SAR Journal of Medicine

Abbreviated Key Title: *SAR J Med*
Home page: https://sarpublication.com/journal/sarjm/home
DOI: 10.36346/sarjm.2022.v03i03.004

ISSN 2707-773X (P)
ISSN 2709-6920 (O)

Original Research Article

Frequency of Red Blood Cells Sensitized with Allo-antibodies in cord blood in Atbara and Eldamer localities, Sudan

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Article History: | Received: 27.05.2022 | Accepted: 18.06.2022 | Published: 22.06.2022 |

Abstract: A direct antiglobulin test (DAT) is not routinely used in developing countries including Sudan. The direct antiglobulin test (DAT) is a screening test for antibodies present in an individual's red cells and is used to diagnose hemolytic disease of the newborn (HDN). The study was to determine the prevalence of DAT among infants at Atbara and Eldamer. The study was designed as a descriptive, cross-sectional study carried out among neonates during January and February 2019 Atbara and Eldamer. ABO group and DAT were carried out among 100 samples of neonatal cord blood. Data were analyzed using SPSS version 22. A total of 100 neonates were recruited for the study. Among 100caces ABO incompatibility status was determined in 74 cases. The higher frequency was (O+, O+)(40%), the lower frequency was (AB+/AB+)(2%) while ABO incompatibility between neonates and mothers was found in 26 (26.0%) cases, The higher frequency of (A/O) incompatibility (15%), the lower frequency was (B+/A+),(AB+/A+)(1%) by the ABO group distribution at the Atbara & Eldamer localities. in this study positive DAT was detected in 2/100 (2.00%) cases (positive DAT was attributed to a typical antibody) two cases are the (O+/O+) group, These two neonates (O RhD positive) and the mothers (O RhD positive), The two cases of alloimmunization were mothers multipara without a history of past transfusions. The study concluded that sensitization with alloantibodies was found among neonatal blood which reflects the importance of detection of allo Abs among cord blood and mothers.

Keywords: Allo-antibodies, a direct Antiglobulin Test, HDN, RBCs Sensitized, Cord Blood.

INTRODUCTION

The direct antiglobulin test (DAT) is a screening test for antibodies present in an individual's red cells and is used to diagnose autoimmune hemolytic anemias as well as the hemolytic disease of the newborn (HDN). A positive DAT in newborns results from the transplacental transfer of IgG antibodies, which are present in maternal serum and directed against antigens on fetal and neonatal red blood cells (RBCs). Such antibodies may destroy neonates' RBCs and shorten their life span, leading to clinical manifestations of HDN and various degrees of hyperbilirubinemia and anemia [1]. The anti-D antibody is responsible for most cases of severe HDN'! Although anti-c, anti-E, anti-K, and a wide range of other antibodies are found in occasional cases. Although antibodies against the ABO blood group system are the most frequent cause of HDN this is usually mild. Within the UK, approximately 500 fetuses develop the hemolytic disease each year and approximately 30 of these cases are fatal [2].

Clinically, HDFN ranges from hydrops fetalis to stillbirth and from mild and compensated to severe life-threatening anemia in the newborn, with the risk of permanent bilirubin-induced neurological damage (kernicterus) [3]. Left untreated, there is an 11% risk of stillbirth, a 24% risk of neonatal death, and a 13% risk of kernicterus; while 33% of newborns would require no treatment and the remaining newborns would have severe hyperbilirubinemia. Anti-D immunoglobulin (anti-D) prophylaxis has reduced the prevalence of

Citation: Nusaiba Mubarak Sirelkhatim Khalafalla, Elharam Ibrahim Abd Allah, Zakaria Albadawi Alagmi, Ghanem Mohammed Mahjaf, Mosab Nouraldein Mohammed Hamad (2022). Frequency of Red Blood Cells Sensitized with Allo-antibodies in cord blood in Atbara and Eldamer localities, Sudan, *SAR J Med*, *3*(3), 41-46.

HDFN from 14 to 0.3%; and phototherapy, exchange transfusion, and intrauterine transfusions have improved survival rates and residual affected children to over 90% in Western countries [3,4 ,5,6]. In low-to-middle-income countries (LMICs), several factors, including high costs, hamper the routine administration of anti-D [7]. According to the U.S. Center for Disease Control and Prevention, in 1996 in the United States were recorded 21 deaths of children which can be attributed to hemolytic disease (Erytroblastosis fetalis) and jaundice. Determination of blood type in ABO Rh D negative pregnant women allows reasonable precautions which limit the risk to the fetus. Erythroblastosis is a very serious medical condition for about 4000 babies a year. In 15% of cases, babies die before birth. Those who survive may suffer from jaundice, which leads to deaf-muteness, speech disturbances, cerebral palsy, and mental retardation [8, 9]. In low-resource countries, screening for D antibodies and other antibodies to detect pregnancies at risk for hemolytic disease of the newborn is not routine practice. The availability of little care medical services during pregnancy prevent women from the incident of HDN. In 2010, an estimated 271,800 to 477,500 newborns were at risk for hemolytic disease of the newborn (HDN) in LMICs [7]. Under-reporting or misrecognition may be a major contributing factor in further determination of the exact impact of HDN in LMICs. In addition, logistic and financial constraints in maternal and newborn care, along with limited diagnostic testing, complicate adequate assessment and prevention of clinical HDN [7]. However, in that cohort, antibody screening was done in only 74.5% of newborns, without the possibility for determination of antibody specificity. This leaves the exact relationship between DAT results and clinical HDN to be established. This study aimed to determine the frequency of positive DATs among newborns.

MATERIALS AND METHODS

Study design: Descriptive cross-sectional study

Study setting:

The study was conducted in Atbara and Eldamar localities. Atbara locality accommodates five hospitals providing maternal services, while Eldamar locality accommodates seven-hospital, both localities have rural and urban areas.

Study population: The study population comprised neonates who delivered either by vaginal or cesarean delivery.

Sample Size: One hundred samples were collected using simple random sampling.

Ethical considerations

All parturients sign an informed consent for indispensable laboratory tests to be performed, including ABO/Rhesus D group and DAT in cord blood. In this respect, I reviewed all results of DAT, which was performed in cord blood samples of selected infants born. Following the guidelines of the Ethics Committee, upon admittance to the River Nile ministry of health

Data collection

The data was collected using the following methods: A questionnaire covered socioeconomic status and Clinical and obstetrical factors. Cord blood samples were collected by puncturing the umbilical vein with a needle and a syringe preserved in EDTA containers.

Data analysis

After completion of data collection and entry, the data were analyzed using SPSS version 22. Qualitative data based on observations, comments, and suggestions were included and incorporated to complement the findings of the quantitative data. Quantitative data was carried out to determine the rates and percentage of the study variables and tabulation of the results was done.

Laboratory result analysis

During this period, a total of 100 cord blood samples were analyzed. from the questionnaire of mother‘s neonates, the following data were evaluated: dat results, infant/maternal abo, and rhesus d group. The following characteristics were recorded: sex, gestational age, mode of delivery, relevant antenatal and delivery data, (maternal medical history), number of abortions, and transfusion of blood for mothers. Cord blood was collected by puncturing the umbilical vein with a needle and a syringe. dat was carried out by washing the samples three times and adding a comb reagent. Abo rh blood group typing was performed using standard blood bank techniques (slide or tube test).

RESULTS

Compatibility status was determined in 74 cases The higher frequency was (O+, O+)(40%), the lower frequency was (AB+/AB+)(2%) while ABO incompatibility between neonates and mothers was found in 26 (26.0%) cases, The higher frequency of (A/O) incompatibility (15%), the lower frequency was (B+/A+),(AB+/A+)(1%) in accordance with the ABO group distribution at the Atbara & Eldamer localities. in this study positive DAT was detected in 2/100 (2.00%) cases. (Positive DAT was attributed to a typical antibody). two cases are the (O+/O+) group, These two neonates (O RhD positive) and the mothers (O RhD positive), The two cases of alloimmunization were mothers' multipart without a history of past transfusions. The percentage of the history of blood transfusion in mothers was (2%) Direct comb test: Positive in two samples (2%) Widely blood group distribution among newborns is:O Rh (+)

Table-1: The distribution of respondent neonates'mothers according to their age in Atbara & Eldamer teaching h ospitals and National Ribat hospital

Age group	N	%
Less than 20 years	6	6.0
20 - 29 years	74	74
30 - 49 years	20	20
Total	**100**	**100**

Table-2: The distribution of respondent neonates 'mothers according to the place where they live

Resident	Frequency	%
Atbra	43	43
Eldamer	56	56
Barber	1	1
Total	**100**	**100**

Table-3: The distribution of neonates according to the gender

Sex of the neonate	Frequency	%
Male	31	31
Female	69	69
Total	**100**	**100**

Table 4: The distribution of respondent women according to the mode of delivery at Atbara & Eldamer teaching hospitals and National Ribat hospital

Mode of delivery	Frequency	%
Vaginal	65	65
Cesarean	35	35
Total	**100**	**100**

Table-5: The distribution of neonate's mother according to their gestational age.

Gestational age	Frequency	%
Less than 37 years	5	5
37 - 40 years	92	92
More than 40 years	3	3
Total	**100**	**100**

Table-6: The distribution of neonate's mother according to their ABO Rh grouping.

ABO-RH Blood group of mother	Frequency	%
A+	23	23
B+	9	9
AB+	2	2
O+	61	61
O-	5	5
Total	**100**	**100**

Table-7: the distribution of neonates according to their ABO Rh grouping

blood group of baby	Frequency	%
A+	25	25
B+	10	10
AB+	1	1
O+	59	59
B-	2	2
O-	3	3
Total	**100**	**100**

Table-(8) the distribution of association between ABO Rh grouping between mothers and neonates

ABO-RH Blood group of mother * blood group of babies Cross tabulation								
		Blood group of babies						Total
		A+	B+	AB+	O+	B-	O-	
ABO – RH Blood group of mothers	A+	10	1	1	10	0	1	23
	B+	0	4	0	5	0	0	9
	AB+	0	0	0	0	2	0	2
	O+	15	5	0	40	0	1	61
	O-	0	0	0	4	0	1	5
Total		**25**	**10**	**1**	**59**	**2**	**3**	**100**

Table 9: The distribution of neonate's mother according to their ABO number of abortion

Number of abortion	Frequency	%
One	12	12
More than one	6	6
Nil	82	82
Total	**100**	**100**

Table 10: The distribution of neonate's mother according to their parity

Parity	Frequency	%
Primigravida	10	10
Multipara	85	85
Grand multipara	5	5
Total	**100**	**100**

DISCUSSION

Although several reports have proposed that routine cord blood DAT testing is not necessary, according to my cross-sectional study, its impact on the newborn cannot be overlooked. Nevertheless, DAT testing cannot replace STB measurements early in life. Furthermore, although atypical antibodies account for the majority of DAT-positive cases, other causes should be also considered. Maternal screening tests and a careful look at the history of drug administration during pregnancy could identify other important but rare causes of DAT positivity, aiding maternal and neonatal management, the findings of my study stress the need for prenatal testing of potential HDFN in newborns of mothers in Atbara and Eldamer. Among the 100 neonates born between January and February 2019, 25 (25.0%) neonates were of group (A +), 10 (10.0%) of group (B +), 59 59.0%) of group (O +), and 1 (1.0%) of group (AB +), 2 (2.0 %), of group (B -) 2 (2.0%), 3 (3.0%) of group (O -). ABO incompatibility between neonates and mothers was found in 26 (26.0%) cases. Of these, 15 neonates of the group (A +) and 5 of the group (B+) were born to group (O +) mothers (A +/O +) 15, (B +/O +),5) The remaining ABO-incompatible cases identified were (B +/A +), 1 cases; (AB +/A +), 1 cases; and (O +/ O -), 4 cases. The ABO neonatal/ maternal compatibility status could be determined in 74 cases (21 neonatal group A, 2 neonatal group AB, 9 neonatal group B, and 42 neonatal group o), as the study was cross-sectional. All the 74 (74,0%) cases were considered ABO compatible (A+/A+) 10, (O+/A+)10 , (O -/A +)1, (B+/B+) 4 (O +/B +) 5, (B -/AB +) 2, (O +/O+) 40, (O-/O +)1, (O-/O-) 1 , as stated above, 100). In my study the wide distribution of the neonate's blood group is O (+), then A (I), B (+), O ( ), B ( ) and finally AB (+). DAT was found to be positive in 2/100 (2.00%) cases. In some cases, DAT positivity was attributed to atypical antibodies. two cases are the (O +/O +) group, These two neonates (O RhD positive) and the mothers (O RhD positive), delivered by cesarean section, one of them at 38*th* week and the other at 39th week of gestation, The two cases of alloimmunization was mothers multipara without a history of past transfusions, who uneventfully delivered females infants. The neonate did not suffer hemolysis discharged in a good health. In this cross-sectional study, was analyzed all DAT results routinely performed in cord blood samples of selective infants born at the Atbara and Eldamer teaching hospitals and National Ribat hospital. The ABO incompatibility between neonates and mothers was 26 cases. The higher frequency of A versus O incompatibility was also to the ABO group distribution in the Atbara & Eldamer teaching hospitals and National Ribat hospital. The incidence of a positive DAT in newborns was 2. 00%, a Percent page similar to that (2.3%) reported by Dillon *et al.*, but lower than that (3.5%) of Hershel *et al.*, [10, 11]. Both studies applied the same methodology as the cross-sectional study. 22, 23 By previous studies, the current one presented ABO incompatibility in 91.43%, positive DAT cases 15, and the incidence of DAT positivity was slightly higher in neonates of group A/O (22.93%) when compared to group B/O (13.40%) [12]. In my study DAT positivity was low, there were no cases of positive DAT was attributed to anti-D passive or immune alloimmunization of the mothers because the percentage of women who had blood group O Rh-negative is low because the frequency of Rh negativity

is low in black than in white and all of them (5 mothers) have taken anti D also no cases were attributed to ABO HDN due to the high percentage of the ABO compatibility between neonates and mothers, in addition, there is a very low percentage (1%) of type-O mothers have a high titer of the antibodies of IgG class against both A which They cross the placenta and cause hemolysis in the fetus, fetal RBCs appear to have less surface expression of A or B antigen, resulting in few reactive sites; hence the low incidence of significant hemolysis in affected neonates, Recent analysis of IgG subclass in ABO-incompatible direct coombs positive neonates showed IgG2 was a predominant antibody which is poorly transferred across the placenta and less efficient in causing hemolysis while IgG1 was noted in 22% of neonates [13]..so that ABO HDN occurs according to the type of IgG sub class The remaining reason of low DAT positivity in this study, there was low percentage of mothers with a history of blood transfusion (2%) and low percentage of mothers with no history of abortion which are factors affecting of immunization of mothers. In this study, DAT positivity was attributed to atypical antibodies and the mother's immunization due to past deliveries. The role of DAT screening in predicting HDN has been debated over the past years [12, 14-16]. The predictive value of the DAT positivity was not assessed in this cross the sectional study, because only the DAT positive cases were taken into consideration. Some limitations of this study need to be mentioned here. First, there is a chance for selection bias, because only women in three of the twelve hospitals in Atbara and Eldamer localities, and no women from birth clinics in the rural part of them were included. Some limitations of this study need to be mentioned here. First, there is a chance for selection bias, because only women in three of the twelve hospitals in Atbara and Eldamer localities, and no women from birth clinics in the rural part of them were included.

CONCLUSION

The study concluded that sensitization with all oantibodies was found among neonatal blood which refl ects the importance of detection of allow Abs among co rd blood and mothers.

RECOMMENDATION

1. Early booking of antenatal care clinics and stressin g the need for prenatal testing of mothers and postn atal testing of newborns and mothers.
2. All pregnant women prefer to have tested for the i mmune antibodies in the serum during follow-up in our hospitals.
3. Direct antiglobulin test, antibodies screening, and a ntibodies titer are recommended to be free of charg e.
4. Finally, prospective studies including cost-benefit o nes with defined endpoints would be helpful.

Funding: There was no specific grant for this research from any funding agencies.

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