 0.82% and 0.18% respectively [3]. Similar findings have been observed among Africans, West Indians and Blacks living in Britain [4,6,7].

Apart from the importance of the Rhesus (Rh) antigens in blood transfusion and Haemolytic Disease of the Newborn [8], Rh proteins are involved in transporting ammonium across the red blood cells (RBC) and are thought to play an important role in maintaining the integrity of the RBC membrane [9,10,11]. Clinically, the Rhesus blood group system is considered to be complex [3] because of the large number of antigens that makes up the system including its nomenclature, expression, the antigens immunogenicity [12] and serious complications for the fetus of a pregnant woman sensitized by transfusion and patient to be transfused. In the Rhesus blood group system, naturally occurring Rhesus antibodies are not found in the serum of persons lacking the corresponding rhesus antigens [13]. Therefore red cell allo-antibodies or irregular antibodies are formed by immunization and are

detectable through indirect anti-human globulin test [14]. The irregular IgG and/or IgM antibodies are produced and can lead to difficult matching of blood, haemolytic disease of the newborn, haemolytic transfusion reaction, and so on.

In resource limited settings as seen in most developing countries, blood grouping procedures are usually limited to the ABO and Rhesus blood group. Pre-transfusion screening of the other important Rhesus antigens including C, E, c, and e as well as irregular antibodies are not carried out routinely. A cross sectional study was therefore performed to determine the distribution of the ABO and Rhesus antigens in individuals resident in the Central Region of Cameroon, as well as determine the prevalence ofirregular antibodies to the Rhesus antigens D, C, E, c and e in the study area.

MATERIALS AND METHODS

Study area

This study was performedat the Yaoundé Central Hospital. This hospital is situated at the heart of Yaoundé. Yaoundé (3º52'N 11º31'E) is the capital of Cameroon, and with a population of approximately 2.5million, the second largest city in Cameroon after Douala. Yaoundé is a very diverse city with people from different works of life and is home to most of the administrative structures in the country.

Study population

In a cross sectional study approved by the Faculty of Health sciences Institutional Review Board of the University of Buea, Cameroon, participants were recruited using the Yaoundé Central hospital between 17th April to June 17th 2013. All participants were required to sign and informed consent which was duly explained to them in English or French. For participants who could not read or write or could not sign the informed consent for one reason or the other, their guardians or next of kin did on their behalf. A standard questionnaire was administered to obtain information on the demographic of the study population.

Sample collection and processing

4ml of whole blood was collected into EDTA and dry tubes each from participants by venipuncture of the ante-cubital or brachial vein. The tubes were centrifuged at 2000 rpm for 5mins to obtain serum and plasma from the dry and EDTA test tubes respectively. Using a Pasteur pipette the plasma were discarded to obtain only RBC.

2% red cell suspension from the EDTA tubes was prepared by placing 0.5ml of red cells in dry tubes. The tubes were then filled with 0.9% normal saline and centrifuged at 8000 rpm for 5mins to obtain packed red cells. Using pasteur pipettes, the supernatant were carefully discarded and the sediment mixed by gently tapping the tubes. This procedure was repeated for 2 more times to obtain washed red cells.

Determination of the ABO and Rhesus D blood group

ABO and Rhesus D (RhD) was determined using a commercially available kit for blood grouping (DIAGAST). In determining ABO and Rhesus blood group, 4 spots of 30µl of washed red cells were placed on clean plate and anti-A, anti-B, anti-AB and anti-D grouping sera were added, mixed, and rocked for 2mins on a mixer. Positive results were shown by haemagglutination.

Reverse ABO blood grouping was performed to confirm the blood groups of the participants by determining the reaction of the participants' serum to known ABO washed red cells.

Determination of Rhesus antigens C (Rh C), E (Rh E), c (Rh c), and e (Rh e)

A commercially available kit (DIAGAST) was used for the test. Using droppers, 1 drop of the reagents was deposited on a clean plate and 25ul of unwashed cell clot was deposited adjacent to each drop and mixed with a stirrer such that 2- 3 diameter mixture was created. The mixture was then incubated for 30 seconds at room temperature. The mixtures were observed for agglutination by gentle side-to-side rolling of the plate. A negative result was shown by no agglutination.

Detection and identification of irregular antibodies

The indirect anti-globulin test (IgG) kit (BIOREX) was used to detect anti-human globulin in washed red cells from donors and the serum of the blood recipients in a typical cross-match test. 3 volumes of the test serum were placed in labeled test tubes. 1 volume of 2% suspension of washed red cells was added into the test tubes and thoroughly mixed. The mixture was then incubated at 37ºC in a water bath for 30 – 45minutes. A drop of the

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mixture was then placed on a clean plate and 2 volumes of anti-human globulin added, mixed and checked for agglutination macroscopically. Where agglutination could not be appreciated, a light microscope was employed.

Statistical analysis

Statistical analysis of data was performed using Stata® version 12.1 (StataCorp LP). Chi square test was used. Statistical significance was set at $P < 0.05$.

RESULTS

126 participants were recruited of which 31 (24.6%) were females and 95 (75.4%) were males. The study participants were between 19 and 72years of age.

ABO blood grouping revealed group O (44.44%) as the most common blood group and group AB (6.34%) as the least (Figure 1).

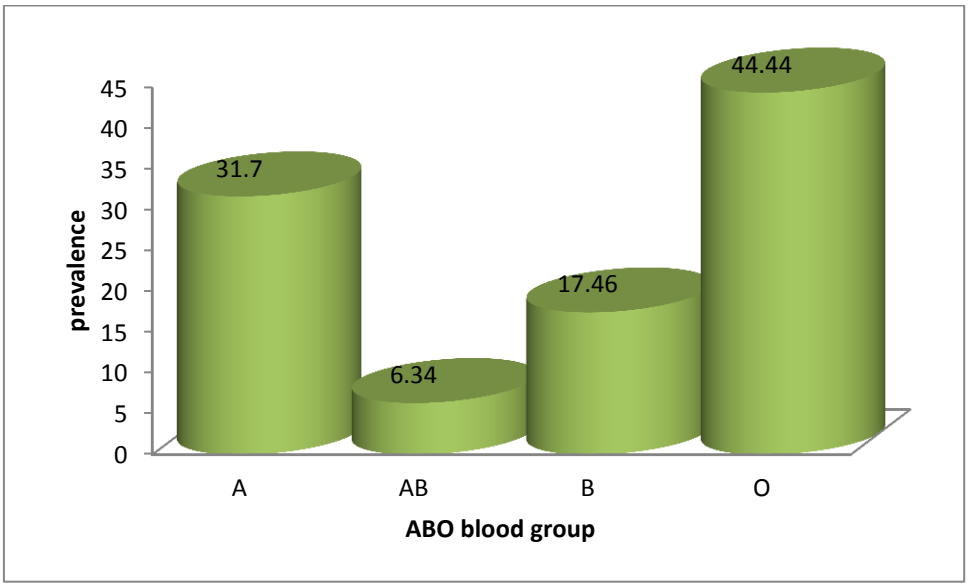

Figure 1: The prevalence of ABO blood groups in study participants

Among the 126 participants, 118 (93.65%) individuals were Rhesus D (Rh D) positive, 8 (6.35%) were Rh D negative. For the other Rhesus antigens, 18 (14.28%) were Rh C positive, 40 (31.75%) were Rh E positive, 125 (99.21%) were Rh c positive and 120 (95.24%) were Rh e positive (Figure 2).

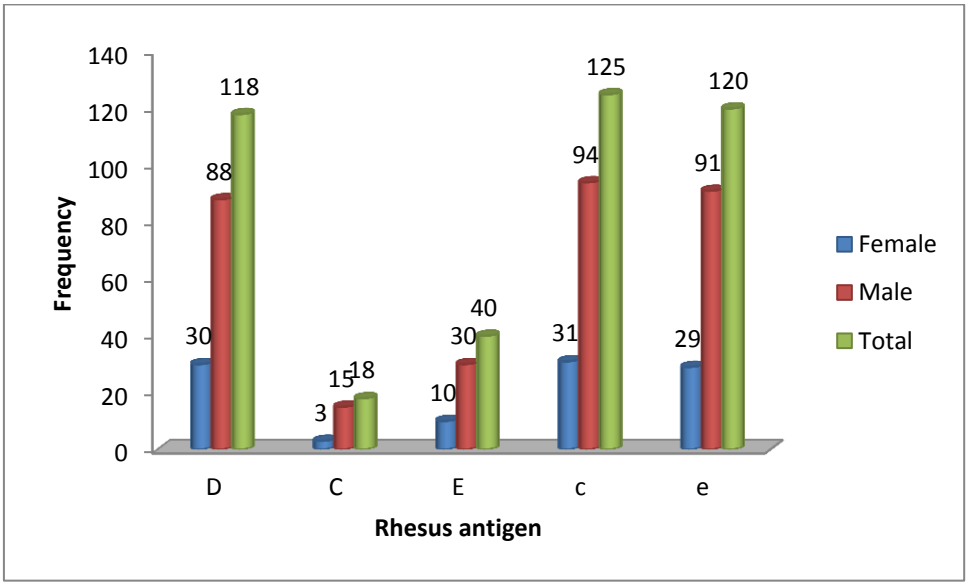

Figure 2: Sex distribution of Rhesus antigens in the study population

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The distribution of the various Rhesus antigens between males and females was not observed to be significant statistically (P=0.955).

The most frequent Rhesus phenotypes among the participants was ccDee (57.15%) and the least frequent phenotypes were CCdee (0.79%) and ccD (0.79%) (Figure 3).

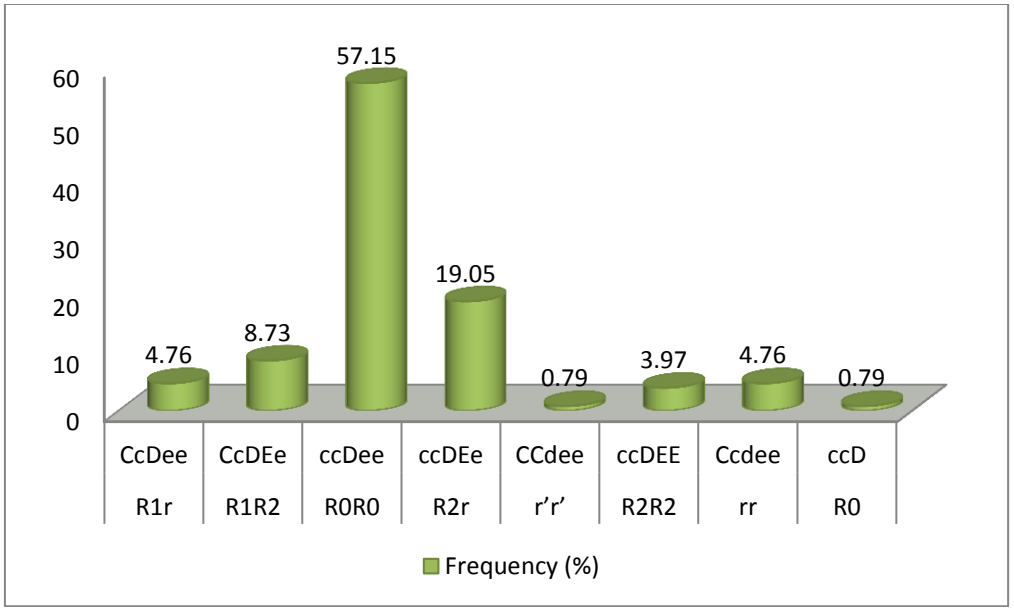

Figure 3: Distribution of Rhesus phenotypes in the study population

Irregular antibodies were determined in cross-match samples between 50 recipients and their donors. 18 of the 50 cross-match samples were incompatible. The prevalence of irregular antibodies in the 50 cross-match samples were 12 (24%) for anti-E, 9 (18%) for anti-C and 2 (4%) for anti-e. None was observed for the other Rhesus antigens.

DISCUSSION

In this study, blood group O (44.44%) was the most prevalent ABO blood group and the least prevalent was blood group AB (6.34%). This observation is very similar to what has been reported in Nigeria[3,15] and in the study among Africans, West Indians and Blacks resident in Britain [4,6,7].

The majority of participants in this study were Rhesus D positive (93.65%) and only a few were Rhesus D negative (6.35%). For the other Rhesus antigens, the most prevalent were Rh c (99.21%), and Rh e (95.24%), meanwhile Rh C (14.28%) was the least prevalent Rhesus antigen. This finding differs from what has been observed among Caucasians and Asians [16], Gaza city population in Palestine[1], and that of the Kalba Region in UAE[17].

In this study, the most frequent Rhesus phenotype was ccDee (57.15%) meanwhile ccD (0.79%) and CCdee (0.79%) were the least prevalent. In a similar study in Port Harcourt in Nigeria, ccDee was also observed to be the most prevalent but contrary to this study, CcDee was observed to be the least prevalent [8].

However, this completely differs from similar studies in Kalba Region in UAE[17] and Turkey [18]. These differences could be due to the fact that populations differ with their genetic makeup – there are 8 different haplotypes of which genetic combination could occur in any order. This further emphasizes the need for studies like this to be performed on different populations in different areas.

In this study, the prevalence of irregular antibodies in blood recipients' serum was 36%. This is very high when compared with values from Nigeria (4.8%) [13], Mexico (10.2%) [19,20,22,23] and even far more higher than values reported in developed countries like Sweden (0.5%), and Netherlands (2.7%) [20,23].

The most frequent and potentially significant non-anti-D antibody in our study was anti-E (24%). Anti-E, which was found to be the most frequent in this study, corroborates with a similar study by Jeremiah and Mordi [13], but is contrary to the study by Bowel et al [21].

Anti-E can be a naturally occurring IgM antibody, however IgG anti-E can be found in the sera of pregnant women with a history of previous transfusions and pregnancies. This immune form of anti-E is able to cause mild to moderate Haemolytic Disease of the Newborn [24]. Haemolytic Disease of the Newborn caused by anti-C is usually mild as the C antigen has weak immunogenicity [21].

CONCLUSION

Group O was the most common ABO blood group in the study population with majority of the participants being Rhesus D positive. Rhesus c and e were the most prevalent Rhesus antigens.

A very high prevalence of irregular antibodies (36%), with anti-E being the most common irregular antibody responsible for the most cases of cross-match failure in blood transfusion in the study area.

These findings are similar to that of many studies performed elsewhere but yet also differ considerably with many other studies performed in other areas. This emphasizes the need for studies like this to be performed on different populations in different areas which could be fundamental in the practice of immunohaematology and blood banking in the area.

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