European Journal of Obstetrics & Gynecology and Reproductive Biology 237 (2019) 89–92

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Prevalence and obstetric outcome of women with red cell antibodies in pregnancy at the Leeds Teaching Hospitals NHS Trust, West Yorkshire, England

Ibraheem Awowole[a,b,*], Kelly Cohen[a], Jennifer Rock[c], Colette Sparey[a]

[a] Fetal Medicine Unit, Leeds Teaching Hospital NHS Trust, West Yorkshire, UK
[b] Feto-maternal Unit, Department of Obstetrics and Gynaecology, Obafemi Awolowo University, Ife, Osun State, Nigeria
[c] Blood Transfusion Unit, Department of Pathology, Leeds Teaching Hospitals NHS Trust, West Yorkshire, UK

ARTICLE INFO

Article history:
Received 18 December 2018
Received in revised form 31 March 2019
Accepted 16 April 2019

Keywords:
Alloimmunization
Haemolysis
In utero transfusion
Pregnancy
Red cell antibodies

ABSTRACT

Objective: The prevalence of red cell antibodies in pregnancy varies with ethnicity and geographical location, while the obstetric outcome depends on the available standard of care. Despite being the tertiary fetal medicine centre in West Yorkshire, the prevalence of red cell antibodies, and the outcome of pregnancies associated with these antibodies at the Leeds University Teaching Hospitals Trust remains unreported. This article aims to provide this information for the purpose of patient education and counselling.

Study design: The data of pregnant women with red cell antibodies between January 2011 and December 2016 was obtained from the Trust's database and reconciled with the Fetal Medicine Unit records using Viewpoint©. Fetal anaemia requiring in utero transfusion (IUT) was defined as a Middle Cerebral Artery Peak Systolic Velocities $\geq$ 1.5multiple of the median expected for gestational age. The mean gestational age at delivery, and perinatal outcomes of the pregnancies were recorded.

Result: Overall, 398 of the 96, 692 pregnant women that were screened had red cell antibodies, giving a prevalence of 1: 242 pregnancies. The Anti- E and Anti-M antibodies were the most common (114 women; 28.6%, and 112 women; 28.1% respectively), but did not cause fetal anaemia in isolation, while anti-D alloimmunization was the predominant indication for in-utero transfusion (IUT). Anti-DE and anti-Kell antibodies had the highest mean number of transfusions per pregnancy. The mean gestational age at delivery was 34 $\pm$ 2weeks. Post-transfusion fetal demise was recorded in two hydropic fetuses, both at a gestational age of 25 weeks; giving a transfusion-related mortality rate of 2.5%.

Conclusion: The prevalence of red cell antibodies at West Yorkshire is lower compared with reports from other Caucasian populations.Nevertheless, these antibodies are important causes of iatrogenic preterm delivery and fetal morbidity. The prognosis is however good with prompt diagnosis and management.

Introduction

Since the discovery of the human ABO blood group in 1901 by Nobel Laureate Karl Landsteiner, and the Rhesus blood group in 1937, in conjunction with Weiner, knowledge of the blood group antigen/antibody interaction has evolved significantly [1]. Several other red cell antigens have been described in humans in addition to the rhesus and ABO antigens, with varying significance in blood transfusion and organ transplantation services. This knowledge has also become especially important in obstetric practice, where the previously limited understanding of feto-maternal haemorrhage and maternal alloimmunization in pregnancy had led to significant morbidity and mortality among fetuses and newborns [2–4].

Red cell antibodies in pregnancy are common, with varying prevalence based on geographical and ethnic distribution. They may be broadly classified into three, including; 1) Antibodies that pose significant fetal and neonatal risks such as anaemia, haemolysis, hyperbilirubinaemia and its sequelae to the fetuses and neonates; 2) Antibodies that mediate blood transfusion reactions and may therefore constitute a challenge to the safe provision of blood for transfusion purposes during pregnancy and delivery, and 3) Antibodies of undetermined significance in pregnancy [4].

The most common red cell antibodies include the Rhesus group (anti-c, -C, -D, -e and -E), Kell (anti-K, Anti-Kp), Duffy (Anti-Fya and

* Corresponding author at: Fetal Medicine Unit, Leeds Teaching Hospitals NHS Trust, LS9 7TF, West Yorkshire, UK.
E-mail address: ibraheem.awowole@nhs.net (I. Awowole).

https://doi.org/10.1016/j.ejogrb.2019.04.016
0301-2115/

-Fyb), Kidd (Anti-Jka and -Jkb), MNS (Anti-M, -N and -S), and Lewis (Anti-Lea and -Leb) antibodies [5,6]. Rhesus alloimmunization is however reported to precipitate the most severe forms of HDFN in medical literature, especially due to maternal anti-D antibody in rhesus negative women bearing rhesus positive fetuses [4,7,8]. About 16% of the Caucasian population are reported to be rhesus D negative, with about 65,000 rhesus positive babies born to these women annually in the UK [9].

Advances in scientific knowledge and clinical care have led to a reduction in the number of women with red cell antibodies in pregnancy. Routinely, pregnant women in the UK are tested for their blood group and red cell antibodies at booking, and at a gestational age of 28 weeks if the initial screening is negative. Postpartum anti-D prophylaxis for rhesus negative women started in the UK in 1969 and antepartum administration following sensitizing events such as vaginal bleeding and amniocentesis started in 1976. Prior to these, the incidence of alloimmunization in rhesus-D negative women following the delivery of rhesus-D positive infants was in the order of 16%, reducing to about 2% following the policy of postpartum administration of anti-D immunoglobulin [2]. With improved understanding of the concept of silent feto-maternal haemorrhage and consequent alloimmunization in pregnancy, Routine Antenatal Anti-D Prophylaxis (RAADP) was recommended by the UK National Institute for Health and Clinical Excellence (NICE) in 2002 for unsensitized rhesus-D negative women, and it is estimated that the rate of alloimmunization has reduced further by ten-fold to about 0.2% [2,9,10]. This has in turn significantly reduced the perinatal mortality rate associated with haemolytic disease of the fetus and newborn [2,11].

The Leeds Teaching Hospitals Fetal Medicine Unit provides tertiary specialist care for women with high risk pregnancies, including rhesus D-negative women and women with prior or ongoing pregnancies complicated by red cell alloimmunization, using a care plan that entails routine screening in pregnancy, RAADP for rhesus negative women, immuno-prophylaxis after sensitizing events, fetal Middle Cerebral Artery (MCA) Peak Systolic Velocity (PSV) doppler monitoring of alloimmunized pregnant women for fetal anaemia, in utero fetal blood transfusion where indicated and postpartum anti-D antibody for rhesus negative women who delivered rhesus positive babies. This article seeks to review the prevalence of red cell antibodies in pregnancy at the Leeds Teaching Hospitals Trust (LTHT), and the perinatal outcomes of alloimmunized women that were managed in pregnancy at the Trust. The outcome of invasive treatments such as in utero fetal blood transfusion where undertaken, was provided for the purposes of patients' counselling.

Materials and methods

The total number of obstetric patients screened during the six-year period between January 2011 and December 2016 was obtained from the Trust's Blood Bank and Transfusion Services database. The subset of women with red cell antibodies was identified from the same database and reconciled with the Fetal Medicine Unit (FMU) database on women with red cell antibodies that were referred for specialist's care using the Viewpoint© software. The indications for referral to the FMU were maternal serum anti-D antibody concentration > 4iu/ml, anti-c >7.5iu/ml, anti-Kell at any level, and women with affected fetuses/babies in previous pregnancies [5]. Fetal anaemia was defined as a Middle Cerebral Artery Peak Systolic Velocities (MCA PSV) greater than 1.5 multiple of the median (MoM) expected for gestational age. Serial weekly monitoring of the MCA PSV was done post-transfusion and a repeat in utero transfusion was undertaken if the MCA PSV rises beyond 1.5 MoM of what is expected for the gestational age. Information about intrauterine fetal blood transfusion for antibody-mediated anaemia was retrieved, and the fetal median pre-transfusion and immediate post-transfusion PSV were determined. The mean gestational age at delivery, and the immediate neonatal outcome of the baby at delivery were reviewed. The IUT-related perinatal mortality was calculated. As this study was conducted retrospectively and completely anonymised such that the data were not attributable to any patient, research approval was not sought.

Results

In the six-year period under review, 398 out of the 96, 692 pregnant women that were screened by the blood bank and transfusion services of the Leeds Teaching Hospital Trust had circulating red cell antibodies in their sera. The prevalence of red cell antibody in pregnancy in the Trust was therefore calculated to be 1: 242 pregnant women. The median parity of the alloimmunised women was 2 (range of 1-6), and twelve of them were managed for alloimmunisation in two or more pregnancies. Two hundred and ninety-nine (75%) of the women had only one red cell antibody, while 20.1%, 3.8% and 1% of them had a combination of two, three and four antibodies respectively. The frequencies of the most prevalent red cell antibodies among the women are depicted on Table 1, with Anti-E antibody being the most common (129 women; 32.4%). The anti-D antibody was however the most common cause of fetal anaemia that was severe enough to warrant blood transfusion.

There were 92 in utero fetal blood transfusions undertaken in the fetal medicine unit during the same period; 80 (87%) of which were due to fetal anaemia resulting from red cell alloimmunization in 29 women (Table 2). The other indications for in utero fetal blood transfusion are beyond the scope of this manuscript. The gestational age at first fetal transfusion ranged between 16 and 33weeks, with a mean of 26 weeks. Overall, the median number of in utero transfusions was 2, with a range of 1-7 tranfusions. There was a significant reduction in the immediate post-transfusion fetal middle cerebral artery peak systolic velocities compared with the pre-transfusion MCA PSV (35.5 cm/s vs 60.0 cm/s respectively: $p < 0.001$).

The gestational age at delivery ranged between 27–35 weeks, with a mean of 34 weeks. There were two intra-uterine deaths among the fetuses that were transfused, with a procedure-related fetal mortality rate of 2.5%. Both fetuses were already hydropic at the time the women engaged with the antenatal care services of the LTHT, and they were the only two fetuses that developed hydrops fetalis. The fetuses had pre-transfusion hemoglobin concentrations of 10 g/Land 12 g/L respectively. The two women had anti-D antibody, and fetal demise occurred within six hours of

Table 1
Prevalence of red cell antibodies among pregnant women at Leeds Teaching hospitals Trust (2011–2016).

No.	Antibody	Frequency	% of women with antibody
1.	Anti-E	114	28.6
2.	Anti-M	112	28.1
3.	Anti-c	55	13.8
4.	Anti-D	50	12.5
5.	Anti-C	30	7.5
6.	Anti-K	27	6.8
7.	Anti-Jka	21	5.8
8.	Anti-Cw	20	5.0
9.	Anti-Lea	17	4.2
10.	Anti-Leb	11	2.7
11.	Anti-Jkb	06	1.5
12.	Anti-e	03	0.7

Table 2
Identified antibodies and frequency of in utero fetal blood transfusions in women with red cell antibodies at the LTHT FMU (2011–2016).

Number	Antibody	Affected pregnancies	Number of transfusions	Mean transfusion per pregnancy.
1.	Anti-D	18	38	2.1
2.	Anti-DE	3	16	5.3
3.	Anti-CD	2	6	2.0
4.	Anti-Kell	2	10	5.0
5.	Anti-cE	2	6	3.0
6.	Anti-CDE	1	1	1.0
7.	Anti-DG	1	2	2.0

IUT, at a gestational age of 25 weeks in both women. All the other babies survived and were discharged after appropriate care in the Neonatal Unit. There was no neuro-morbidity observed in any of the neonates at the time of discharge from the Neonatal Care Unit.

Comments

The prevalence of red cell antibody among the pregnant women managed at LTHT between 2011 to 2016 was 1:242 pregnancies; a lower prevalence compared with the 1.80 reported among Caucasian populations from the Netherlands [4]. Although the exact reason for this disparity is uncertain, the multi-ethnic population in Leeds may be partly responsible. Among the 398 alloimmunized women, only 29 (7.3%) had fetal anaemia that was severe enough to require in utero fetal blood transfusion, indicating that most fetuses in pregnancies complicated by alloimmunization would not be affected, or only mildly affected.

The anti-D antibody was the most common cause of fetal anaemia that was severe enough to warrant blood transfusion. Although anti-E and anti-M antibodies were predominant among the women, none of these two antibodies precipitated fetal anaemia when present in isolation. The anti-E antibody however precipitated significant fetal anaemia when it co-existed with other antibodies such as the anti-c or anti-D antibody, in line with previous reports [4,12]. The Anti-DE antibody combination however appears to be the most potent in this study, as pregnancies complicated by this combination had the highest mean number of fetal blood transfusions per pregnancy. Next to the anti-DE antibody combination is the anti-Kell antibody, which is capable of precipitating fetal anaemia irrespective of the maternal serum concentration.

The Middle Cerebral Artery (MCA) Peak Systolic Velocity (PSV) has been shown by Mari et al to reliably diagnose moderate to severe fetal anaemia in utero with a sensitivity of 100%, a false positive rate of 12%, with positive and negative predictive values of 65% and 100% respectively [13]. The middle cerebral artery peak systolic velocity cut-off of 1.5Mom was used at the LTHT FMU as proposed by Mari, eliminating the need for cordocentesis or amniocentesis in the serial monitoring of the fetuses at risk. There is also the additional benefit of monitoring fetuses exposed to the anti-Kell antibody, in whom amniocentesis is not a reliable method of monitoring, as the anaemia in such fetuses is induced by suppression of haemopoiesis rather than haemolysis. Fetal blood was not routinely taken for blood count post-transfusion, as the immediate fetal post-transfusion MCA doppler velocimetry also provided a reliable assessment of the fetus.

IUT in this study was largely safe, with an associated perinatal mortality rate of 2.5%. This is comparable to the 2% mortality rate quoted by NICE, and 1–3% reported by the RCOG [4,9,14]. This is however remarkably high when compared with the background stillbirth rate of 3.9/1,000 total birth in the UK [15]. As the number of fetal deaths were two, it was impossible to conduct any reasonable analysis for the predictors of adverse fetal outcome following IUT. The two fetuses were however hydropic pre-transfusion, with early onset of fetal anaemia.

While the use of RAADP has proven beneficial, almost 40% of rhesus-D negative women delivered rhesus-D negative babies as well; such women, estimated to be approximately 40,000 annually in England and Wales, therefore wouldn't have needed RAADP or additional doses of prophylactic anti-D immunoglobulin following sensitizing events in such pregnancies [16,17]. The use of Non-Invasive Prenatal Diagnosis (NIPD) of fetal blood group using cell-free fetal DNA (cfDNA) in maternal circulation [4] to determine the presence of fetal D, C,c,e,E and K antigens has therefore been introduced into the antenatal care plan of women at risk of red cell alloimmunization in the United Kingdom. The technique has a high diagnostic accuracy, with a sensitivity of 99.94% and specificity of 97.74% [16]. With this practice, only rhesus-D negative women carrying rhesus-D positive babies are exposed to Anti-D immunoglobulin in pregnancy. Evidence from Bristol concluded that this policy is cost-effective and has reduced the need for RAADP in rhesus-D negative women by 29%. It is anticipated that the high accuracy of NIPD for fetal blood group may soon eliminate the need for routine cord blood testing of babies born to rhesus-D negative completely, with further savings in manhour and cost [16]. In line with this, the use of cfDNA has been recently introduced into the routine antenatal care package line at the LTHT.

Conclusion

Red cell antibody in pregnancy remains a significant cause of iatrogenic preterm delivery and fetal morbidity. While the anti-M and Anti-E antibodies were the predominant antibodies in pregnancy, the anti-D antibody remains the most significant cause of severe fetal anaemia. The use of MCA velocimetry remains reliable for monitoring fetuses at risk of anaemia in pregnancy. Intensive monitoring of alloimmunized women in pregnancy is recommended to facilitate early diagnosis, treatment and prevention of morbidity.

Funding

The authors did not receive any funding for this reseach

Author's contribution

IA, KC, JR and CS all contributed to study conception, design and data collection. All the authors reviewed the manuscript and made critical contributions to the final manuscript.

Acknowledgement

None.

References

[1] Bowman J. Thirty-five years of Rh prophylaxis. Transfusion 2003;43:1661–6.
[2] Qureshi H, Massey E, Kirwan D, Davies T, Robson S, White J, et al. BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn. Transfus Med 2014;8–20.
[3] Kumpel BM. Lessons learnt from many years of experience using anti-D in humans for prevention of RhD immunization and haemolytic disease of the fetus and newborn. Clin Exp Immunol 2008;154(1):1–5.
[4] Royal College of Obstetricians and Gynaecologists. The management of women with red cell antibodies during pregnancy. London: RCOG; 2014.
[5] Chapman B, Duguid Kelsey, Knowles Murphy, Williamson, et al. Addendum for guidelines for blood grouping and red cell antibody testing during pregnancy. Transfusion Med 1999;9(1):99.
[6] White J, Qureshi H, Massey E, Needs M, Byrne G, Daniels G, et al. Guideline for blood grouping and red cell antibody testing in pregnancy. Transfus Med 2016;26:246–63.

[7] Howard H, Martlew V, McFadyen I, Clarke C, Duguid J, Bromilow I, et al. Consequences for fetus and neonate of maternal red cell allo-immunisation. Arch Dis Child Fetal Neonatal Ed 1998;78:F62–6.
[8] Filbey D, Hanson U, Wesström G. The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: a 12 year study in central Sweden. Acta Obstet Gynecol Scand Suppl 1995;74(9):687–92.
[9] National Institute for Health and Care Excellence. Routine antenatal anti-D prophylaxis for women who are rhesus D negative. NICE; 2008.
[10] National Institute for Health and Clinical Excellence. Full guidance on the use of routine antenatal anti-D prophylaxis for Rh D negative women. NICE; 2002.
[11] Pilgrim H, Lloyd-Jones M, Rees A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. NIHR health technology assessment. .
[12] Nordvall M, Dziegiel M, Hegaard HK, Bidstrup M, Jonsbo F, Christensen B, et al. Red blood cell antibodies in pregnancy and their clinical consequences: synergistic effects of multiple specificities. Transfusion 2009;49(10):2070–5.
[13] Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ, et al. Noninvasive Diagnosis by Doppler Ultrasonography of Fetal Anemia Due to Maternal Red-Cell Alloimmunization. N Engl J Med 2000;342:9–14.
[14] Maxwell DJ, Johnson P, Hurley P, Neales K, Allan L, Knott P. Fetal blood sampling and pregnancy loss in relation to indication. Br J Obstet Gynaecol 1991;98 (9):892–7.
[15] Draper ES, Gallimore ID, Kurinczuk JJ, Smith PW, Boby T, Smith LK, et al. MBRRACE-UK perinatal mortality surveillance report, UK perinatal deaths for births from January to December 2016. The Infant Mortality and Morbidity Studies, Department of Health Sciences: University of Leicester; 2018.
[16] Soothill PW, Finning K, Latham T, Wreford-Bush T, Ford J, et al. Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. Brit J Obstet Gynaecol. 2015;122:1682–6.
[17] Kent J, Farrell AM, Soothill P. Routine administration of Anti-D: the ethical case for offering pregnant women fetal RHD genotyping and a review of policy and practice. BMC Pregnancy Childbirth 2014;14:87.