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The role of folate receptor autoantibodies in preterm birth

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ABSTRACT

Objective: Cellular uptake of folate is mediated by folate receptor (FR)α. Prior studies indicate that a FRα autoantibody (FRAb) is implicated in poor pregnancy outcomes. The aims of this study were to determine the prevalence of FRAbs in women with preterm and term pregnancies, and to investigate the role of maternal FRAbs in preterm birth.

Methods: This prospective observational study included 23 mothers and 25 preterm infants (two twin births) born at gestational age (GA) ≤32 wk and/or birth weight ≤1500 g (group 1) and 25 mother–term infant pairs (infants born at GA ≥37 wk, group 2). Blocking and binding FRAbs in maternal and in cord blood were determined. The association between maternal FRAbs and pregnancy outcome was measured using multiple logistic regression, adjusted for maternal age and previous preterm birth.

Results: The prevalence of FRAbs was 65.2% in women with preterm birth, which was twofold higher than in those with term pregnancy (28%; relative risk [RR], 2.3; 95% confidence interval [CI], 1.2–4.7). The prevalence of FRAbs in preterm infants (64%) was significantly higher than in term infants (24%; RR, 2.7; 95% CI, 1.3–5.7). Pregnant women with positive FRAbs had 4.9 times higher odds of having preterm birth (odds ratio, 4.9; 95% CI, 1.4–17.7), adjusted for maternal age and previous preterm birth.

Conclusions: These findings suggest that the presence of FRAbs might be a contributing factor to preterm birth, which could be prevented with appropriate testing and therapeutic interventions. Further studies are warranted to investigate the possible mechanisms of fetal sensitization resulting in FRAb production in utero and its possible clinical correlates.

Introduction

Preterm birth, defined as <37 wk gestation at birth [1], is a major public health concern [1], with reported rates ranging from 5% to 20% worldwide [2,3]. As the consequence of associated comorbidities, prematurity is a major factor contributing to neonatal mortality, with lifelong consequences in surviving infants [1]. Health care costs related to preterm birth and its attendant complications approximate $26.2 billion annually in the United States [1,4].

Folate (folic acid), also known as vitamin B_9, is an essential vitamin exerting important functions in DNA synthesis, DNA repair, and methylation, as well as acting as a cofactor in certain biological reactions [5]. These metabolic functions play a major role in pregnancy and fetal development [5]. Cellular uptake of folate is mediated by folate receptor (FR)α. The transfer, uptake, and binding of folate can be inhibited by the presence of FRα autoantibodies (FRAbs). FRAb-associated decreases in folate bioavailability may result in reduced central nervous system folate levels. The potential consequences include increased rates of central nervous system developmental anomalies (e.g., neural tube defects [NTD]) [6–8], cerebral folate deficiency syndrome [6,9], infantile autism [6,10], and global neurodevelopmental delay [6,7,9]. Periconceptional folate supplementation has shown to decrease the incidence of NTD and improve pregnancy outcome [11–13]. Epidemiologic studies in the United States have

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demonstrated an inverse relationship between low concentrations of serum folate and greater risk for premature delivery and low infant birth weight [14]. Since 1998, when folic acid fortification of flour was initiated, the prevalence of NTD has declined by 19% [15]. Published data also indicate decreased prevalence of low infant birth weight and premature birth following folic acid fortification in the United States [16].

To the best of our knowledge, the effects of suboptimal folate uptake and use during pregnancy resulting from increased FRAb levels have not been examined, to date. In particular, the present study sought to determine whether the presence and concentration of circulating FRAbs in pregnant women contribute to adverse gestational outcomes and/or preterm birth. Additionally, by controlling for other maternal factors known to contribute to preterm labor, an additional aim was to investigate the possible association between maternal FRAbs and preterm birth.

Methods

Participants

This was a prospective observational study. Between March 2012 and November 2013, we enrolled 50 consecutive pregnant women who gave birth at the State University of New York (SUNY) Downstate Medical Center and their infants. Group 1 (preterm birth) included mother–infant pairs with gestational age (GA) ≤32 wk and/or birth weight (BW) ≤1500 g. Group 2 (term birth) included mother–infant pairs with GA ≥37 wk. Mothers with known or suspected immune disorders and their infants were excluded. Ethical approval was obtained from the Institutional Review Board at the SUNY Downstate Medical Center. All women provided written informed consent.

Group 1 comprised 23 mothers and 25 infants (two twin births); the mean GA was 28.5 ± 2.5 wk, and 13 of the 25 (52%) infants were male. The mean BW of all preterm infants was 1188 ± 375 g, and none of these infants were diagnosed with intrauterine-restricted growth (IUGR, defined as estimated fetal weight <10th percentile for GA and fetal abdominal circumference <2.5th percentile [17]). Group 2 comprised 25 mother–term infant pairs; the mean GA was 38.6 ± 1.1 wk, and 11 of the 25 (44%) infants were male. The mean BW of all term infants was 3190 ± 410 g.

Clinical data and blood sample collection

Maternal clinical variables, including demographic characteristics and medical history, were recorded. Maternal and cord blood specimens were collected in tubes containing potassium EDTA, centrifuged at 5000*g* for 5 min, and plasma was stored in aliquots at −80°C until use. All specimens were deidentified after collection. The researcher who performed the assays was unaware of each sample's origin.

Analytical techniques

Blocking and binding FRAbs in maternal and cord blood were determined as previously described [9,18]. Blocking FRAbs are defined as those that block the binding of folic acid when a serum is preincubated with apo-FRα. The titer of these FRAbs in serum was measured by inhibition of radiolabeled folic acid binding to a known amount of purified human milk apo-FRα. The blocking antibody could be either immunoglobulin (Ig) G or IgM, and this method does not identify any specific antibody type. Binding IgG-type FRAbs are defined as antibodies directed against any epitope on the FRα other than the folate-binding site. These FRAbs were quantified by an enzyme-linked immunosorbent assay (ELISA)–based measurement of FRAbs that bind to epitopes on purified human milk apo-FRα [18]. Additionally, as a surrogate for serum folate status in mothers and their infants [19,20], homocysteine (Hcy) concentrations in maternal and cord blood samples were determined using a homocysteine ELISA method (Axis-Shield).

Statistical analysis

Data were analyzed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA), and were reported as the mean ± SD or proportions. Groups were compared using χ^2 test or Fisher's exact test for categorical variables, and using *t* test or Mann-Whitney test for continuous variables. Hcy concentrations in the mothers and their infants were also compared using paired *t* test. Relative risk (RR) and 95% confidence interval (CI) were calculated to measure the degree of relationship between maternal baseline characteristics and the presence of FRAbs. Additionally, the association between maternal FRAbs and pregnancy outcome was measured using multiple logistic regression to estimate the odds ratio (OR) and 95% CI, adjusted for maternal age (≥35 y versus <35 y) and history of preterm birth (yes/no). $P < 0.05$ was considered statistically significant for all tests.

Results

Table 1 describes maternal baseline characteristics in both preterm and term cohorts. All but three mothers in group 2 had been taking prenatal multivitamins during pregnancy; and none reported taking any other forms of vitamin supplementation. All women had normal serum Hcy levels, although the mean of Hcy concentrations was significantly higher in group 2 than in group 1 mothers (Table 1). Among mother–infant pairs whose blood samples were both available for Hcy assay, no significant difference was found in Hcy concentrations between maternal and cord blood in either group (5.96 ± 1.43 versus 5.68 ± 1.86 μmol/L in group 1, respectively, $P = 0.55$; and 7.78 ± 0.72 versus 7.89 ± 1.03 μmol/L in group 2, respectively, $P = 0.69$).

Figure 1 shows the prevalence of FRAbs in maternal blood (Fig. 1A) and in cord blood (Fig. 1B). The prevalence of FRAbs in women with premature birth was greater than twofold, when compared with mothers giving birth at term (65.2% versus 28%, respectively; RR, 2.3, 95% CI, 1.2–4.7). Furthermore, in the preterm birth cohort, women with previous preterm births were 1.7 times more likely to have positive FRAbs (95% CI, 1.2–2.4). Similar to these maternal findings, the prevalence of FRAbs in preterm infants was significantly higher than in term infants (64% versus 24%, respectively; RR, 2.7; 95% CI, 1.3–5.7). However, FRAb titers in maternal and cord blood did not vary significantly between the two groups. Among women with positive FRAbs, the mean of maternal binding and blocking FRAbs (pmol/mL) was 0.52 ± 0.32 and 0.36 ± 0.14 in group 1 and 0.39 ± 0.21 and 0.51 in group 2, respectively (NS). Although binding (IgG-type) FRAb was identified in the cord blood of 4 of 25 (16%) preterm infants compared with none of the term infants, this difference did not reach a level of statistical significance (Fig. 1B; $P = 0.11$). However, significant gestation-related differences were found with respect to the presence of blocking FRAb. Thus, blocking FRAb was present in 77.8% of the preterm infants, and in only 24% of the term infants (Fig. 1B).

The association between maternal FRAbs and preterm birth was examined using multiple logistic regression analysis shown in Table 2. Pregnant women with positive FRAbs had 4.9 times

Table 1
Maternal baseline characteristics in preterm and term cohorts

	Group 1 (preterm birth) (n = 23)*	Group 2 (term birth) (n = 25)	*P* value
Age (y), mean ± SD	28.9 ± 6.5	28.2 ± 6.6	NS
Ethnicity, n (%)			NS
Black	21 (91.4)	25 (100)	
Asian	1 (4.3)	0 (0)	
White	1 (4.3)	0 (0)	
Previous preterm birth, n (%)	3 (13)	2 (8)	NS
Medication use, n (%)			
Prenatal multivitamin	23 (100)	22 (88)	NS
Folate supplement	0 (0)	0 (0)	NS
Anemia during pregnancy, n (%)	13 (56.5)†	4 (16)†	0.006
Hcy levels (μmol/L), mean ± SD (range)	6.17 ± 1.46 (2.6–8.1)	7.78 ± 0.72 (6.3–9.1)	<0.001

* 23 mothers (two twin births).
† None were diagnosed with macrocytic anemia.