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Original Article

Contribution of Histologic Chorioamnionitis and Fetal Inflammatory Response Syndrome to Increased Risk of Brain Injury in Infants With Preterm Premature Rupture of Membranes

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ABSTRACT

BACKGROUND: To determine the association of histologic chorioamnionitis (HCA) and fetal inflammatory response syndrome (FIRS) with brain injuries in infants born to mothers with preterm premature rupture of membranes. **METHODS:** A total of 103 singleton infants born to mothers with preterm premature rupture of membranes were enrolled. The placental inflammation was confirmed by HCA, and FIRS was defined in fetuses with preterm labor and an elevation of the fetal plasma interleukin-6 concentration. Examination of brain images was conducted to confirm the existence of brain injuries. Based on placental HCA and umbilical cord blood interleukin-6 level, all patients were divided into three groups: $HCA^{-}FIRS^{+}$, $HCA^{+}FIRS^{-}$, and $HCA^{+}FIRS^{+}$. **RESULTS:** Among all infants with preterm premature rupture of membranes, 53.40% were exposed to HCA, 20.38% experienced FIRS, and the overall incidence of brain injuries was 38.83%. The incidence of brain injury in $HCA^{-}FIRS^{+}$, $HCA^{+}FIRS^{-}$, and $HCA^{+}FIRS^{+}$ groups were 20.83%, 41.18%, and 76.19%, respectively. HCA at the advanced grades and stages was associated with increased risk of brain injury. Umbilical cord blood levels of interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and granulocyte-colony stimulating factor (G-CSF) in premature infants with brain injuries were significantly higher than in those without brain injuries. Infants diagnosed with both HCA and FIRS showed significantly higher levels of IL-8, TNF-α, and G-CSF than those with HCA alone. **CONCLUSIONS:** Preterm infants exposed to severe chorioamnionitis had an increased risk of brain injury. IL-6, IL-8, TNF-α, and G-CSF in cord blood were associated with brain injuries in preterm infants and may be used as extradiagnostic criteria.

Keywords: histologic chorioamnionitis, fetal inflammatory response syndrome, brain injury, premature infants, cytokine

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Introduction

The incidence of premature birth was 7.6% to 12% of all births in developed countries, and ≥15% of all births in low- to middle-income countries. Alarmingly, the incidence of premature birth continues to rise.[1,2] Substantial evidence indicates that intrauterine infection is a common cause of preterm birth.[3,4] Intrauterine infection is found in approximately 40% of patients with preterm premature rupture of membranes (PPROM) and is a risk factor for impending preterm delivery and adverse pregnancy and neonatal outcomes.[5] Fetal inflammatory response syndrome (FIRS) is a condition featured by systemic activation of the fetal innate immune system, defined as a fetal plasma interleukin-6 (IL-6) concentration greater than 11 pg/mL. IL-6 can elicit biochemical, physiologic, and immunologic changes, including stimulation of C-reactive protein production, activation of the acute-phase plasma protein response and T-cell and natural killer cell responses. Affected fetuses have multiorgan involvement, with a higher morbidity rate after gestational age adjustment, and are more likely to have a subsequent spontaneous preterm delivery in cases of preterm PROM.[6] Maternal inflammation (chorioamnionitis) is often followed by systemic FIRS.[7] This inflammation signal is likely transmitted across the blood–brain barrier and

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initiates a neuroinflammatory response.[8,9] Studies have found that the risk of brain injuries, such as severe intraventricular hemorrhage (IVH) or periventricular leukomalacia (PVL), was significantly increased in preterm infants exposed to chorioamnionitis.[10,11] However, the association of combined placental and fetal inflammation with brain injuries in preterm infants has not been well investigated. In the present study, we aim to explore the association of placental and fetal inflammation with brain injury in preterm infants by examining placental histopathologic and proinflammatory cytokine profiling, thus providing clinical guidance for the prevention and early diagnosis of brain injuries in preterm infants.

Materials and Methods

Study population

The study population consisted of a prospective cohort of singleton preterm infants born at the Affiliated Hospital of Jiangsu University (Zhenjiang, China), from August 2012 to October 2013. The infants were eligible if they were delivered earlier than 34 weeks with PPROM. Exclusive criteria comprised the following: (1) maternal complications such as gestational diabetes, pregnancy-induced hypertension syndrome, placental abruption, placenta previa, and pregnancy-complicating heart disease; (2) clinical evidence of inherited metabolic diseases, neurological or other malformations. All studies were approved by the ethics committee of the Affiliated Hospital of Jiangsu University, and informed consent was obtained from the parent or legal caregiver of each infant.

Histologic examinations

The placentas were examined according to routine protocol. Standard sections for each placenta included the chorion amnion, the chorionic plate, and the umbilical cord. Samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of tissue blocks were stained with hematoxylin and eosin. Two pathologists who had no knowledge of clinical information reviewed the slides.

Definitions and study procedures

Maternal and infant clinical data were collected from the infants chart from enrollment to hospital discharge. Gestational age was estimated by means of menstrual history and ultrasound examinations performed before 14 weeks of gestation. Maternal chorioamnionitis was diagnosed histologically by pathology. PPROM was diagnosed when membrane rupture occurred before the onset of spontaneous labor by visualization of amniotic fluid loss. Maternal steroids included two doses of dexamethasone, which were administered parenterally to induce lung maturation. All preterm infants received their first cranial ultrasonography examination after admission, then subsequent examinations at least once a week until discharge to their local hospital, and their last examination was at 40 weeks postmenstrual age. Cranial magnetic resonance imaging scans were carried out to further confirm the presence of brain injury for those who displayed no abnormalities in cranial ultrasound imaging. Preterm infants with either IVH or PVL were diagnosed with brain injury. IVH was classified based on a previous study by Papile et al.[12] in four grades: grade I, hemorrhage restricted to the germinal matrix; grade II, IVH without ventricular dilatation; grade III, IVH with ventricular dilatation; and grade IV, parenchymal hemorrhage. PVL was graded as described previously[13]: grade I, periventricular areas of increased echogenicity present for $\geq$7 days; grade II, periventricular areas of increased echogenicity evolving into small localized frontoparietal cysts; grade III, periventricular areas of increased echogenicity evolving into extensive periventricular cystic lesions involving the occipital and frontoparietal white matter; and grade IV, areas of increased echogenicity in the deep white matter evolving into extensive subcortical cysts.

Umbilical cord blood cytokine measurement

Umbilical vein blood was obtained from all infants and centrifuged at 2000 rpm for 10 minutes at 4°C within 2 hours after birth. Serum was collected and stored at −80°C for cytokine measurements. Serum levels of IL-6, interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and granulocyte-colony stimulating factor (G-CSF) were measured using a liquid chip kit (Merck Millipore) in accordance with the manufacturer's instructions.

Histologic chorioamnionitis and FIRS diagnostic criteria

Histologic chorioamnionitis (HCA) was diagnosed, staged, and graded based on the criteria described previously with slight modifications.[14] HCA was defined as neutrophil infiltration of amniotic membranes, umbilical cord, or chorionic plate. Based on the number of neutrophil infiltrations into the amnion, chorion, and decidua, the grade of HCA ranged from I to III (grade 0 is less than 5 neutrophils, grade I is 5 to 9 neutrophils; grade II is 10 to 19 neutrophils; grade III is greater than 20 neutrophils, all recorded at ×20). The stage of HCA ranged from I to III (0 is none, 1 is neutrophils collecting in the subchorionic space, 2 is neutrophils into the chorionic plate, 3 is neutrophils up to the amnionic epithelium). FIRS was diagnosed based on criteria described previously[6]: umbilical cord blood IL-6 greater than 11 pg/mL. Based on the placenta histopathology and umbilical cord IL-6 levels, all the patients were further divided into three subgroups: $HCA^{-}FIR^{-}$ (infants without HCA and umbilical cord blood IL-6 less than 11 pg/mL), $HCA^{+}FIR^{-}$ (infants diagnosed with HCA and umbilical cord blood IL-6 less than 11 pg/mL), and $HCA^{+}FIR^{+}$ (infants diagnosed with HCA and umbilical cord blood IL-6 greater than 11 pg/mL).

Statistical analysis

Continuous data are presented as mean ± S.D.; dichotomous data are expressed by frequency and associated percentage. One-way analysis of variance was used for comparison among all different groups. If the analysis of variance was significant, SNK-q testing of differences between groups was performed using the least significant difference multiple comparison test. Categorical and nominal values were compared using the chi-square test or the Fisher exact test where appropriate. The umbilical cord blood cytokine levels of the brain injury group and the levels of those who exhibited no brain damage were compared using a *t* test. To assess predictors of placental inflammation, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression. ORs were considered to represent a significant association if the CIs did not include 1.00. Significance was accepted at $P < 0.05$ (two sided). All analyses were performed using SPSS version 11.6 software.

Results

Clinical characteristics of the premature infants

Of 103 preterm children enrolled in this study, 61 were male and 42 were female; average birth weight was 2105.9 ± 475.9 g; mean gestational age was 32.8 ± 1.6 weeks. General information is detailed in Table 1. Based on placental histopathologic examination and umbilical cord blood levels of IL-6 described in Table 2, 55 (53.4%) were confirmed to have HCA, 21 (20.38%) were diagnosed with FIRS (20.38%), 48 (46.60%) had $HCA^{-}FIRS^{-}$ (46.60%), 34 (33.01%) had $HCA^{+}FIRS^{-}$, and 21 (20.39%) were afflicted with $HCA^{+}FIRS^{+}$. No statistical significances were observed in sex, birth weight, gestational age, antenatal steroids, and delivery mode among the three groups.

The association of preterm brain injuries with HCA grading and staging

Incidences of preterm brain injury in infants with HCA grades I, II, and III were 40% (10 of 25), 61.11% (11 of 18), and