A Pilot Prospective Study of Fetomaternal Hemorrhage Identified by Anemia in Asymptomatic Neonates

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Abstract

Background—Fetomaternal hemorrhage (FMH) is a poorly understood condition in which fetal erythrocytes transfer to the maternal circulation via a faulty placental barrier. Little is known about the true incidence, epidemiology, or pathophysiology of FMH in the general pregnant population as existing studies are based on retrospective cohorts and manifest diagnosis and selection bias.

Objective—To evaluate the practicability of a prospective study of fetomaternal hemorrhage in the general population based on antepartum maternal blood testing and neonatal anemia.

Study Design—Prospective cohort study.

Result—Nineteen pregnant women were enrolled prior to the term delivery of twenty well infants. Five neonates were unexpectedly anemic on first postnatal testing. Antenatal maternal blood samples associated with 2 of 5 anemic newborns had positive Kleihauer-Betke testing while no newborn with a normal postnatal blood count had an associated abnormal Kleihauer-Betke test.

Conclusion—Clinically significant FMH may be more common than previously thought. Prospective epidemiological study of FMH is feasible.

Keywords

fetomaternal hemorrhage; neonatal anemia; perinatal epidemiology; prospective cohort

INTRODUCTION

Fetomaternal hemorrhage (FMH) results from a pathologic failure of separation between the fetal and maternal circulations. Although clinically insignificant fetal-to-maternal blood transfer occurs in a majority of pregnancies during delivery,(1) pathologic FMH prior to the onset of labor is believed to be a rare event.(2–5)

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The focus of FMH research has historically been the mother, with detection goals aimed at limiting sensitization to the blood group D antigen in D-mismatched fetal-maternal pairs.(1, 6–12) Definitions of severity of FMH are derived from the estimated volume of blood transferred, with the goal of administering adequate rho(D) immune globulin to the postpartum mother to prevent isoimmunization in future pregnancies. Although FMH is not a cause of direct maternal morbidity or mortality, FMH can cause significant morbidity or mortality to the fetus. The clinical impact of FMH on the fetus/neonate is not strictly related to the volume of blood lost, and therefore does not correlate with existing classifications of mild/moderate/severe FMH. This is because the time course of the hemorrhage is critically import to the fetus; a large volume of blood lost over a long period of time can be well-tolerated due to functional compensatory mechanisms of fetal hematopoiesis and intravascular volume regulation, while a smaller volume of blood lost acutely can be devastating.(4, 5, 13–15)

Diagnosis of FMH requires specific blood testing of the mother – the Kleihauer-Betke acid elution test (KB)(16) or flow cytometry for fetal cells in the maternal circulation – not routinely performed in the perinatal period. Change in alpha-fetoprotein (AFP) level over the course of pregnancy has also been suggested as a potential biomarker for FMH,(17) but AFP level is not regularly ascertained in the third trimester. Fetomaternal hemorrhage is therefore most commonly diagnosed after an adverse fetal or neonatal event has occurred, indicating the need for testing.(5, 18) This raises the possibility of significant under-diagnosis of mild to moderate cases of the disease.(4, 5, 18) If perinatal care providers do not order FMH testing in response to neonatal anemia identified soon after birth, the diagnosis is missed. Making the correct diagnosis of FMH is important for risk stratification of the affected neonate, for family planning and increased obstetric surveillance in future pregnancies, and to establish epidemiologic predictors for the condition. As anemia in infancy is associated with deficits in executive functioning, attention, school performance, social success and emotional health from middle childhood through adulthood,(19, 20) detection of congenital anemia related to FMH could be clinically important to the affected child.

As previous studies of FMH resulting in neonatal harm have relied on retrospective evaluation of clinically identified cases, FMH has been categorized as a rare condition, unsuitable for prospective study. However, it is now clear that retrospective studies suffer from significant bias related to under-diagnosis of the condition.(4, 5) Prospective studies are needed to establish the epidemiology of FMH as early identification of pregnancies at risk for FMH could significantly reduce fetal/neonatal harm. If identified prior to the onset of labor, fetal/neonatal illness due to anemia from FMH may be successfully managed by intrauterine fetal transfusion and/or labor-free delivery.(21–23) If epidemiological predictors of FMH were known, targeted screening could be used to identify pregnancies that would benefit from intervention.

We conduced a pilot prospective study of FMH identified in asymptomatic mother-baby pairs. Blood testing for FMH was performed on maternal specimens collected at term prior to the onset of labor or during early labor. The study was designed to pilot practicability of the observational protocol, with the assumption that studying mother-baby pairs with mild FMH can provide important insight into those rarer pregnancies affected by severe FMH.

Based on our prior retrospective work,(4, [5]) our hypothesis was that occult but clinically significant FMH is more common than previously thought, such that a prospective epidemiological study of FMH is feasible.

SUBJECTS AND METHODS

Women presenting to the labor floor of our urban teaching hospital for imminent delivery between 34-0/7 and 41-6/7 weeks gestation were eligible. Imminent delivery was defined as anticipated birth of the fetus within 48 hours of presentation to the labor floor. Women with a prenatal diagnosis of any fetal anomaly were excluded. Women unable to complete the consent process due to advanced labor or fetal distress requiring immediate intervention were excluded. Each woman was enrolled in the study, including formal written informed consent, prior to antepartum blood draw, such that a study blood specimen could be obtained by clinically indicated phlebotomy. Study blood specimens were banked for batch testing after the birth of the baby. This study was approved by the Icahn School of Medicine Program for the Protection of Human Subjects and registered at ClinicalTrials.gov prior to enrollment of the first participant (NCT01232387).

Neonates born to enrolled mothers had a complete blood count and reticulocyte count ascertained with first clinically indicated phlebotomy after birth. Maternal blood obtained prior to delivery was tested for the presence of fetal cells by KB testing, the FMH laboratory test available at our hospital, for all neonates found to be anemic and a statistical subset of those found not to be anemic. Alpha-fetoprotein (αFP) level was also measured in the antepartum blood specimen.(24) The obstetrical medical record was abstracted for clinical information about the pregnancy including second trimester αFP level and antepartum maternal complete blood count. Enrolled women completed a survey focused on demographic, family, and lifestyle factors that could have impacted the pregnancy.

RESULTS

The clinical and demographic characteristics of the study population are presented in Tables 1–3. Study families were majority white, educated, and of high socioeconomic status. All study subjects had good prenatal care and delivered viable term newborns. No study mother-baby pair demonstrated the pre-determined clinical indicators of anemia unrelated to FMH: maternal vaginal bleeding in the third trimester, positive maternal Coombs test, known isoimmunization, cord compression at the time of delivery (prolapsed cord, tight cord around the neck or body), or neonatal blood loss (unclamped cord, laceration). All newborns were cared for in the level I nursery after birth, and all were discharged home with the mother. Due to the method of patient recruitment, planned Cesarean birth was common. Five neonates were found to be anemic based on published gestational age appropriate norms. (25) A positive KB test on antenatal maternal blood was associated with neonatal anemia in 2 of 5 anemic neonates. No neonate with a normal hemoglobin concentration had a positive KB test.

Details of the two cases marked by positive KB testing are as follows:

Case 1

A 42 year-old G2P1001 woman presented for pre-operative testing the day before her scheduled repeat Cesarean delivery with known breech presentation of the fetus. She had a known unicornuate uterus; her first pregnancy ended with Cesarean delivery for breech presentation 40 months prior to the index delivery. Family history was remarkable only for type I diabetes mellitus of the baby's father. The pregnancy was achieved by spontaneous conception. Vaginal bleeding occurred at 7 weeks gestation and a subchorionic hematoma was noted on sonogram. Both bleeding and the hematoma resolved without intervention. Genetic testing via chorionic villus sampling and anatomy scan were normal. Maternal blood type was O+, antibody negative. Blood for FMH testing was drawn. Antepartum maternal hemoglobin and hematocrit were normal, 12.2 g/dL and 35.7% respectively.

Delivery occurred at 39-1/7 weeks gestation. There were normal fetal movements and painless irregular uterine contractions. Fetal heart tracing was category I. The fetus was breech and the placenta was posteriorly placed. A low transverse Cesarean delivery was performed. The male neonate emerged vigorous. Apgar scores were 9 at 1 and 5 minutes. Birth weight was 2.81kg (11th percentile), head circumference was 34cm (8th percentile), and length was 47cm (34th percentile). The baby's blood type was O+ and Coombs test was negative.

The baby had mild asymptomatic anemia with hemoglobin 14.8 g/dL and hematocrit 43.2% on day of life (DOL) 3. The platelet count was 292×10^3 per μL and reticulocyte count was 4.5%. KB testing of the antepartum maternal blood sample was positive with 0.05% fetal cells present. Antepartum αFP was 163.1 ng/mL, compared to the second trimester value of 38.8 ng/mL. The postpartum and neonatal hospital courses were unremarkable.

Case 2

A 32 year-old G2P1001 woman presented for pre-operative testing the day before her scheduled repeat Cesarean delivery with vertex presentation of the fetus 51 months after an uncomplicated primary Cesarean delivery at term. Family history was unremarkable. The pregnancy was achieved by spontaneous conception. It progressed to term without complication. Anatomy scan in the second trimester was normal. No genetic testing was completed. Maternal blood type was B+, antibody negative. Blood for FMH testing was drawn. Antepartum maternal hemoglobin and hematocrit were normal, 10.3 g/dL and was 32.1% respectively.

Delivery occurred at 39-1/7 weeks gestation. There were normal fetal movements and painless irregular uterine contractions. Fetal heart tracing was category I. The fetus was vertex and the placenta was anteriorly placed. A low transverse Cesarean delivery was performed. The female neonate emerged vigorous. Apgar scores were 9 at 1 and 5 minutes. Birth weight was 3.04kg (24th percentile), head circumference was 33.5cm (27th percentile), and length was 50cm (46th percentile). The baby's blood type was B+ and Coombs test was negative.

The baby had mild asymptomatic anemia with hemoglobin 14.9 g/dL and hematocrit 45.8% on DOL 3. The platelet count was 292×10^3 per μL and reticulocyte count was 4.0%. KB

testing of the antepartum maternal blood sample was positive with 0.14% fetal cells present. Antepartum αFP was 92.3 ng/mL, compared to the second trimester value of 24.5 ng/mL. The postpartum and neonatal hospital courses were unremarkable.

DISCUSSION

This pilot study challenges the belief that clinically significant FMH is a rare event. Previous population-based studies relying on retrospective clinical data indicate an incidence of clinically significant FMH of 1–2 per 10,000 births.(2, [3], [5]) These studies all rely on a diagnosis of FMH made in the clinical setting of extreme neonatal compromise based on KB testing of maternal blood obtained postpartum. The present study, in contrast, evaluated "normal" term pregnancies in which the newborn had occult anemia with no clear etiology. We found FMH resulting in neonatal anemia in 2 in 10 mother-baby pairs.

Unlike neonates described in previous studies, the neonates in this report were well-appearing. Mild congenital anemia such as demonstrated by our cases, however, is not benign. It is well-known that iron-deficiency anemia in infancy, a significant risk for those with perinatal blood loss, is associated with decrements in cognitive and behavioral function, school performance, and social and professional achievement into adulthood in a dose-dependent manner.(19, [20]) Although the level of anemia seen in this study is mild, frequently occurring mild to moderate congenital anemia could have important implications on a population level.(26–[28])

Our study demonstrates the feasibility of conducting a prospective epidemiological study of FMH in the general pregnant population using KB testing. Although, antepartum phlebotomy eliminated potential confounding by blood transfer at placental separation, change in AFP over pregnancy was not significantly different between cases and controls, making AFP an ineffective biomarker for FMH diagnosis in our population. If the incidence of FMH seen in our study is confirmed in larger populations, the study sample size needed to identify predictors of FMH would not be prohibitive. Epidemiological, molecular, and pathological data gained from such a study could significantly improve our understanding of the pathophysiology of GMH and positively impact our ability to target screening to populations at risk for FMH.

Although based on a small population, this pilot prospective study corroborates previous retrospective work indicating that FMH resulting in neonatal morbidity is a rarely diagnosed, rather than rarely occurring event.(4, [5]) Prospective study of FMH is urgently needed to better define clinical and demographic risk factors for FMH that could enable early detection. Identification of neonates at risk for congenital anemia could enable interventions to both reduce the significant morbidity and mortality associated with severe FMH, and address mild congenital anemia prior to the emergence of more subtle deficits.

Acknowledgments

Abbreviations

αFP	Alpha-fetoprotein
DOL	day of life
FMH	fetomaternal hemorrhage
KB	Kleihauer-Betke acid elution test

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Table 1

Parental/Family Characteristics

	Mother	Father
Age, years	35.4 [28–42]	35.5 [29–43]
Educational Attainment		
middle school	1 (5.9)	0 (0)
high school	0 (0)	0 (0)
some college	0 (0)	0 (0)
completed college	7 (41.2)	7 (43.8)
graduate school	9 (52.9)	9 (56.2)
Ethnicity		
White	12 (63.2)	13 (81.3)
Black	1 (5.3)	1 (6.3)
Hispanic	2 (10.5)	1 (6.3)
Asian	2 (10.5)	1 (6.3)
Mixed	2 (10.5)	0 (0)
Annual Income		
<$25K	1 (6.3)	
$25–50K	0 (0)	
$50–100K	2 (12.5)	
100–200K	3 (18.7)	
>$200	10 (62.5)	

Number (percent) or mean [range] as appropriate

Some participants declined to provide some demographic information

Table 2

Pregnancy Characteristics

Maternal blood type Rh+	19 (100)
Maternal antepartum hemoglobin, g/dL	11.9 [8.7–14.0]
Maternal antepartum hematocrit, %	35.0 [25.3–40.3]
Median second trimester αFP, ng/mL (range)	25.2 [10.5–50.1]
Median antepartum αFP, ng/mL (range)	154.5 [67.5–334.5]
Median fold increase in αFP during pregnancy (range)	4.7 [1.15–22]
Uterine anomaly	1 (0.05)
Placental abruption	0 (0)
Maternal illicit drug or tobacco use	0 (0)
Twin gestation	1 (5)
Length of gestation, weeks	39.0 [37.6–41.1]
Cesarean birth	15 (78.9)
Scheduled Cesarean birth	12 (63.2)
Mother-fetus ABO incompatibility	2 (10)
Positive Kleiheur-Betke test	2 (10)

Number (percent) or mean [range] as appropriate unless otherwise specified

Table 3

Neonatal Characteristics

Ethnicity	
White	9 (56.3)
Black	1 (6.3)
Hispanic	1 (6.3)
Asian	1 (6.3)
Mixed	4 (25.0)
Male	10 (50)
Birth weight, kg	3.32 [2.55–4.28]
Birth weight percentile	48 [5–95]
Birth length, cm	50.7 [47.0–54.5]
Birth length percentile	58 [8–55]
Birth head circumference, cm	34.7 [32.5–37.5]
Birth head circumference percentile	55 [9–99]
Median Apgar score at 1 minute (range)	9 [7–9]
Median Apgar score at 5 minutes (range)	9 [9-9]
Hemoglobin, g/dL	18.3 [12.9–21.9]
Hematocrit, %	53.3 [36.8–64.0]
Reticulocyte %	4.4 [3.1–9.4]

Number (percent) or mean [range] as appropriate unless otherwise specified