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Duffy Red Cell Phenotypes among Pregnant Women in Sokoto, North Western Nigeria

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Abstract

Background: Duffy blood group system is one of the clinically significant blood group systems. Duffy antibodies can cause Haemolytic Disease of Foetus and Newborn (HDFN) and Haemolytic Transfusion Reaction (HTR). Knowledge of the distribution of red cell antigens can help to prevent alloimmunisation and haemolytic transfusion reaction among pregnant women as well as facilitate the optimum stocking of blood banks. In this present study, we investigated the prevalence of Duffy antigens among pregnant women in Sokoto, North Western, Nigeria.

Method: One hundred and sixty two (162) pregnant women aged 18-45 years (Mean age 27.19 ± 4.72 years) attending antenatal clinic in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto were screened for the presence of Duffy blood group antigens using the conventional tube method and anti-Fya and Fya reagents (Lorne Laboratories, UK).

Result: Out of the 162 pregnant women tested 82 (50.6%) were Hausa, 26 (16%) were Igbo, 23(14.2%) were Fulani and 20 (12.3%) were Yoruba while the minority ethnic groups were 11(6.8%). The distribution of Duffy antigens was compared based on the ethnic groups of subjects. The prevalence of Fya was highest among the minority ethnic groups (9.09%) and lowest among the Yoruba ethnic group (0%). Similarly the prevalence of Fyb was highest among minority ethnic groups (9.09%) followed by the Fulani ethnic group (8.69% and Ibo (7.69%). Lowest prevalence was observed among the Yoruba ethnic group (0%). Majority of the subjects were multigravidae 122 (75.3%) compared to primigravidae 40 (24.7%). The distribution of Duffy antigens among subjects studied indicated a Fya, Fyb and Fya (a+ b+) prevalence of 7 (4.3%), 9 (5.6%) and 1 (0.61%) respectively. A significant number of subject tested negative for Duffy antigens. Of the 162 pregnant women tested, 155 (95.7%), 153 (94.4%) and 148 (91.37%) tested negative for Fya, Fyb and Fy (a-b-) respectively.

Conclusion: This study indicates that blood group antigens can be distributed differently within different nationalities. Duffy phenotypes observed among pregnant women in this study is similar to previous reports among Blacks but at variance with report among Caucasians and Asians. We recommend that detailed routine phenotyping for all clinically significant red cell antigen including Duffy antigen be carried out routinely among all pregnant women in Nigeria. There is also the need to routinely screen all pregnant women for alloantibodies to facilitate the selection of antigen negative units for those with clinically significant alloantibodies who require a red cell transfusion. This can potentially optimise the obstetric management of HDFN and prevent HTR among pregnant women particularly those who have a clinically significant alloantibody.

Keywords: Duffy; Red cell phenotypes; Pregnant women; Sokoto; North Western Nigeria

Introduction

Approximately 600 blood group antigens have been described so far. These are inherited stable characteristic and therefore are useful in paternity testing, transfusion medicine, prevention and management of HTR and HDFN [1]. The Duffy antigen located on the surface of red blood cells was first discovered in 1950 and named after the multiply transfused haemophiliac in whose serum contained the first example of anti Fy (a) antibody. The protein encoded by this gene is a glycosylated membrane protein and a non-specific receptor for several chemokines. The protein is also the receptor for the human malarial parasites *Plasmodium vivax* and P*lasmodium knowlesi* [2-4].

In areas of West Africa, there is a high frequency of the Fy (a-b-) phenotype. This has prevented *P. vivax* malaria from becoming endemic in West Africa [5]. Antibodies against Duffy antigens have all been implicated as the cause of transfusion reaction [6-8]. Maternal-foetal incompatibilities within the Duffy blood group system is a common cause of HDFN. The Duffy antigens are known to cause maternal immunization and subsequent HDFN [9-11].

There is paucity of data on the prevalence of Duffy blood group antigens among pregnant women in Sokoto, Nigeria. The risk of anti-Duffy- related HDFN in this environment is unknown. Therefore the present study is aimed at determining the prevalence of Duffy these antigens among pregnant women in Sokoto, Nigeria. Data generated will help improve the obstetrics care offered to pregnant women in the area and may help justify the need to routinely screen pregnant women in the area for clinically significant red cell antigens as well as help blood bank in the area to stock optimum levels of Duffy negative units.

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Materials and methods

Study site

The selected area for this study is Usmanu Danfodiyo University Teaching Hospital (UDUTH) which is located in Wamakko Local Government within Sokoto Metropolitan city in Sokoto State. Sokoto State is located in the extreme Northwest of Nigeria, near the confluence of the Sokoto River and Rima River. With an annual average temperature of 28.3^{0}C (82.9^{0}F). Sokoto is, on the whole, a very hot area. However, maximum day time temperatures are for most of the year generally under 40^{0}C (104.0^{0}F). The warmest months are February to April when daytime temperatures can exceed 45^{0}C (113.0^{0}F). The rainy season is from May to October during which showers are a daily occurrence. There are two major seasons, wet and dry which are distinct and are characterized by high and low malarial transmission respectively. Report from the 2007 National Population Commission indicated that the State had a population of 3.6 million [12].

Study population

The study population consists of 162 consecutively-recruited pregnant women aged 18-45 years and mean age 27.19 ± 4.72 years attending antenatal clinic (ANC) at Usmanu Danfodiyo University Teaching Hospital Sokoto, Sokoto State. Verbal informed count was obtained from each subject after counselling. Ethical approval was obtained from the ethical committee in UDUTH (UDUTH/HERC/2013/NO.145).

Study design

This present study is a case study and included 162 consecutively-recruited pregnant women aged 18-45 years who were investigated for their Duffy blood group antigen status in the blood transfusion laboratory of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, North Western, Nigeria.

Sampling method and testing procedure

Three millilitres of EDTA anticoagulated blood was collected aseptically from each subjects and used for the determination of Duffy antigen status. When testing was delayed, the specimen was stored at 2-80′C. All blood samples were washed at least 3 times with Phosphate Buffered Saline (PBS) before being tested. Standard conventional tube method was used for all testing. The manufacturer's standard operating procedure (SOP) was followed strictly. Duffy phenotype was determined using Lorne Laboratories (UK) anti-Fya and anti-Fyb reagents. Indirect Antiglobulin (IAT) method was used for Fya and Fyb phenotype determination. The principle is based on the ability of anti-Fya and Fyb antibody reagents to cause agglutination (clumping) of test red cells, which carry the Fya and Fyb antigen, in the antiglobulin phase of testing. No agglutination generally indicates the absence of the Fya and Fyb antigens. In summary, the test red cell was washed three times in phosphate buffer saline solution, a 3% suspension of washed red cell was made, equal volume (1 volume) of 3% washed red cell and anti-Fya or Fyb (Lorne Laboratories (UK) was placed in a labelled test tube, the mixture was mixed thoroughly and incubated at 370′c for 15 minutes, it was then washed one more time with PBS and supernatant was decanted, two volumes of AHG was added then mixed and centrifuged for 20 seconds at 1000 rcf and the sediment was re-suspended and was observed macroscopically and microscopically for agglutination.

Inclusion criteria

All pregnant women who meet the following eligibility criteria; age (18-45 years), confirmed pregnant by a qualified gynaecologist, attending ANC in UDUTH, resident in Sokoto metropolis and willingness to offer informed consenting after counselling were recruited into the study.

Exclusion criteria

The following women who do not meet the eligibility criteria; non-pregnant women, non-consenting pregnant women, pregnant women who have had a recent red cell transfusion in the last 3 months.

Statistical analysis

The data collected was recorded on an Excel spread sheet and later subjected to statistical analysis using statistical software SPSS Version 18.0 (Chicago Illinois). Statistical analysis included descriptive statistics of mean and bivariate analysis of t-test and chi-square. Correlation was compared using linear regression analysis. Differences were considered significant when $p \leq 0.05$.

Result

One hundred and sixty two (162) pregnant women aged 18-45 years (Mean age 27.19 ± 4.72 years) attending antenatal clinic in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto were screened for the presence of Duffy blood group antigens. Out of the 162 pregnant women tested, 82 (50.6%) were Hausa, 26 (16%) were Igbo, 23 (14.2%) were Fulani and 20 (12.3%) were Yoruba while the minority ethnic groups were 11 (6.8%).The distribution of Duffy blood antigen among subjects studied indicated a Fya, Fyb, Fya (a+ b+) prevalence of 7 (4.3%), 9 (5.6%) and 1 (0.61%) respectively. A significant number of subject tested negative for Duffy antigens. Of the 162 pregnant women tested, 155 (95.7%), 153 (94.4%) and 148 (91.37%) tested negative for Fya, Fyb and Fy (a-b-) respectively. Table 1 show the distribution of Duffy antigens among the subjects.

The distribution of Duffy blood group antigen was compared based on the ethnic groups of subjects. The prevalence of Fya was highest among the minor ethnic groups (9.09%) and lowest among the Yoruba ethnic group (0%). Similarly, the prevalence of Fyb was highest among the minor ethnic groups (9.09%) followed by the Fulani ethnic group (8.69%) and Ibo (7.69%). The lowest prevalence was among the Yoruba ethnic group (0%). The distribution of Duffy blood group antigens based on ethnicity is shown in table 2.

Subjects were categorized based on parity. Majority of the subjects were multigravidae 122 (75.3%) compared to primigravidae 40 (24.7%). Subjects were stratified based on trimester. A significant number of women were in the 2nd trimester 11 (68.5%) compared to third trimester 38 (23.5%) and first 13 (8.0%). Table 3 show the distribution of subjects based on parity and trimester of pregnancy.

Antigens	Frequency	Percentage (%)
Fya+	7	4.3
Fyb+	9	5.6
Fy (a+b+)	1	0.61
Fya-	155	95.7
Fyb-	153	94.4
Fy (a-b-)	148	91.4

Table 1: The distribution of Duffy antigens among subjects.

Ethnic Group	Frequency (%)	Fya positive	% Fya positive	Fyb Positive	% Fyb positive
Hausa	82 (50.6)	4	4.87	4	4.87
Fulani	23 (14.2)	1	4.34	2	8.69
Yoruba	20 (12.3)	0	0	0	0
Igbo	26 (16.0)	1	3.85	2	7.69
Others	11 (6.8)	1	9.09	1	9.09

Table 2: Distribution of Duffy antigen among subjects based on ethnicity.

Variable	Frequency	Percentage (%)
Parity		
Primigrvidae	40	24.7
Multigravidae	122	75.3
Trimester		
First	13	8.0
Second	111	68.5
Third	38	23.5

Table 3: The distribution of subjects based on parity and trimester.

Discussion

The Duffy blood group system is one of the clinically significant blood group systems. Antibodies of this blood group system have the ability to cause HTR as well as cross the placenta barrier into the foetal circulation and cause HDFN. In this present study, we investigated the prevalence of Duffy blood group antigens among pregnant women in Sokoto, North Western, Nigeria.

In this study, we observed Fya and Fyb prevalence of 4.3% and 5.6% respectively among our cohort of 162 pregnant women in Sokoto, North Western Nigeria. Our finding is consistent with a previous report which observed Duffy antigens among 2.38% of Sudanese subjects [13]. Our finding is also in agreement with previous report which indicated that Fya and Fyb antigens are found relatively frequently in Caucasians (66% and 83%) respectively and Asians (99% and 18.5%) but far less commonly in Blacks (10% and 23%) [11]. Our observed prevalence is significantly lower than a prevalence reported in a previous study to determine the presence of clinically significant blood group antigens in the Lao population which indicated an Fy(a+b-) prevalence of 80.82% [14]. Similarly, the prevalence of Fya and Fyb antigen observed among Caucasians, Chinese and Blacks was (66.0%, 99.0% and 10.0%) and (83.0%, 9.2%, 23.0%) respectively [11]. Previous study on the distribution of blood group systems in Thai blood donors indicated that Fya is very common among Asian populations with occurrences of about 90.8%, 81.5%, and 69% in Chinese, Japanese and Thai subjects respectively [15]. Blood group antigens can be distributed differently within different nationalities. In the Yemenite Jews, the frequency of the Fy allele is 0.5879 [16]. The incidence of Fy (a+b-) in northern India among blood donors is 43.85% [17]. The frequency of this allele varies from 0.1083 to 0.2191 among Jews from the Middle East, North Africa and South Europe. The incidence of Fya among Ashkenazi Jews is 0.44 and among the non-Ashkenazi Jews it is 0.33. The incidence of Fyb is higher in both groups with frequencies of 0.53 and 0.64 respectively [18]. In the Chinese ethnic populations-the Han and the She people, the frequencies of Fya and Fyb alleles were 0.94 and 0.06 and 0.98 and 0.02 respectively [19]. In Nouakchott, Mauritania, an overall 27% of the populations are Duffy positive. 54% of Moors are Duffy antigen positive (Poular, Sonike and Wood) while only 2% of Black ethnic groups are Duffy positive [20]. A study in an urban Tunisian population indicates that Fyb is the most common Duffy allele in West African Blacks [21].

The prevalence of Duffy phenotype obtained in this study is lower compared to that observed among Caucasians and Asians. The reason for the low prevalence of Duffy phenotype among pregnant women in Sokoto North Western Nigeria may be as a result of high malaria endemicity in this area. Duffy negative status is suggested to play a role in resistance to malaria infection [22]. Fy (a-b-) has been reported with higher frequencies in countries where there is a high incidence of *Plasmodium vivax* malaria [23]. On erythrocytes the Duffy antigen acts as a receptor for invasion by the human malaria parasites *P. vivax* and *P. knowlesi*. Duffy negative individuals whose erythrocytes do not express the receptor are believed to be resistant to merozoite invasion although *P. vivax* infection has been reported in Duffy negative children in Kenya, suggesting a role in resistance to disease, not infection [23]. The protection to *P. vivax* malaria conferred by the absence of the Duffy antigen appears to be very limited at best in Madagasca. About 72% of Madagascan populations are Duffy antigen negative while 8.8% of the Duffy antigen negative individuals were asymptomatic carriers of *P. vivax* [22]. Malaria has also been found in Angola and Equatorial Guinea in Duffy negative individuals [24]. *P. vivax* malaria has also been detected in Duffy antigen negative individuals in Mauritania [25]. Similar infections have been reported in Brazil [26] and Kenya [23]. Additional cases of infection in Duffy antigen negative individuals have been reported from the Congo [27] and Uganda [28]. A study in Brazil of the protection against *P. vivax* offered by the lack of the Duffy antigen found no differential resistance to *P. vivax* between Duffy antigen positive and negative individuals [2].

Duffy blood group system is a clinically significant system because of its role in HTR and HDFN. Anti-Fya has been incriminated in HDFN in a previous report [9]. There is increasing advocacy that pregnancy in which anti-Fya is detected as significant titres (>64) should be closely monitored in a similar way as pregnancy where other clinically significant antibodies are present. Moreover, in the presence of high or rising antibody titres, if the father is homozygous and functional assay suggest the antibody is active, then foetal genotyping should be offered to help plan the future management of that pregnancy [9].

The Duffy blood group system holds fourth rank, after the ABO, Rhesus and Kell systems, on the clinical importance scale of group systems with regards to HTR and HDFN. Plasmapheresis and intrauterine exchange perfusion have dramatically improved the prognosis of this disease. A previous study, Dufour et al. [29] reported a case of severe post-transfusion anti-Duffy (Fya) allo-immunization which required a treatment of four intrauterine exchange transfusions. The child was born at 32 weeks of amenorrhoea and he benefited from an exchange perfusion at birth. The outcome was fully satisfactory. The first case of haemolytic disease of the newborn due to anti-Fya antibodies in Italy was described by Agosti and Moroni [30]. The maternal alloimmunization was related to a previous blood transfusion. Foetal condition was monitored by measuring bilirubin in amniotic fluid on three different occasions during pregnancy. The infant developed a mild haemolytic disease that required treatment only with phototherapy. Similarly, the first case of intrauterine transfusion due to anti-Duffy antibody was reported in 1989 [31]. Anti-Fy (a) has the potential to lead to significant foetal haemolysis resulting in HDFN. Management guidelines developed for D sensitization are appropriate for pregnancies complicated by anti-Fy (a) alloimmunization [32,33]. The alloantibodies, which frequently develop and are encountered during compatibility testing, are primarily against antigens related to the Duffy blood group system [34].

In this study, we observed Fy (a-b-) phenotype among 91.35% of our cohort of pregnant African women in Sokoto, Nigeria. Our finding

is in agreement with previous report which indicated that the Fy (a-b-) phenotype is present in two-third of African-American Blacks but is very rare in Caucasians [11]. Our finding is however at variance with a previous report [35] which obtained a 46.69% Fy (a-b-) prevalence among a total of 115 blood group O donors from three different blood banks of South Gujarat who were typed for Duffy blood group system. Also, blood group antigens were characterized in 522 blood donors in Mashhad, Iran and reported Fy (a-b-) phenotype among 3.4% of subjects [36]. Differences in racial distribution of the Duffy antigens was first discovered in 1954 when it was found that the majority of Blacks (68% in African Americans and 88-100% in African Blacks) had the erythrocyte phenotype Fy (a-b-) [37]. Our finding is also consistent with a previous report [38] which observed 98.2% prevalence of Fy (a-b-) phenotype among their cohort of donors in Malawi. In Grande Comore (also known as Ngazidja) the frequency of the Fy (a-b-) phenotype is 0.86 [39].

In our study, we observed the Duffy phenotype Fy (a+b+) among 0.61% of our cohort of 162 pregnant women in Sokoto, Nigeria. Our finding is at variance with a previous report which observed Fy (a+b+) phenotype among 50.4% of their cohort of 123 randomly selected regular Maldivian blood group O donors who were phenotyped for Duffy antigens in Malaysia [40]. Previous report indicates that the Fy (a+b-) was common among Malays and Chinese, whereas among Indians, the Fy (a+b+) was more common. Indians showed a higher Fy (a-b+) expression than Malays and Chinese. Fy (a-b-) is by all means considered to be a rare phenotype in Chinese, Japanese, and Thai subjects [11]. In previous report among Thai donors, no Fy (a-b-) was found (only two donors, one Malay and one Chinese) had this phenotype.

Conclusion

This study indicates that blood group antigens can be distributed differently within different nationalities. Duffy phenotypes observed among pregnant women in this study is similar to those previously report among Blacks but at variance with reports among Caucasians and Asians.

Limitations

The sample size for this study was relatively small compared to the huge population of women in Nigeria. It however gives a picture of the prevalence of Duffy antigen among pregnant women in Sokoto, North Western Nigeria. There is however the need to carry out a large randomized countrywide study to determine the prevalence of these antigens among Nigerians. Cost implications as well as access to reagents was a limiting factor that affected the number of subjects included in this study. Secondly, we used the conventional manual tube method for the determination of the Duffy antigen status of our subjects. Other previous reports used the more sensitive method using commercial antisera and gel card. The Gel and glass beads-based card method has several advantages over the conventional tube method. The advantages include; paediatric friendly because they require small volume of patient specimen, unlike the tube methods, no washing of the antiglobulin is required, it is more standardized and does not require the subjective red cell suspension preparation required with the conventional tube method, results are more objective (clear, eye and analyser readable) and does not require use of microscopes as required in tube testing, it is easy to automate unlike conventional tube method and thus very useful for high throughput laboratories. It also facilitates large batch testing with no risk of possible mix-up of patient sample common with tube testing and it is more sensitive and specific and has the capacity to detect individuals with very weak reactions unlike conventional tube method.

Also the use of manual tube method and observation of agglutination to indicate the presence of blood group antigens after the addition of the antisera can possible underestimates the prevalence of the antigen because of its low sensitivity compared to the gold standard (genotyping). However this technology is not present in most settings in developing countries because of the huge cost implication and trained manpower-related challenges.

Recommendation

Knowledge of the distribution of red cell antigens can help to prevent alloimmunisation and haemolytic transfusion reaction among pregnant women and multi-transfused patients by facilitating the provision of antigen negative blood for pregnant women and transfusion- dependent patients with alloantibodies. It can also facilitate the optimum stocking of blood banks in the area based of the relative prevalence of the Duffy and the various clinically significant red cell antigens in the population. We recommend that detailed phenotyping for all clinically significant red cell antigen including Duffy antigen be carried out routinely among all pregnant women in Nigeria. There is also the need to routinely screen all pregnant women for alloantibodies at antenatal booking to identify women at risk for HDFN as well as facilitate the selection of antigen negative units for those with clinically significant alloantibodies who may require a red cell transfusion during pregnancy or delivery. This will facilitate the optimum obstetric management of HDFN in pregnant women who have a clinically significant alloantibody.

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