Does Placental Inflammation Relate to Brain Lesions and Volume in Preterm Infants?

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Objectives To evaluate the association between histologic inflammation of placenta and brain findings in ultrasound examinations and regional brain volumes in magnetic resonance imaging in very-low-birth-weight (VLBW) or in very preterm infants.

Study design VLBW or very preterm infants (n = 121) were categorized into 3 groups according to the most pathologic brain finding on ultrasound examinations until term. The brain magnetic resonance imaging performed at term was analyzed for regional brain volumes. The placentas were analyzed for histologic inflammatory findings.

Results Histologic chorioamnionitis on the fetal side correlated to brain lesions in univariate but not in multivariate analyses. Low gestational age was the only significant risk factor for brain lesions in multivariate analysis ($P < .0001$). Histologic chorioamnionitis was not associated with brain volumes in multivariate analyses. Female sex, low gestational age, and low birth weight z score correlated to smaller volumes in total brain tissue ($P = .001$, $P = .0002$, $P < .0001$, respectively) and cerebellum ($P = .047$, $P = .003$, $P = .001$, respectively). In addition, low gestational age and low-birth-weight z score correlated to a smaller combined volume of basal ganglia and thalami ($P = .0002$).

Conclusions Placental inflammation does not appear to correlate to brain lesions or smaller regional brain volumes in VLBW or in very preterm infants at term age. *(J Pediatr 2008;152:642-7)*

Up to 40% to 80% of very preterm infants have been exposed to infection at the time of delivery.[1,2] Accordingly, intrauterine infection may be one important causal factor underlying the process leading to very preterm delivery.[1-4] Exposure to infection has also been reported as an independent risk factor of brain injury in preterm neonates.[5-9]

Maternal fever, clinical and histologic chorioamnionitis, and funisitis increase the infant's risk for neurologic deficits such as cerebral palsy in term infants.[10-12] Funisitis, a fetal response to chorioamnionitis, correlated with germinal matrix/intraventricular hemorrhages (IVH), although no correlation between histologic chorioamnionitis and abnormal brain findings was found.[13] The preterm infant brain may be particularly vulnerable to the deleterious effects of chorioamnionitis.[5] For example, up to 20% of the preterm infants exposed to chorioamnionitis develop periventricular leukomalacia.[14] Furthermore, clinical and histologic chorioamnionitis was associated with IVH,[5,6,15,16] white matter lesions,[5,7,8,17,18] and cerebral palsy.[19-22]

Even though clinical chorioamnionitis has an impact on the outcome of preterm, as well as term infants, the role of histologic chorioamnionitis is more unclear and controversial. There are studies where no association was found between histologic chorioamnionitis and brain lesions[23-25] or neurologic outcome of preterm infants.[26,27] However, other studies reported that inflammatory placental lesions increase the risk for brain injury both in term[10] and preterm infants.[6,7,15,17,21] High levels of cord blood proinflammatory cytokines correlate to brain damage.[16] Recent research[28] exploring genotypes enhancing cytokine responses also suggest that cytokines may have a role in the process leading to brain damage in preterm infants.

In several studies, preterm infants examined at term have smaller regional cerebral and cerebellar volumes compared with term infants.[29-31] For example, infants born preterm have been reported to have decreased volumes of the cortical gray matter, particularly in sensorimotor and parietooccipital regions of the brain[31] and in deep nuclear gray matter.[29] The risk for reduced brain volumes was associated with decreasing gesta-

CRP	C-reactive protein	PROM	Premature rupture of membranes
IVH	Intraventricular hemorrhage	VLBW	Very low birth-weight
MRI	Magnetic resonance imaging		

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Table I. Characteristics of the study infants (n = 121) and excluded infants (n = 71)

Characteristics	Study infants	Excluded infants
Male	69 (57%)	32 (45%)
Singleton	90 (74%)	52 (73%)
Twins	25 (21%)	19 (27%)
Triplets	6 (5%)	0 (0%)
Caesarean section	76 (63%)	36 (51%)
Gestational age (weeks)	28 ± 4 (2 ± 6) [22 ± 5, 36 ± 1]	28 ± 1 (3 ± 3) [22 ± 6, 35 ± 1]
Birth weight (g)	1047 (306) [485, 1820]	1059 (434) [384, 2070]
Birth weight z score	−1.47 (1.48) [−4.7, 2.2]	−1.33 (1.68) [−4.6, 3.4]
Birth weight z score < −2	39 (32%)	17 (24%)
Apgar at 5 min < 5	19 (16%)	24 (34%)
Severe preeclampsia	26 (21%)	10 (14%)
Group B *Streptococcus* colonization of the mother (n = 71)	27 (22%)	10 (14%)
Maternal clinically diagnosed infection	35 (29%)	25 (35%)
Temperature at delivery > 38° C	7 (6%)	2 (3%)
Premature rupture of membranes >18 h	33 (27%)	14 (20%)
CRP > 40 mg/L during 7 days before delivery[35]	19 (16%)	13 (18%)
Neonatal steroid treatment	13 (11%)	6 (8%)
Neonatal death	7 (6%)	19 (27%)

Means (SD) and [minimum, maximum] are presented for gestational age, birth weight, and birth weight z-score.

tional age[29,30] and brain lesions.[30,32-34] Perinatal factors leading to the reduction in brain volumes in premature infants are not well known. Our hypothesis was that chorioamnionitis could be one of the factors disrupting the normal brain development and leading to loss in brain volume. The aim of this study was to evaluate the association between histologic inflammation of placenta and lesions and regional volumes of the brain in VLBW or very preterm infants.

METHODS

Patient Population

This study is a part of a larger multidisciplinary project PIPARI (Development and Functioning of Very Low Birth Weight Infants from Infancy to School Age). Inclusion criteria for the study were (1) infant's birth weight ≤1500 g or gestational age below 32 weeks, (2) infant's parents spoke and understood written Finnish or Swedish, and (3) families lived inside the hospital catchment area. The exclusion criteria were (1) the placental sample was not available and (2) brain imaging was not available in surviving infants. 72 infants were excluded because of missing placenta (n = 71) or brain imaging studies (n = 1). The final study population consisted of 121 infants born between January 2002 and March 2006 in Turku University Central Hospital, Turku, Finland. Parental consent was obtained after verbal and written information. This study was approved by the Ethical Committee of the Hospital District of the South-West Finland in June 2001.

The clinical data (Table I) were collected prospectively as a part of the PIPARI protocol. Gestational age was estimated according to the first-trimester ultrasonography performed routinely in Finland. The mother was defined as having a clinical infection if her C-reactive protein (CRP) value was pathological, she had fever, or premature rupture of membranes (PROM) more than 18 hours before the delivery. If PROM was the only sign of clinical infection without histologic chorioamnionitis, the mother was excluded from the clinical infection group. CRP, shown to be reliable in detecting chorioamnionitis,[35-38] was measured only for clinical suspicion of an infection (n = 63). PROM was included because it correlates with histologic chorioamnionitis,[39,40] and it gives an opportunity for an infection to spread from the lower genital tract into the fetal membranes and amniotic fluid.[41]

Histologic Analysis

The placenta was routinely inspected by a midwife or an obstetrician after birth and thereafter immediately immersed in 10% aqueous solution of formalin for fixation. The size, weight, insertion of umbilical cord, and possible gross pathologic features were recorded. In the pathology laboratory, one of the authors (H.K.) investigated the placenta, the membranes, and the umbilical cord and sampled tissue for the histologic process. At least 2 nonconsecutive sections were studied from every placenta, umbilical cord, and fetal membranes. In the case of twin or triplet pregnancies, all placentas, cords, and membranes were sampled. Extra samples were taken if warranted by macroscopic impression. The histologic specimens were prepared in a routine manner through an ascending series of ethanol, xylene, and a descending series of ethanol and embedded in paraffin. Tissue sections of 3 to 5 μm were cut and stained with hematoxylin and eosin.

All 121 specimens were analyzed by 2 authors (M.R., H.K.) blinded to clinical and neuroradiologic findings. Chorioamnionitis was divided into maternal and fetal components, and each case was assigned a stage and a grade as described by Redline et al[42] (see definitions in Appendix 1;