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Maternal and fetal oxidative stress and intrapartum term fever

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Abstract

OBJECTIVE—The association between maternal chorioamnionitis and fetal oxidative stress has not been well established.

STUDY DESIGN—A nested case control study was performed within a prospective cohort of term nulliparous women: 20 cases (intrapartum fever of >100.4°F) and 20 afebrile controls. Oxidative stress was assessed using ThioGlo-1 (TG-1; Calbiochem, San Diego, CA) fluorescent sulfhydryl detection. Median levels (± interquartile range) of protein-thiol sulfhydryls were compared.

RESULTS—In early labor, maternal oxidative stress (lower protein sulfhydryls) was significantly higher in those women who subsequently had intrapartum fever develop (79.87 ± 22.88 vs 127.73 ± 43.79 counts/second per μg protein; $P < .001$). In contrast, cord serum sulfhydryls were not different between groups (75.77 ± 14.00 vs 75.04 ± 17.83 counts/second per μg protein; $P = .99$)

CONCLUSION—Our data suggest that the term human fetus is protected from maternal oxidative stress associated with intrapartum fever. However, maternal oxidative status in early labor is associated with subsequent intrapartum fever. Optimal fetal neuroprotection will require a more precise knowledge of pathogenic mechanisms.

Keywords

fetal neuroprotection; fever; inflammation; intrapartum fever; oxidative stress

Fetal exposure to chorioamnionitis (hyperthermia and inflammation) at term has long been accepted as a potent risk factor for neonatal encephalopathy and cerebral palsy. Chorioamnionitis accounts for between 11% and 22% of cases of cerebral palsy in near-term and term infants, and carries an odds ratio of 9.3 for otherwise unexplained cerebral palsy.[1,2] The current diagnosis of chorioamnionitis is largely based on maternal fever; fundal tenderness cannot be used after epidural analgesia and purulent vaginal discharge is a subjective finding. Maternal oral temperature is the best indicator of intrauterine temperature

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but underestimates it by an average of 0.8°C.[3] In turn, fetal core temperature is approximately 0.75°C higher than fetal skin/intrauterine temperature.[4] Therefore, the widely used definition of intrapartum fever (38.0°C) is generally associated with fetal brain temperatures of 39.5°C or higher.

Term infants have a low risk of neonatal encephalopathy of approximately 0.12%.[5] The observed risk of encephalopathy with fetal exposure to maternal fever alone is 1.13% and the risk with fetal exposure to acidosis (cord pH <7.05) alone is 1.58%. However, the combination of maternal fever and neonatal acidosis results in a substantial increase in the risk of neonatal encephalopathy to 12.5%, independent of neonatal sepsis.[5] This observation has led to the hypothesis that maternal fever is associated with increased levels of fetal oxidative stress and depleted cellular reserves, increasing fetal susceptibility to hypoxic injury. Further, antioxidants have been shown to decrease fetal injury and provide neuroprotection in lipopolysaccharide (LPS)-based animal models of chorioamnionitis, especially when used prophylactically.[6–13] These promising data have led to several ongoing Phase I/II studies of the safety and efficacy of N-acetyl cysteine (NAC), a compound with both antioxidant and antiinflammatory properties for the prevention of neonatal death, sepsis, and neuronal injury in the setting of chorioamnionitis in human subjects (http://www.clinicaltrials.gov). Our objective was to determine whether intrapartum maternal fever was associated with increased levels of maternal and/or fetal oxidative stress. Further, we sought to examine the relationship between maternal and fetal oxidative stress.

Materials and Methods

A nested case control study was performed. The initial cohort consisted of nulliparous women who were candidates for a trial of labor. Women with active infections, autoimmune conditions, or those taking antiinflammatory agents were excluded. Subjects were enrolled prospectively at ≥37 0/7 weeks' gestation. Maternal blood was collected at enrollment and in early labor after the onset of regular, painful contractions. Maternal oral temperatures were collected hourly in labor. Cord blood was collected at delivery. All blood samples were processed to serum and stored at −80°C. Maternal data on gestational age at enrollment, height, weight, race, and ethnicity were collected prospectively. Intrapartum and neonatal data were collected from detailed chart review and from our research quality perinatal database (PINS). A total of 607 subjects were enrolled. Subjects with available aliquots of paired maternal and cord serum were identified. Subjects were excluded if significant hemolysis was identified visually in any of the collected samples. A nested case control study was performed by selecting 20 cases (intrapartum fever of >100.4°F) and 20 afebrile controls. Selection was random and was performed by the study coordinator who had no knowledge of the hypothesis. Sample dates ranged from Feb. 5, 2006, to March 12, 2009.

Oxidative stress was assessed through detection of protein-thiol redox status using the maleimide reagent ThioGlo-1 (TG-1; Calbiochem, San Diego, CA) that produces a highly fluorescent adduct after its reaction with sulfhydryl groups.[14–15] Development of fetal oxidative stress results in oxidation of DNA, lipids, and proteins. Reduced protein thiol (sulfhydryl) relatively easily undergoes oxidation to sulfenic, sulfinic, and sulfonic state (the 2 later states are irreversible). Thus, a decrease of protein sulfhydryls is considered as an

appropriate measure of oxidant stress induction.[16–17] Briefly, samples for analysis were prepared using size-exclusion chromatography with BioSpin-6 microcolumn (BioRad, Berkeley, CA) to eliminate low molecular weight (<6 kDa) thiols. Then 20 μL eluent was added to 2 mL of 40 mM PB (pH = 7.4) in a quartz cuvette of spectrofluorometer (PTI, Piscatway, NJ) under constant stirring at 37°C. The emission (513 nm, excitation at 379 nm) of each sample was recorded for 1 minute (background) before and 2 minutes after an addition of 5 μM (final concentration) of ThioGlo-1. Each sample fluorescence saturation value corresponds to a concentration of free sulfhydryls. At the end of each experiment, 1 μM reduced glutathione was added to the sample to ensure that saturation was not associated with the concentration of TG-1. TG-1 fluorescence was normalized for sample protein concentration.

Statistical analysis was performed using SPSS 17.0 (SPSS Inc, Chicago, IL). Median levels (± interquartile range [IQR]) of protein-thiol sulfhydryls were compared using the Mann-Whitney *U* test. Paired nonparametric analysis was performed using the Wilcoxon signed ranks test. Correlation was assessed using the Spearman *R* test. Maternal and neonatal characteristics were compared by using the Student *t* test or Mann-Whitney *U* test as appropriate for continuous variables and Fisher's exact test for categorical variables. A *P* value of < .05 was considered statistically significant. Power analysis determined that 20 samples per group would provide an 80% power to detect a 26% difference in levels of fetal oxidative stress.

Results

Maternal and neonatal characteristics for cases and controls are shown in the Table. Data on neonatal sepsis evaluation and blood culture results were missing for one neonate born to a febrile mother. No significant underlying demographic differences were noted between women who remained afebrile and those who subsequently developed intrapartum fever. Overall rates of epidural analgesia were very high in both groups. There were no differences in maternal temperature at admission or maternal temperature in early labor at the time of blood sampling. Not surprisingly, intrapartum fever was associated with labors that were a median of 3.6 hours longer. There was a significantly higher rate of meconium passage in fetuses of febrile parturients, which suggests an increase in fetal stress associated with maternal fever. There were no significant demographic differences in neonates by fever status. As expected, intrapartum fever was associated with an increased rate of neonatal sepsis evaluation and neonatal length of stay; however, no neonate had culture proven sepsis. There was 1 infant with neonatal seizures in the fever group. Although intrapartum fever is a known risk factor for neonatal seizures,[5,18–19] the rate of neonatal seizures in this study was not statistically significant. The level of oxidative stress in this infant was less than the median oxidative stress for the remainder of the group.

There was no difference in levels of maternal oxidative stress at prelabor enrollment (102.1 ± 15.1 vs 91.0 ± 35.2 counts/second per μg protein; *P* = .53; median ± IQR; data not shown). In early labor, maternal oxidative stress (lower levels of protein sulfhydryls content) was significantly higher in those women who subsequently had intrapartum fever develop (Figure 1: 79.87 ± 22.88 vs 127.73 ± 43.79 counts/second per μg protein; *P* < .001). In

contrast, fetal serum sulfhydryls were not different between groups (Figure 2: 75.77 ± 14.00 vs 75.04 ± 17.83 counts/second per μg protein; $P = .99$). There was no significant correlation between maternal and fetal levels of oxidative stress ($r = -0.24$; $P = .17$). Labor itself was associated with oxidative stress when paired enrollment and labor specimens were compared ($P < .001$).

Comment

Our data suggest that the term human fetus is protected from maternal oxidative stress associated with intrapartum fever. The strengths of our study include the prospective collection of maternal and cord blood samples and the meticulous and uniform ascertainment of maternal hourly maternal temperature during labor. This study design reduced the likelihood of misclassification of fever status. The second strength of our study is that we were able to demonstrate significant differences in measures of oxidative stress in maternal labor samples. This finding demonstrates that our assay is valid; that is, that we were able to detect differences in oxidative stress if they were present. Further, our power analysis ensured that we would be able to detect a clinically significant difference in fetal oxidative stress levels. Therefore, our finding of a lack of difference in oxidative stress in the fetal compartment was not due to methodologic or statistical flaws. The main weakness of our study is that it was limited to term pregnancies. Therefore, these results may not be applicable to preterm neonates. It is possible that the mechanism that allows for relative protection from oxidative stress in the neonatal compartment is not yet developed in preterm pregnancies. One investigation in preterm infants reported an association between neonatal sepsis and increased oxidative stress.[20] Further, our work cannot determine whether there is a threshold for maternal fever at which protective mechanisms are overwhelmed and fetal oxidative stress occurs. Finally, because maternal blood was not collected at the time of delivery, we cannot determine whether the differences in maternal oxidative stress observed in early labor persisted, resolved or worsened after the development of clinical fever.

Our data suggest that labor itself induces relative oxidative stress compared with paired maternal samples before the onset of labor. However, we also demonstrate that increases in maternal oxidative stress with the onset of labor are significantly greater in women who will go on to have subsequent intrapartum fever develop. Although previous publications have reported on the association between increased oxidative stress and the severity of febrile illness,[21–23] to our knowledge this is the first report of increases in oxidative stress preceding clinical febrile morbidity. Our data cannot be used to determine whether the observed increases in oxidative stress are causative or occur in association with other factors that predispose to maternal fever. However, it is biologically plausible that increased maternal oxidative stress in early labor plays a causative role in subsequent fever risk. NAC and other antioxidants have been shown to inhibit LPS-induced nuclear accumulation of NF-κB and influence toll-like receptor 4 (TLR-4) dependent activation of NF-κB.[24–26] However, reactive oxygen species alone do not consistently activate an inflammatory response in all cell lines.[27] This suggests that although oxidative stress alone may not be a adequate inciting factor to induce maternal fever, maternal oxidative stress may potentiate proinflammatory activation.

The failure of maternal intrapartum fever to induce fetal oxidative stress suggests that: (a) animal models of fetal oxidative stress are not readily applicable to humans; (b) antioxidant fetal neuroprotection is mediated by the antiinflammatory properties of tested antioxidants; or (c) neural oxidative stress cannot be measured in peripheral samples. We acknowledge that cord blood samples do not necessarily reflect oxidative stress in the fetal brain. However, it would be biologically plausible to assume that, given the protective mechanisms in place to preserve blood flow to the fetal brain, peripheral oxidative stress should precede neural compartment oxidative stress. If the neuroprotective effects of antioxidants are indeed mediated by their antiinflammatory properties, then this suggests that targeted antiinflammatory agents may be more effective than antioxidants. However, antioxidants have a potential role in reducing the complications of catastrophic perinatal asphyxia, a condition that is known to be associated with increased oxidative stress levels both in the periphery and in the cerebrospinal fluid.[28] Fortunately, isolated fetal hypoxia is a relatively infrequent cause of neonatal encephalopathy in the absence of maternal fever.[5] Therefore, it is logical that election of candidates for fetal neuroprotection should focus on early prediction of the more common risk of intrapartum fever. This study demonstrates that there are underlying differences in maternal oxidative stress in early labor before subsequent maternal fever. Therefore, measures of maternal oxidative stress in early labor may prove to be useful in the future as 1 marker in assessing risk of adverse neonatal outcomes associated with maternal fever. Further animal work is needed to determine the exact relationship between maternal and fetal oxidative stress in animal models. Optimal fetal neuroprotection will require more precise knowledge of pathogenic mechanisms.

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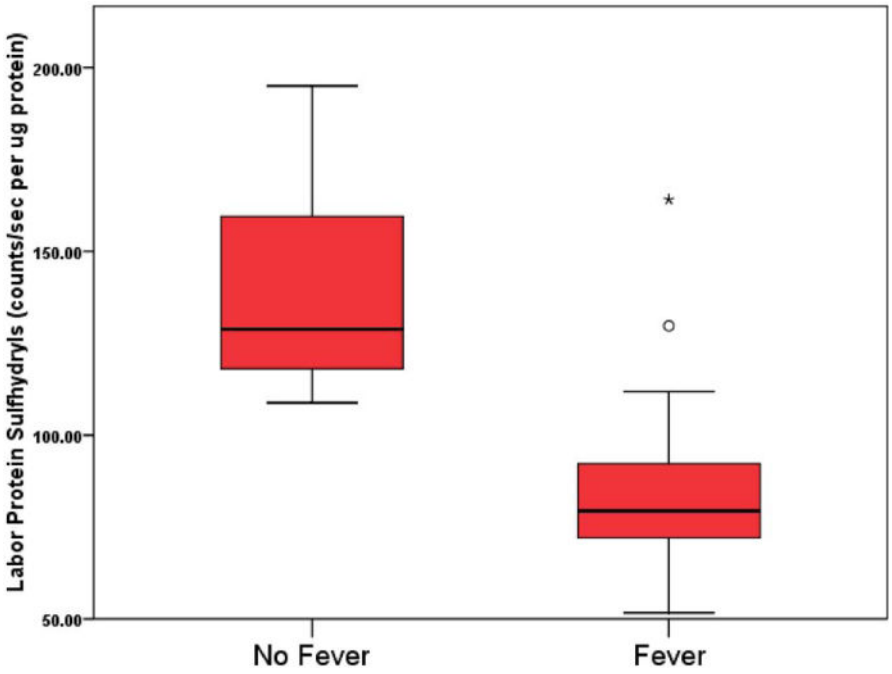

FIGURE 1. Maternal levels of protein sulfhydryls in early labor, stratified by eventual intrapartum fever status

Decreased protein sulfydryl levels correspond to higher levels of oxidative stress. Median levels in the 2 groups were compared using the Mann-Whitney *U* test. A significant difference in median levels was seen between groups ($P \leq .001$).

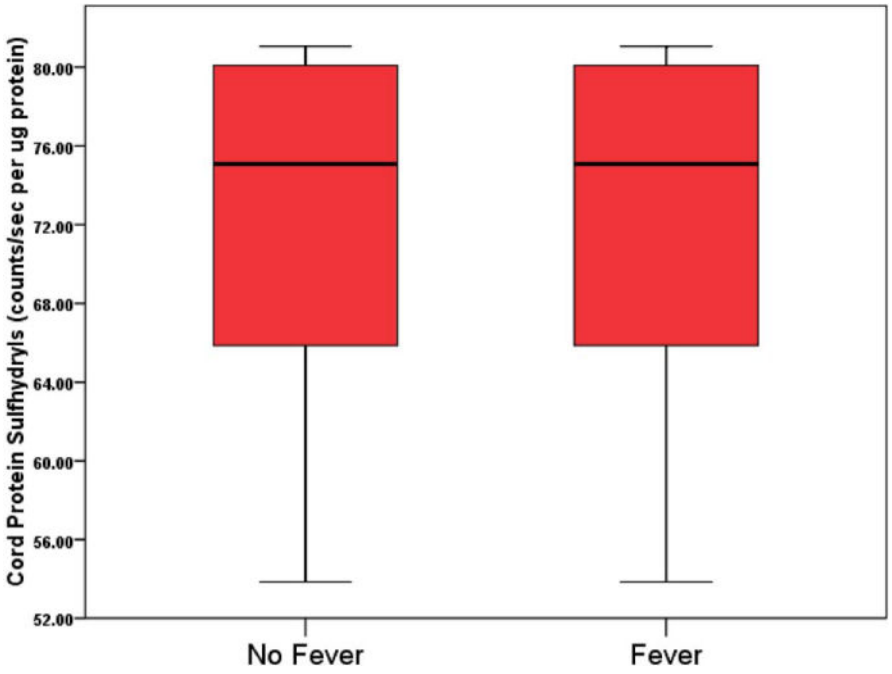

FIGURE 2. Cord blood levels of protein sulfhydryls in neonates born to women with and without intrapartum fever

Decreased protein sulfydryl levels correspond to higher levels of oxidative stress. Median levels in the 2 groups were compared using the Mann-Whitney *U* test. No significant difference was seen ($P = .99$).

TABLE

Maternal and neonatal status by intrapartum fever status

Variable	Fever group (n = 20)	Afebrile (n = 20)	*P* value
Maternal age, y	25.8 ± 1.1	24.4 ± 1.1	.36
Race, %			.9
White	25	30	
Black	25	20	
Hispanic	50	50	
Gestation at enrollment, wk	37.9 ± 0.2	38.0 ± 0.17	.61
Gestation at delivery, wk	40.2 ± 0.2	39.2 ± 0.2	.27
Admit dilation, cm	3.0 (0–8.0)	4.0 (1.0–5.0)	.34
PROM before labor, %	5	0	.6
GBS positive, %	35	30	.5
Epidural analgesia, %	100	95	.5
Maternal BMI	30.5 ± 2.7	31.2 ± 1.9	.82
Admission temperature, °F	98.3 ± 0.2	98.2 ± 0.2	.93
Temperature early labor, °F	98.4 ± 0.2	98.0 ± 0.2	.13
Meconium, %	55	15	.02
Duration of labor, min	826 (369–1285)	610 (308–1164)	< .001
Birthweight, g	3476 ± 125	3356 ± 111	.46
Male infant, %	60	45	.26
5-min Apgar <7, %	5.3	5	.74
Neonatal sepsis evaluation, %	84.2	15	< .0001
Positive blood culture, %	0	0	> .99
Neonatal seizures, %	5	0	.52
Neonatal LOS, d	3.0 (2.0–5.0)	2.0 (1.0–5.0)	.002

Means are presented as mean ± standard error, medians are presented as median with range.

BMI, body mass index; *GBS*, Group B Streptococcus; *LOS*, length of stay; *PROM*, premature rupture of membranes.