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Positive Cord Blood Direct Anti Globulin Test (DAT) is Strongly Associated with Parity and Maternal Age among Rh Negative Mothers in Maiduguri, Nigeria

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Abstract

Background: Rh D negative blood in mothers carrying Rh D positive fetus is usually associated with the hemolytic disease of the new born. The rate of alloimmunization *in vivo* among Rh D negative mothers in Maiduguri, Nigeria has not been determined.

Materials and methods: We determined the ABO blood group of the Rh D negative mothers by hemagglutination method. Structured questionnaires were administered to Rh negative mothers for demographics, parity and transfusion status. Direct Anti Globulin (DAT) test was performed on 50 cord blood samples using standard procedures.

Results: Twelve (24.0%) of the cord blood samples were DAT positive (χ^2=13.52; p<0.001). The positive DAT was found to be significantly associated with maternal age (χ^2=7.58; p<0.02) and parity (χ^2=10.16; p<0.01). ABO blood group was not found to be significantly associated with positive DAT (χ^2=1.046; p>0.05). Women who were 31 years and above had a 50% positive DAT while grandmultigravida (4 children and above) were more sensitized than the others. A significant proportion of the mothers (24.0%) had previous abortion while 26.0% of the women had previously received blood transfusion.

Conclusion: There is a high prevalence of positive cord blood DAT in this part of the world. There is need to establish intervention programmes in terms of neonatal screening and immunoprophylaxis for the benefit and protection of the neonates, the family and the health care system in Nigeria.

Keywords: Direct antiglobulin test; Cord blood; Rh negative mothers Maiduguri; Nigeria

Introduction

The Rhesus negative group refers to the genotype in which the three major genes (CDE) are absent. Thus, the genotype of the Rhesus negative individual is 'CDE' [1]. In our routine blood transfusion and antenatal services, only the D antigen is tested due to its ability to cause Rhesus incompatibility more than the other Rhesus antigens [2]. The Rh D negative is found in approximately 15% of the Caucasians and much earlier studies done in Africa suggested that frequencies of 20 to 30% existed in Middle and West African countries [3]. In separate studies conducted in Nigeria, a percentage prevalence of Rh D negative was found to be 5% in Port Harcourt and 5.56% in Calabar respectively [4,5].

A sensitized Rh negative mother produces anti-Rh IgG antibodies that cross the placenta and the risk factors for antibody production has been reported to include second and later pregnancies, feto-maternal incompatibility in ABO system, paternal zygosity, maternal toxaemia and antigen load. Rh D antigen associated hemolytic disease occurs when the maternal antibodies to the Rh D on the fetal/infant red cells crosses the placental barrier to cause acquired immune-mediated hemolysis of the new born (i.e. red cell destruction) [6]. The overall strategy of the study of hemolytic disease of the newborn (HDN) include the determination of the direct test of human antiglobulin or direct Coomb's test (DAT) [7]. This procedure allows us to identify the presence of anti erythrocyte antibodies of IgG isotope, originating in maternal serum on the surface of the erythrocytes of the fetus or newborn [8,9].

The British Committee for Standardization in Haematology (BCSH) recommends that infant born to Rh D negative mothers should have umbilical cord blood ABO and Rh tested [10]. If the baby is Rh D positive, maternal samples should then be taken for assessment of feto-maternal hemorrhage so that adequate anti RhD can be given to the mother.

In Nigeria, there is no established guideline for management of Rh D negative mothers and we did not encounter any report that highlights the rate of isoimmunization in our Rh D negative population. It therefore becomes necessary for a study such as this to be conducted in order to ascertain and identify the percentage of babies who are at risk of hemolytic disease of the new born. The objective of the study is to report the occurrence of the reactivity of DAT on cord blood of children born to Rh negative mothers.

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Patients and Method

Subjects

We included 50 apparently healthy Rhesus negative pregnant mothers who attended the antenatal clinic of the University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. The pregnant women were all Rh D negative and in their third trimester. They were all followed up till delivery time. Pregnant mothers who were Rh D negative but undergoing drug therapy with penicillin, cephalosporin or other broad antibiotics were excluded. Anyone with the history of having taken anti-Rh D prophylaxis was excluded. The third group of those excluded from the study was HIV positive subjects on anti-retroviral therapy. The study received ethical approval from the University of Maiduguri Teaching Hospital ethical committee in accordance with the Helsinki declaration. Thereafter, informed consent was received from each participant before samples were collected from them.

Sample collection

Performance of the DAT was achieved using whole blood samples obtained by a puncture of the umbilical vessel at the end of the placenta immediately after delivery. The umbilical cord was clamped at the end attached to the neonate. Another cord clamp was placed 8 to 10 inches away from the first. Isolated section was cut and the fetal blood sample was collected into ethylene diamine tetra acetic acid (EDTA) container. The bottle was labeled with patient identity including name and hospital number. The umbilical cord blood was centrifuged immediately at 4000 rpm for 2-3 minutes to enable the red cells to sediment. Supernatant plasma was removed leaving the fetal red cells in the tube. The fetal red cells were washed in physiological saline to remove Wharton Jelly after which the cells were prepared for further testing. Blood samples from Rh negative mothers were collected by venipuncture into EDTA container and used for ABO and Rhesus grouping.

Methods

ABO blood group of mothers

We determined the ABO blood group by hemagglutination with test tube technique. For the direct phase, commercial reagents were used that contained monoclonal antibodies with anti-A, anti-B and anti-AB specificity (Gamma Biologicals, Licon).

Direct Antiglobulin Test (DAT) on cord blood

Two tubes (12×75 mm) were labeled 'test' and 'control' and a drop of a 3% suspension of cord blood added to them. The cells in the tube were washed three times with saline and the saline was completely decanted after the last wash. To the tube labeled test was added 2 drops of AHG reagent (Monoclonal anti-IgG, Gamma Biologicals, Licon) while 2 drops of 3% of bovine albumin was added to the tube labeled 'control'. Both tubes were centrifuged at 3000 rpm for15 to 20 seconds. Following centrifugation, the cell pellet was completely re-suspended by gentle tipping and rolling of the tube. Agglutination was read macroscopically and scored. In doubtful cases, the presence or absence of agglutination was confirmed with low power magnification. For control, to all negative tubes was added 1 drop of control cells weakly sensitized with IgG, it was mixed, centrifuged and observed for agglutination. A mixed field weakly positive reaction at this stage indicates that the AHG had been added to the tube and it was still reactive. All reactive results at this stage were considered valid.

Statistical analysis

The results of each blood group and the DAT reaction are presented as frequencies and proportions using Statistical Package for Social Sciences (SPSS) (version 18, Chicago, Ilinois, USA). The intensity of the association between positive DAT age, parity, and blood groups of mothers was established by chi-square analysis. A p value less than or equal to 0.05 was considered significant.

Results

We evaluated the ABO blood groups of 50 Rhesus D negative mothers and DAT in 50 cord blood samples in Maiduguri, Nigeria. Table 1 shows the characteristics of the 50 Rhesus D negative mothers. Majority of the women, 20 (40.0%) were under 25 years of age. 21 (42.0%) of them were primigravidae while the rest 29 (58.0%) were multigravidae. 19 (38.0%) were of blood group O, 16 (32.0%) group A, 11 (22.0%) group B and 4 (8.0%) group AB. The prevalence of positive cord blood DAT and association with demographic characteristics of the study population is as shown in table 2. 12 (24.0%) of the cord blood was found to be positive (χ^2=13.53; p<0.001). This was found to be significantly associated with maternal age (χ^2=7.58; p<0.02) and parity (χ^2=10.16; p<0.01). Blood group was not found to be associated with positive DAT test (χ^2=1.046; p=0.790). Women who were 31 years and above had a 50% positive DAT while those who had 4 children and above were more sensitized than the others.

Discussion

The hemolytic disease of the new born (HDN) is a disorder which

Characteristics	N	%
Age Group (years)		
< 25	20	40.0
25 - 30	16	32.0
31+	14	28.0
Average Age (SD) =27.46 (0.91)		
No of Children		
1	21	42.0
2	10	20.0
3	12	24.0
4+	7	14.0
Blood Group		
A	16	32.0
AB	4	8.0
B	11	22.0
O	19	38.0

Table 1: Characteristics of Study Participants.

Characteristic	N	DAT of Cord blood		χ^2 (df)	P-value
		Positive	Negative		
Overall	50	12 (24.0%)	38 (76.0%)	13.52 (1)	<0.001****
Age Group (yrs)					
< 25	20	2 (10.0%)	18 (90.0%)		
25 - 30	16	3 (18.8%)	13 (81.3%)		
31+	14	7 (50.0%)	7 (50.0%)	7.58 (2)	0.023*
Parity					
1	21	3 (14.3%)	18 (85.7%)		
2	10	2 (20.0%)	8 (80.0%)		
3	12	2 (16.7%)	10 (83.3%)		
4+	7	5 (71.4%)	2 (28.6%)	10.16 (3)	0.017*
Blood group					
A	16	3 (18.8%)	13 (81.3%)		
AB	4	1 (25.0%)	3 (75.0%)		
B	11	2 (18.2%)	9 (81.8%)		
O	19	6 (31.6%)	13 (68.4%)	1.046 (3)	0.790 ns

Significant level: * = *p*<0.05, ***** = *p*<0.0001; ns= not significant (p>0.05).

Table 2: Cord blood DAT and association with the demographic characteristics of the study participants.

is principally associated with the antigen D of the Rhesus system and the ABO system incompatibility. In the typical Nigerian population, the prevalence of Rh antigens have been reported [4,5] but so far no report has been encountered that highlighted the incidences of DAT in Rhesus D negative mothers except a study by Worlledge et al. as far back as 1968 [11].

In this study, it is shown that a significant proportion of the Rhesus D negative mothers were found to be sensitized with 24% of the cord blood being DAT positive. The 24% of prevalence rate of positive DAT is so high and significant when compared with what is reported elsewhere in the literatures. A prevalence rate of 1-9% had earlier been reported by Dinesh [2] and Cianciarullo [12] in White populations. This high prevalence rate of positive cord blood DAT places women at high risk of developing the hemolytic disease of the new born.

DAT is a useful tool in early prediction of jaundice or hyperbilirubinaemia. Studies have shown that up to 23% of infants with positive DAT require phototherapy [13]. In another study of the infants receiving phototherapy, 15.1% show a DAT positive [14]. Detection of positive DAT on cord blood enables the newborn at risk of clinically relevant jaundice to benefit from programs aimed at identifying risk or early detection of various adverse clinical conditions such as hyperbilirubinemia, encephalopathy, prolonged hospitalization, intense hemolytic anemia or others [15]. Inclusion of DAT in the neonatal screening allows the early intervention with prophylactic phototherapy [15].

Apart from HDN, *in vivo* phenomenon associated with a positive DAT could be due to a number of factors such as transfusion, drug induced and autoimmune hemolytic anaemia [16]. With a high percentage of women having received previous blood transfusion and with the multiple pregnancies, it is possible that alloantibodies in the recipient Rh D negative mothers must have sensitized their red cells to cause *in vivo* sensitization. The limitation of this study is that antibody detection and identification was not done to identify the antibodies that coated the cord blood cells. The neonates were not also followed up to ascertain whether they developed hyperbilirubinaemia. The rationale of this study was just to determine the cord blood DAT for early detection of alloimmunization [11]. This study will also form a pivot upon which further studies will be based.

Conclusion

It has been established in this study that there is a very high rate of alloimmunization among the Rh D negative mothers in this part of the world. The prevalence of cord blood DAT is very high and this calls for the establishment of intervention programmes for the benefit and protection of the neonates, the family and the healthcare system in Nigeria.

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