Hemolytic Disease of the Fetus and New-born and Intrauterine Blood Transfusion among Palestinian Pregnant Women

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Abstract

Background: Intrauterine transfusion (IUT) is considered to be the most successful relief of fetal anemia resulting from Hemolytic Disease of Fetus and New-born (HDFN). This study aims to determine the frequencies of RBC alloantibodies that might cause fetal hemolysis and evaluate the perinatal outcome of IUTs in Palestine.

Materials and Methods: We conducted a retrospective-cohort study of pregnant women who requited IUT procedure at Al-Makassed Hospital in East Jerusalem. We reviewed Blood-Bank records between 2003 and 2013. Data were collected on all RBC-alloimmunized pregnancies requiring IUTs including the age of pregnant women, blood typing, antibody identification, and antibody titers. Also, we collected clinical data from the patients' files about the obstetric history and current pregnancy.

Results: A total of 222 IUTs were performed during the study period in 65 alloimmunized pregnancies. Of all cases, 95.4% were associated with anti-D, 36.9 % with anti-C, and 10.8% with anti-E. Other non-Rh antibodies included mainly Kell, Kidd (10.8%), Luth and Lewis. The median number of transfusions needed per pregnancy was 3. The survival rate in the study was 90% and 27.3% of cases were hydropic; survival rate was significantly higher for fetuses without hydropic fetalis.

Conclusions: IUT can improve perinatal outcome in alloimmunized pregnancies. In Palestine, policies should be drawn to introduce this procedure to more Palestinian Hospitals to increase its accessibility to the patients. In addition, secondary prevention of anti-D associated sensitization by rhesus immune globulin to reduce the incidence of HDFN should be more efficaciously implemented.

Keywords: RBC-alloimmunization, intrauterine transfusion, hydrops fetalis, hemolytic disease of the fetus and new-born, natal outcome.

Introduction

Maternal red blood cell (RBC)-alloimmunization is the primary cause for hemolytic disease of the fetus and new-born (HDFN). Severe hemolysis results in fetal anemia leading to complications ranging from hepatosplenomegaly, cardiomegaly, cardiac decompensation, and hydrops fetalis to fetal loss if the anemia is not treated (Lindenburg, van Kamp, & Oepkes, 2014; Santiago et al., 2010; Singla, Kumar, Roy, Sharma, & Kachhawa, 2010; Zwiers, van Kamp, Oepkes, & Lopriore, 2017).

More than 50 RBC-antigens have been implicated in HDFN including ABO-antigens and Rh-antigens (Fan, Lee, Wikman, Johansson, & Reilly, 2014; Lindenburg et al., 2014). Mild to severe cases of fetal hemolytic disease could be caused by anti-D, -c, -C, -e, -E, or Kell, Kidd, Duffy, MNS, Lutheran, Diego, Xg, P (Babinszki & Berkowitz, 1999). Anti-Rh(D) was the major alloantibody to cause HDFN, but the introduction of RhD immune globulin (RhIg) as a secondary prevention mechanism

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for HDFN reduced the risk of alloimmunization by anti-D (Delaney & Matthews, 2015). Thus, prenatal screening for red cell antibodies was recommended for all pregnant mothers (British Committee for Standards in Haematology, 1996).

Before the introduction of intrauterine blood transfusion (IUT) for the treatment of HDFN prenatally, the only option was premature delivery for severely affected babies. In 1963, Lily was the first to describe intraperitoneal IUT. Further, intravascular IUT was not introduced until the 1980s and started to be considered a safer alternative (Lindenburg et al., 2014; Zwiers et al., 2017). However, IUT still poses a risk considering the stress on the cardiovascular system of the fetus caused by the procedure. IUT carries a risk of adverse events between 1-3% (Delaney & Matthews, 2015; Yinon et al., 2010).

Palestine is a small country with around five million population. The birth rate is estimated to be 28.8 per 1,000 population and the fertility rate is 4.1 per woman in childbearing age (15-49 years) (Ministry-of-Health, 2016). The Ministry of Health (MOH) is the main provider of healthcare for Palestinians and is followed by the UNRWA and non-governmental organizations (NGOs). There are five blood banks in Palestine, four of which are under the supervision of MOH and the last is Al-Makassed Blood-Bank, which is a non-governmental hospital located in East Jerusalem and provides care to Palestinians on referral basis. Routine antenatal antibody screening is performed only for Rh(D)-negative mothers to screen for anti-D antibodies. Al-Makassed Hospital is the only one performing IUT procedure and provides it to a small percentage of in need Palestinian pregnant women. In this study, we aim to report the causes of alloimmunization among Palestinian pregnant women and further to evaluate the outcome of IUTs performed at Al-Makassed Hospital from 2003 till 2013 for fetal alloimmune anemia.

Materials and Methods

Ethical approval

The study was approved by the Institutional Review Boards (IRBs) of Al-Quds University and Al-Makassed Hospital.

Data Collection

This is a retrospective-cohort study of pregnant women who required IUT procedure for HDFN at Al-Makassed Hospital, East Jerusalem, between 2003 and 2013. Cases were identified through the blood bank records as pregnant women with requests for blood issuing for IUT. A total of 41 women were included in the study. Data regarding blood typing, antibody identification, antibody titers, blood preparation for transfusion and issued blood quantities were collected from the blood bank archive. Clinical data of the patients were taken from their medical files to evaluate the IUT outcome.

IUT protocols

Data regarding the procedures and the guidelines of IUT were provided by the Obstetrics and Gynaecology Department at Al-Makassed Hospital. Fetal anemia is currently diagnosed by the middle cerebral artery peak systolic velocity (MCA-PSV). The hospital guidelines follow the criteria reported by Mari et al. (Mari et al., 2000) in predicting moderate to severe fetal anemia by considering 1.5 multiples of median on MCA-PSV as a cut-off value. The evaluation of the presence of ascites and hydrops is performed using ultrasonography.

Antibody screening and identification were performed using indirect coombs with commercial cells and enzymes according to the manufacturer instructions. Antibody titration was performed before each IUT and cross-match test was performed with maternal serum. All blood units used for transfusion were O-negative leukocyte-depleted blood, washed, and phenotyped to be negative for the antigens against which the mother is immunized and compatible with final hematocrit ranging between 75% and 80%. However, blood units were not tested for cytomegalovirus. Transfusion was done without fetal immobilization except in cases having a posterior placenta in which a muscle relaxant (pavolon) was given. Fetal blood transfusions were performed under maternal local

anesthesia using a 22-gauge needle. When fetuses were hydropic, intravascular transfusion was done. However, intraperitoneal transfusion was done on anemic fetuses who have no hydrops. One shot of monocef antibiotic was given to the pregnant women before the procedure in order to face any possible bacterial infection that could be acquired during the transfusion. Second transfusion was performed within 7-14 days. Transfusion volume was calculated as described by Giannina et al (Giannina, Moise, & Dorman, 1998).

Statistical analysis of the data was performed using Microsoft Excel 2010. Data were reported as medians and ranges for continuous variables and counts (n) and percentages (%) for categorical variables. Chi-square test and Mann-Whitney were used for comparisons, a p-value <0.05 was considered significant.

Results

During the period between 2003 and 2013, 41 RBC alloimmunized women were treated at Al-Makassed Hospital for HDFN. The median age of women was 30 years with a range of 20-43 years. The majority were A- blood type (52.5%), five women were noted to have history of miscarriage or stillbirth and four had history of early neonatal death (ENND) (Table 1).

Table 1: Baseline characteristics of cases with RBC alloimmunization (n=41).

Characteristic		Frequency n (%)
Blood Group, n (%)	A-	21 (52.5)
	A+	1 (2.5)
	AB-	5 (12.5)
	B-	7 (17.5)
	O-	6 (15)
History of IUFD		5 (12.2)
History of ENND		4 (9.8)

Abbreviations: IUFD: intrauterine fetal death; ENND: early neonatal death

A total of 222 IUTs were performed in 65 fetuses during the period of the study. Each fetus received a median of three IUTs (range of 1-9). Of the 65 alloimmunized pregnancies, 95.4% were associated with anti-D, 36.9% with anti-C, and 10.8% with anti-E. In addition, 9.2% were associated with anti-Kell antibodies, 4.6% with anti-MNS antibodies, and 10.8% with anti-Kidd antibodies. While 55.6% of pregnancies were associated with one single type of antibodies, 44.4% had more than one antibody type involved. The antibody titers at the time of first IUT ranged from 1:128 to 1:4000. For 38.5% of the pregnancies, there was a previous history of IUT. Furthermore, the median gestational age at first transfusion was 20 weeks (range 17-25) with 61.5% of pregnancies receiving the first transfusion before week 22 (Table 2).

Table 2: Characteristics of alloimmunized pregnancies (n=65), Palestine, (2003-2013).

Characteristic		Value
Maternal age, years, median (range)		30 (20 - 43)
Titer, median (range)		1:1024 (1:128 – 1:4000)
Number of IUTs, median (range)		3 (1 - 9)
GA at1st IUT, weeks, median (range)		20 (17 - 25)
Rh-antibodies, n (%)	Anti-D	62 (95.4)
	Anti-C	24 (36.9)
	Anti-E	7 (10.8)
Kell-antibodies, n (%)	Anti-K	6 (9.2)
MNS-antibodies, n (%)	Anti-S	2 (3.1)
	Anti-s	1 (1.5)

Kidd-antibodies, n (%)	Anti-Jk[a]	4 (6.2)
	Anti-Jk[b]	3 (4.6)
Duffy-antibodies, n (%)	Anti-Fy[a]	1 (1.5)
Lewis-antibodies, n (%)	Anti-Le[b]	2 (3)
Luth-antibodies, n (%)	Anti-Lu[a]	2 (3)
History of IUT		25 (38.5)
Hydropic status	Hydropic	3 (27.3)
	Non-hydropic	8 (72.7)

Abbreviations: IUT: intrauterine transfusion; GA: gestational age

The obstetric and neonatal outcomes of pregnancies are summarized in table 3. Three pregnancies were hydropic, one of which resulted in fetal loss leaving a survival rate of 90%. Furthermore, 60% of fetuses were delivered at 33 weeks of gestation. The median fetal weight was 2.26 Kg and ranging from 1.23 to 2.59. Survival rates differed significantly between hydropic and non-hydropic fetuses (p-value=0.047) but not birth weight. Further, neither did maternal age show any association with hydropic status, nor maternal blood group, gestational age at first IUT, number of transfusions, number of positive antibodies, and titer levels did (Table 4).

Table 3: Obstetric and natal outcome of study group.

Characteristic		Value
Natal outcome, n (%)	IUFD	1 (10)
	Livebirth	9 (90)
Birth weight, Kg, median (range)		2.26 (1.23 - 2.59)
GA at birth, weeks, median (range)		33 (31 - 33)

Abbreviations: IUFD: intrauterine fetal death; GA: gestational age

Table 4: Comparison of characteristics and outcomes of hydropic and non-hydropic fetuses.

Characteristic		Hydropic	Non-hydropic	P-value
Maternal age, years, n (%)	<=30	2 (66.7)	6 (75)	0.782
	>30	1 (33.3)	2 (25)	
No. of IUTs, mean (range)		5.3 (4 - 6)	4.8 (3 - 9)	0.341
Perinatal survival, n (%)	IUFD	1 (50)	0 (0)	**0.047**
	livebirth	1 (50)	7 (100)	
Maternal blood group, n (%)	A-	0 (0)	3 (37.5)	0.15
	B-	2 (66.7)	5 (62.5)	
	O-	1 (33.3)	0 (0)	
GA at1st IUT, mean (range)		21 (18 - 25)	20.4 (17 - 23)	0.918
Titer, mean (range)		683 (512 - 1024)	1226 (128 - 2048)	0.245
Birth weight, mean (range)		1.5 (-)	2.1 (1.2 - 2.6)	0.275

Abbreviations: IUT: intrauterine transfusion; IUFD: intrauterine fetal death; GA: gestational age

Discussion

In this study, we described a cohort of 65 pregnancies treated with IUT at Al-Makassed Hospital, a Palestinian hospital located in East Jerusalem and the only one that provides IUT service for Palestinians in the West Bank on referral basis. The findings of this study showed that IUT is a relatively safe procedure that improved the outcome of alloimmunized pregnancies with a survival

rate of 90% for the procedure. It is estimated that 10% of alloimmunized women develop severe fetal anemia (Santiago et al., 2010). Survival rates between 80-93% have been reported for this procedure (Lindenburg et al., 2014).

In spite of the availability of RhIg as a prophylactic treatment for sensitization with anti-D, this study showed that anti-D is still one of the major causes of HDFN in Palestine. In fact, the three hydropic pregnancies and the stillbirth in this study were associated with anti-D. Other studies reported anti-Rh antibodies (mainly anti-D) as the major cause of severe HDFN with rates varying between 18.4% in New York (Geifman-Holtzman, Wojtowycz, Kosmas, & Artal, 1997), 70% in Israel (Weisz et al., 2009), 83% in Finland (Sainio et al., 2015) and 89% in the Netherlands (van Kamp et al., 2004). Furthermore, anti-Kell and other non-Rh antibodies such as Duffy, Kidd and S were also found to be associated with HDFN, although they were rare (Babinszki & Berkowitz, 1999; Geifman-Holtzman et al., 1997; Sainio et al., 2015; Weisz et al., 2009; Zwiers et al., 2017). These findings could be explained by the lack of resources and the inadvertent omissions in administration as well as antenatal sensitization prior to RhIg given at 28 weeks' gestation (Moise, 2005). Moreover, proper administration of RhIg can reduce the risk of alloimmunization by anti-D antibodies from 16 to <0.1% (Delaney & Matthews, 2015). A Swedish study showed that maternal alloimmunization with anti-D, -E, -C, -c and -Le[a] antibodies was associated with increased odds of stillbirth and preterm birth. Furthermore, anti-Kell associated fetal anemia, which was found in around 10% of alloimmunized pregnancies in our study, generally develops earlier in gestation. This has been attributed to the presence of Kell antigens on erythroid precursor (Lindenburg et al., 2013).

Fetal hydrops is considered a negative prognostic factor of the outcomes of fetal anemia (Lindenburg et al., 2013). In this study, survival rates of hydropic fetuses were significantly lower. Previous studies reported that performing the first IUT before the 20th week was associated with higher risk of perinatal loss (Lindenburg et al., 2013), although in this study we couldn't establish an association. In this study, we examined the association between maternal history for past stillbirths and perinatal mortality and the development of hydrops fetalis in pregnancies. A detailed maternal history is considered useful to determine pregnancy outcomes (Delaney & Matthews, 2015), nevertheless, no association was found here. Other studies reported hydrops at first transfusion among between 11-38% of the fetuses (Sainio et al., 2015; van Kamp et al., 2004; Weisz et al., 2009) with significantly lower survival rates associated with hydrops fetalis (van Kamp et al., 2004).

Conclusion

This study is the first study to examine pregnancy outcomes in RBC-alloimmunized women who required IUT in Palestine. Due to the small sample size and the amount of missing data due to loss of follow-up, the findings of this study should be interpreted cautiously. Regardless of that, based on the findings of this study, we advocate timely detection of anti-RBC antibodies in order to avoid complications that can compromise the pregnancy, specifically hydrops fetalis. Further, the study shows that IUT is a relatively safe procedure with high survival rates. Introduction of IUT to the healthcare services provided in the West Bank proves to be important to maximize the ability to treat HDFN since the service is currently available at Al-Makassed Hospital only, which is not easily accessible for Palestinians living in the West Bank and Gaza Strip due to the political situation. Clearly, anti-D is still a major cause of HDFN in Palestine despite the availability of the postnatal immunoprophylaxis which indicates the necessity to draw policies and guidelines and to increase awareness towards this health issue.

Disclosure of conflicts of interest

The authors declare that they have no competing interests.

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Abbreviations

ENND: Early Neonatal Death

GA: Gestational Age

HDFN: Hemolytic Disease of Fetus and New-born

IUFD: Intrauterine Fetal Death

IUT: Intrauterine Transfusion

RhIg: RhD immune globulin

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