

High Intestinal Permeability in Low Birth Weight Infants with Perinatal Encephalopathy

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Abstract

The aim of this work was to estimate intestinal barrier damage, based on structural changes of the brain, in low birth weight infants with hypoxic encephalopathy. Neonates without changes on the neurosonograms were included in the group 1. Neonates with intraventricular hemorrhage on the neurosonograms comprised the group 2. Neonates with intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) comprised the group 3. In the group 2, the median I-FABP levels (6.2 ± 1.3 ng/ml) were significantly higher than those of the groups 1 and 3 (2 ± 0.88 ng/ml, 3.6 ± 2.31 ng/ml, respectively) within the first days of life. The I-FABP levels tended to increase until the 7th-10th day of life. High levels of LBP (45.24 ± 1.4 ng/ml) in the 7th-10th day of life were found in infants in the group 3. This difference was significant compared to the levels found in the other groups ($p < 0,05$). Infants with perinatal hypoxic brain injury have different features of intestinal barrier damage. High serum concentrations of I-FABP in the group 2 might indicate ischemic changes of the gut wall in infants with IVH. Increases in LBP levels in newborns with PVL demonstrate the inflammatory character of gut injury.

Introduction

Neonatal hypoxia occurs frequently and is usually accompanied by serious complications in the affected infant, especially when the infant is of low birth weight (8, 17). Although considerable efforts have been directed towards evaluating alterations in the neurological, cardiopulmonary and renal function of these infants (5, 20, 24), the intestinal function in response to hypoxia in the neonate has not been fully evaluated. Mucosal organs, such as the intestine, are highly vascular and have extensive metabolic demands. For this reason, they are particularly susceptible to diminished blood flow and resultant tissue hypoxia (16). It has been shown that mature enterocytes located at the tips of the villi are most susceptible to ischemia-reperfusion. This finding has classically been explained by their constant state of hypoxia due to the countercurrent exchange mechanism of oxygen in the villus microvasculature, wherein oxygen from arterial blood entering the villus diffuses to neighboring venules, traveling from the tip to the base of the villus. As a result of this phenomenon, a steep oxygen gradient is present in the intestinal villus, with a substantially lower oxygen concentration in the villus tip than at the crypt (4). One of the serological markers of gut ischemia is the intestinal fatty acid binding protein (I-FABP) (18). I-FABP plays important roles in the translocation and metabolism of long-chain fatty acids. Due to its small size, I-FABP leaks rapidly out of is chemically damaged necrotic cells, leading to increased I-FABP serum levels (9).

Disruption of the intestinal epithelial barrier could lead to endotoxin translocation and secondary lipoprotein-binding protein (LBP) expression (1). It is known that LBP has the paradoxical dual functions of sensitizing the immune system to endotoxin and enhancing the neutralization of endotoxin by high-density lipoprotein (23, 26).

The aim of this work was to estimate intestinal barrier damage based on structural changes of the brain in low birth weight infants with hypoxic encephalopathy.

Methods

Subjects: The study population consisted of infants with hypoxic ischemic encephalopathy (HIE) who were admitted to the K. Farajova Pediatrics Institute NICU from January to November 2011. The Problem Commission on Pediatric Research at Azerbaijan Medical University approved this study.

This study was supported by Science Development Foundation Under the President of Azerbaijan Republic.

The exclusion criteria were weight greater than 2500 g and less than 1500 g, chromosomal anomalies, major congenital malformation of any organs or systems, and intrauterine infections.

Gestational age was determined by the first day of the last menstrual cycle, and when possible, was confirmed or corrected by the first sonographic examination with growth measurements taken before the 10th week of gestation. Intrauterine growth restriction (IUGR) was diagnosed if the birth weight was below the 10th percentile, according to Di Battista et al. (6).

Data concerning birth weight, gestational age, Apgar scores at 1 and 5 min of life, blood gases, neurosonography, type of feeding, and signs of feeding intolerance were included in the individual data cards for each infant. The severity of neonatal encephalopathy was estimated based on the clinical behavior over 24 hours of life using the Sarnat Score (22). Necrotizing enterocolitis (NEC) diagnoses were based on clinical findings (bile-stained, gastric residuals) consistent with Bell's staging and were confirmed by radiologic evidence of NEC (15). Neonatal death was defined as death within 28 days of postnatal life.

Study design: According to the results from the cranial ultrasounds, the infants with HIE were assigned to one of the following groups: neonates without change on the neurosonograms (group 1); neonates with evidence of IVH on the neurosonograms (group 2); neonates with evidence of IVH and periventricular leukomalacia (PVL) on the neurosonograms (group 3).

Ultrasound inspection: Transfontanellar neurosonography was performed between days of life 1 and 3 and, subsequently, at the discretion of the neonatologist, using 5 and 7.5 MHz sector transducers. IVH was graded according to Papile's classification (19). PVL was diagnosed as an echolucent area or areas of persistent echogenicity in the periventricular region of the brain in coronal and sagittal views (11).

Blood collection: Blood samples were collected in EDTA-containing tubes on the 1st-3rd and 7th-10th days of life. The samples were separated by centrifugation and stored at -70°C until the assay was performed.

Serum I-FABP and LBP concentrations in serum samples was measured using a commercially available enzyme-linked immunosorbent assay (HyCult Biotechnology, Uden, The Netherlands) according to the manufacturer's instruction. The minimum detection level for I-FABP was 0.047 ng/ml and for LBP was 0.00044 ng/ml.

Statistical analysis: The data in the study groups were tested for a normal distribution and found to be nonparametric. Differences among the study groups were calculated using the Mann-Whitney *U*-test. Correlations were calculated by the Spearman rank-order correlation coefficient. The quality variables were compared using Fisher's exact test. In all instances, significance was established at $p < 0.05$.

Results

As shown in table, the mean gestational age, Apgar scores, and birthweight of all three groups did not differ significantly. Significant differences ($p < 0.005$) were found with respect to SGA and death between group 1 and groups 2 and 3. The mean values of granulocytes were significantly higher in groups 2 and 3 compared to group 1. The mean pH was higher in the patients in group 1 compared with the patients in groups 2 and 3. Patients with PVL often showed evidence of NEC and required total parenteral nutrition.

This observation was found to be statistically significant compared with groups 1 and 2. Group 3 infants also characterized with high frequency of IVH 2nd grade and severe HIE.

Biochemical markers: As shown in Figure 1, I-FABP concentrations in infants with structural changes of the brain were significantly higher than in the control infants. We found low I-FABP levels in the 1st-3rd days of life of newborns in group 1. The levels of I-FABP in the group 1 newborns rose significantly in the 7th-10th day of life, approaching the levels of the group 2 newborns. We found significantly higher I-FABP levels from the 1st day of life in group 2 infants compared with group 1 and group 3 newborns ($p < 0.05$). The newborns in group 3 had significantly lower levels of I-FABP during the neonatal period compared with the levels of IFABP at the 7th-10th day of life for newborns in groups 1 and 2.

LBP concentrations did not differ in the 1st-3rd day of life across the three study groups, and were significantly higher in the study groups than in the control infants. We also found this marker to be increased in the 7th-10th day of life in infants in group 3 compared to infants in groups 1 and 2 ($p < 0.05$).

We analyzed mean I-FABP and LBP concentrations (including 1st-3rd and 7th-10th days parameters) in died infants of all study groups (figure 2). We found a significant increase in I-FABP levels in died newborns compared with surviving infants ($p < 0.05$) and LBP levels did not change depending on mortality.

Correlation between I-FABP and LBP levels. As shown in Figure 3, in groups 1 and 3, this relationship was negative but not statistically significant. We did not find a correlation between I-FABP and LBP in the infants in group 2. Most of the infants in groups 2 and 3 were born by caesarean section. We performed an additional comparison between the I-FABP and LBP levels of the infants born by vaginal delivery and those born by caesarean section to determine the role of delivery type in the levels of these biomarkers. Mean I-FABP (3.5 ng/ml for vaginal delivery and 3.14 ng/ml for caesarean section) and LBP (31.3 ng/ml for vaginal delivery and 33.2 ng/ml for caesarean section) concentrations were not different based on the delivery type for either study group.

Discussion

The fetal circulatory response to hypoxia is a rapid centralization of blood flow in favor of the brain, heart and adrenal glands at the expense of almost all peripheral organs. Among the internal organs, the intestine is likely the most sensitive to hypoxia-ischemia (27). Ischemic injury is complicated by reperfusion injury via superoxide radicals or by bacterial translocation and the subsequent development of multiorgan dysfunction (3).

Intestinal ischemia, even for a short period of time, is followed by changes in plasma levels of I-FABP due to intestinal villi damage (9). I-FABP has also been evaluated as a potential marker for diagnosing and staging NEC (2). The urinary I-FABP/Cr ratio in infants with NEC reflects the extent of the disease (7). In our study, there was no significant difference in serum I-FABP on the 1st day of life between the control group and group 1. The increase in the concentration of I-FABP on the 7th-10th days may be attributed to reperfusion-induced intestinal injury in response to enteral feeding, intestinal colonization and contact with bacterial components.

A high level of circulating I-FABP in infants with IVH on the 1st day of life and increasing levels of I-FABP during the second week of life reflect the membrane integrity damage of the epithelial cells of the villi and the loss of enterocytes. Elevated plasma I-FABP values were associated with poor outcomes in newborns. In our study, significantly higher levels of I-FABP (7.82 ± 1.6 ng/ml) were observed in infants who died, while lower levels were observed in surviving infants (4.74 ± 1.5 ng/ml) ($p < 0.05$). Reisinger et al. showed that high levels of I-FABP are associated with unfavorable outcomes in neonates with NEC at the time of re-feeding (21).

Although the clinical features of NEC are often observed in infants with PVL, the I-FABP levels were lower in the infants with PVL than in the other groups. The lower levels may be related to the disturbance of intrauterine gut maturation in infants with PVL because I-FABP is primarily limited to mature enterocytes of the small and large intestine (12, 13). Gut wall ischemia, bacterial overgrowth in the small bowel from antibiotic use, and deficiency in efficient peristalsis due to parenteral nutrition, especially in groups 2 and 3 (as shown in table), led to endotoxin translocation and, secondarily, to LBP expression.

In this study, we found significantly elevated levels of LBP in plasma samples from neonates with PVL compared to neonates in groups 1 and 2. There are two possible explanations for this increase.

First, infants with PVL have a markedly disrupted intestinal barrier that leads to systemic inflammation and LBP overexpression. This study demonstrated that infants with clinical features of intestinal motility disturbances have significantly ($p<0.05$) high LBP levels (39.1 ± 6.4 ng/ml) in comparison with newborns without these signs (28.8 ± 2.1 ng/ml).

Second, some studies have indicated that in addition to ischemia-reperfusion injury, cytokine-induced brain injury associated with maternal or fetal infection may also play an important role in the pathogenesis of PVL. Recent studies have shown that the administration of LPS to pregnant rats causes LPS-induced white matter injury with enhanced expression of cytokines, such as IL-1 β , TNF- α , and IL-6, in fetal brains (14). Gianine established that maternal lipopolysaccharide exposure increased the frequency and severity of intestinal injury in a neonatal NEC rat model (10). This study has one limitation because we did not evaluate the levels of maternal endotoxin, and we do not have any data that can contribute to the prior study on maternal lipopolysaccharide exposure. The blood from infants with PVL in our study had a high concentration of granulocytes. We hypothesize that the release of LBP in the PVL infants is involved in the defense against endotoxins. In addition to its role in the neutralization and clearance of endotoxins, a basal concentration of LBP is known to enhance the sensitization of the immune system to endotoxins by catalyzing the binding of endotoxins to granulocytes (25).

We found different features of intestinal barrier damage based on different structural changes in the brains of infants with perinatal hypoxic injury. We detected ischemic changes in the gut wall in infants with IVH, which were confirmed by increased circulating I-FABP levels. High concentrations of LBP indicate inflammatory injury by enterocytes in infants with PVL. Evidence of elevated I-FABP and LBP concentrations associated with necrotizing enterocolitis enables these compounds to be used as diagnostic markers for the severity of NEC in newborn infants with perinatal encephalopathy.

Table: Neonatal and maternal characteristics of the study groups.

Neonatal characteristics	Group 1 N=7	Group 2 N=40	Group 3 N=6
Gestational age (wk)	33.9 (33-36)	35.17 (32-38)	35.6 (33-36)
Birth weight (g)	1971.4 (1655-2301)	1897.4 (1330-2500)	2050.0 (1370-2490)
Male gender	2 (28.6)	23 (57.5)	3 (50)
SGA	1 (14.8)	16 (40)	3 (50)*
APGAR score at 1 min	5.33 (4-6)	5.0 (3-6)	5.0 (3-5)
APGAR score at 5 min	6.33 (5-7)	5.5 (4-6)	5.3 (3-7)
Total parenteral nutrition	2 (28.6)	10 (25.0)	3 (50.0)*^
Antibiotic therapy	1 (14.3)	35 (87.5)*	6 (100)*
Blood pH	7.31 (7.28-7.32)	7.29 (7.27-7.32)	7.27 (7.25-7.30)
Granulocytes. %	51.5 (49-64)	68.4 (64-71)	81.2 (79-84)*
Neonatal mortality	–	18 (45)*	2 (33.3)*
NEC	–	7 (17.5)*	2 (33.3)*
1st grade IVH	–	26 (65.0)*	1 (16.7)
2nd grade IVH	–	13 (32.5)	5 (83.3)*
3rd grade IVH	–	1 (2.5)	–
Mild (moderate) HIE	5 (71.4)	26 (65)	1 (16.7)*^
Severe HIE	2 (28.6)	14 (35)	5 (83.3)*
Maternal characteristics			
Age, year	25.6 (19-30)	25.8 (19-33)	20.2 (17-32)
Maternal preeclampsia	–	5 (12.5)	1 (16.7)
Anemia	6 (85.7)	25 (62.5)	3 (50.0)
Caesarean section	3 (42.9)	7 (17.5)	1 (16.7)

Data are expressed as the mean (range) and as n (%). * $p<0.05$ compared with group 1 infants; ^ $p<0.05$ compared with group 2 infants.

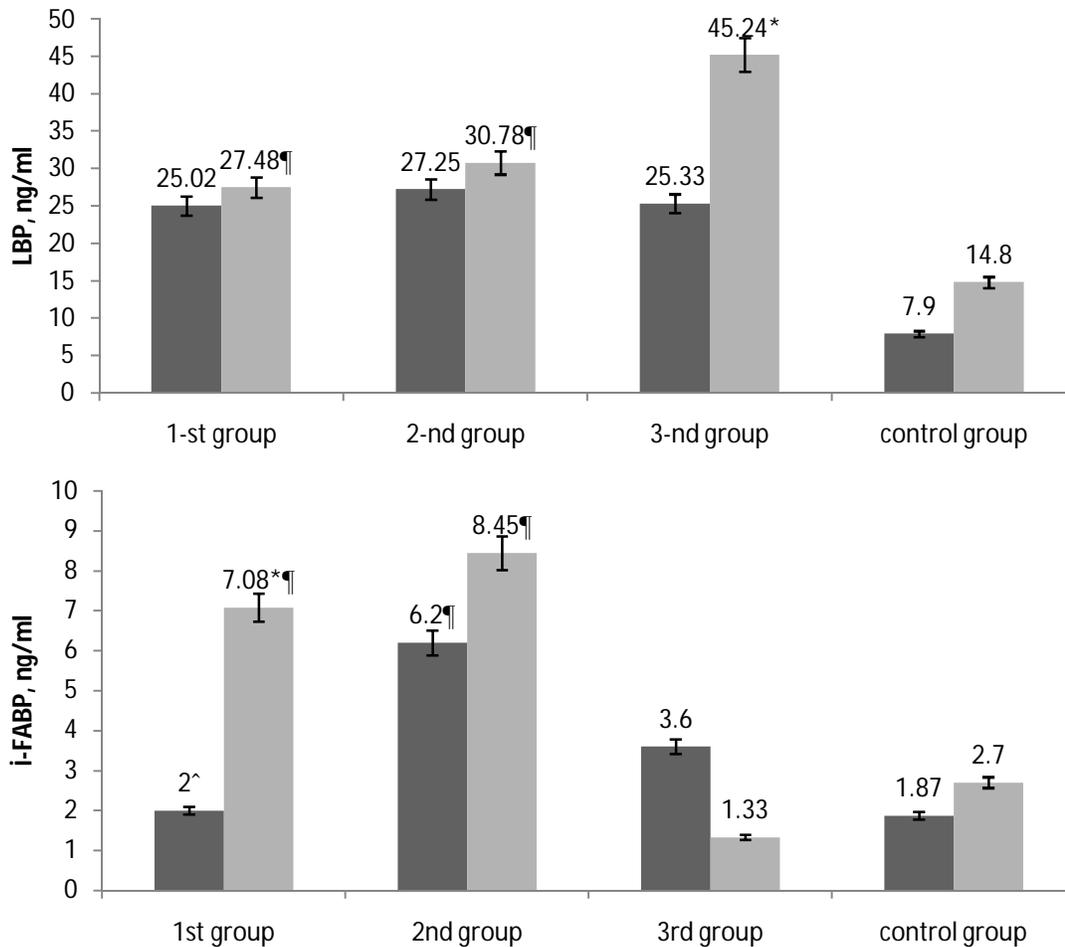


Figure 1. Mean total concentrations of LBP and i-FABP in the study groups. The black bar represents the results from the 1st day of life. The gray bar represents the results from the 1st-3rd day of life. * $p < 0.05$ vs. 7th-10th day of life results, [^] $p < 0.05$ vs. group 2 results, [¶] $p < 0.05$ vs. group 3 results.

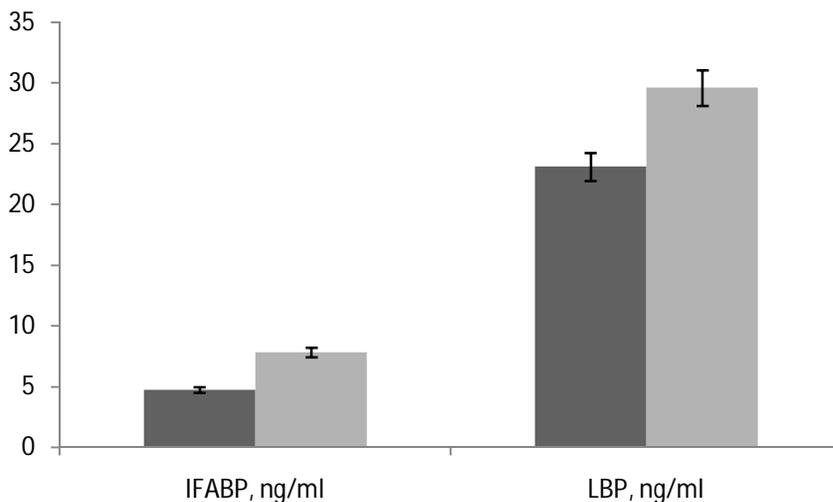


Figure 2. Mean total concentrations of I-FABP and LBP in died and surviving infants. The black bar represents the results of surviving newborns. The gray bar represents the parameters of died infants. * $p < 0.05$ vs. surviving infants.

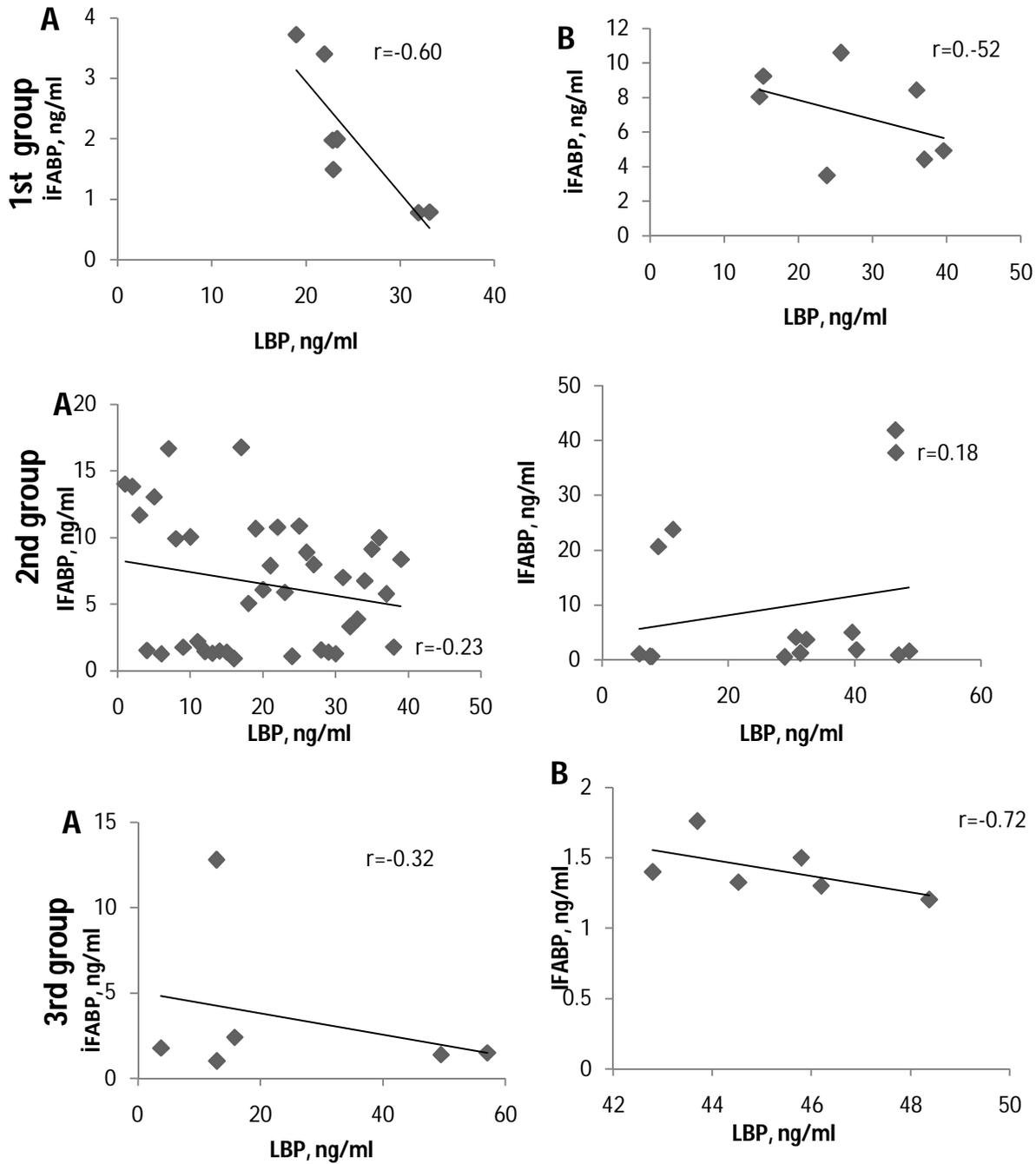


Figure 3: Correlation analysis between LBP and I-FABP measurements in the study groups.

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