

# Comparison of umbilical cord interleukin-6 in preterm infants with premature rupture of membranes and intact membranes

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## ABSTRACT

**الأهداف:** يعد التمزق المبكر للأغشية معقولاً لدى ثلث المواليد الخدج وهو أحد أهم العوامل للإصابة بالإنتان بعد الولادة. قارنا في هذه الدراسة الالتهاب المتوسط في دم الحبل للمواليد الخدج مع التمزق المبكر للأغشية والأغشية السليمة.

**الطريقة:** تم إدراج ٨٩ مولوداً خديجاً كانت ولادتهم بين الأسبوع ٢٧-٣٤ من الحمل بمستشفى غيم بمدينة مشهد بإيران في هذه الدراسة وذلك في الفترة ما بين يونيو ٢٠٠٥م إلى مارس ٢٠٠٦م وتم الحصول على بلازما الحبل السري عند الولادة. تم تقسيمهم إلى مجموعتين (٤٥ مريضاً مصابون بالتمزق المبكر للأغشية و٤٤ مريضاً أغشيتهم سليمة). تم قياس مستويات عنصر انتريوكين-٦ (آي إل-٦) وتفاعل سلسلة البروتين (سي آر بي) في بلازما الحبل بواسطة طريقة (إليسا).

**النتائج:** بلغ معدل مستويات بلازما الحبل في المواليد الخدج المصابين بالتمزق المبكر للأغشية والأغشية السليمة ٢٠٧١ و ٣٣٣ جرام/مللتر على التوالي (نسبة الخطأ = ٠.٠٠٠). بلغ معدل تفاعل سلسلة البروتين في دم الحبل لدى المواليد المصابين بالتمزق المبكر للأغشية والأغشية السليمة ١٠٢ و ١٠٦ ميكروجرام/مللتر على التوالي (نسبة الخطأ = ٠.٤١). كانت نوبات الإنتان المبكرة أكثر تكراراً في المواليد المصابين بالتمزق المبكر للأغشية من المواليد الخدج ذوي الغشاء السليم (٣٨٪ مقابل ١٠٪، نسبة الخطأ = ٠.٠٠١). كان متوسط مستويات عنصر آي إل-٦ للدم في الحبل لدى المواليد المصابين بالتمزق المبكر للأغشية أعلى بشكل ملحوظ من المواليد المصابين بالإنتان (٤١٤٢٨ مقابل ٤٠٤٤ جرام/مللتر نسبة الخطأ = ٠.٠٠٠).

**خاتمة:** ازدادت مستويات عنصر آي إل-٦ لدى المواليد الخدج المصابين بالتمزق المبكر للأغشية في دم الحبل وكانت أعلى بشكل ملحوظ من المواليد الذين تعرضوا لنوبة مبكرة من الإنتان.

**Objective:** To compare inflammatory mediators in the cord blood of premature newborn infants with premature rupture of membranes (PROM) and intact membranes.

**Methods:** Eighty-nine premature neonates with gestational age of 27-34 weeks that delivered in Ghaem Hospital in Mashhad, Iran from June 2005 to March 2006 were enrolled in a prospective observational study, and their umbilical cord plasma was collected at birth. They were allocated into 2 groups (45 patients with PROM, and 44 neonates with intact membranes). Interleukin-6 (IL-6) and C-reactive protein (CRP) levels were measured in cord plasma by the enzyme linked immunoassay (ELISA) method.

**Results:** Mean cord plasma IL-6 levels in preterm neonates with PROM was 205.71 pg/ml, and in neonates with intact membranes was 33.3 pg/ml for IL-6 ( $p=0.000$ ). The mean cord blood CRP level in newborns with PROM was 10.2 µg/ml, and in those with intact membranes was 1.6 µg/ml ( $p=0.41$ ). Early onset sepsis was more frequent in infants with PROM than premature infants with intact membrane (38% versus 10%,  $p=0.001$ ). In neonates with PROM, the mean cord blood IL-6 level was significantly higher in septic newborns (414.28 versus 40.44 pg/ml,  $p=0.000$ ).

**Conclusion:** The premature newborn infants with PROM had increased IL-6 levels in cord blood, which was significantly higher in neonates that developed early onset sepsis.

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Premature rupture of membranes (PROM) is the spontaneous rupture of the chorioamnion and leakage of amniotic fluid before term and onset of labor. The incidence of PROM in term deliveries is 3%, and in preterm deliveries is 18.5%.<sup>1,2</sup> Spontaneous preterm labor accounts for 40-50% of all preterm deliveries, with the remainder resulting from PROM (25-40%) and obstetrically indicated (20-25%) preterm delivery.<sup>3</sup> The exact etiology of PROM remains unknown. Possible predisposing factors include advanced maternal age, non-white race, multiple gestation, instrumentation of cervix, smoking, low maternal socioeconomic class, weakness of membranes, and nutritional deficiencies.<sup>1,3-7</sup> Current evidence strongly suggests that maternal genital tract infection such as vaginitis or endocervicitis may cause PROM.<sup>1,2</sup> Perhaps the proteolytic enzymes produced by bacteria and the inflammatory responses of the host weaken the membranes and causes thinning out and disintegration of the membranes.<sup>1,5,7</sup> The important complications of PROM in mother are sepsis due to intra amniotic infection (chorioamnionitis) or post partum endometritis,<sup>1,4,8</sup> deep vein thrombosis, and maternal stress. Fetal and neonatal complications of PROM are prematurity, respiratory distress syndrome (RDS), congenital anomalies,<sup>1,3,4,9</sup> pulmonary hypoplasia,<sup>3,10</sup> cord compression, and fetal hypoxia.<sup>9</sup> Newborns with PROM have an increased risk (4-33%) of infection.<sup>3,5,6,8,9</sup> Increased neonatal morbidity and mortality are associated with lower gestational age at birth and infectious complications of PROM. In this study, we evaluated pro inflammatory cytokines in cord plasma of preterm newborns with PROM to show the relationship between fetal inflammatory response, PROM, and neonatal sepsis.

**Methods.** This prospective observational study was conducted in the University Hospital of Ghaem in Mashhad, Iran from June 2005 to March 2006. The study population was delivered premature newborn infants with gestational age of 27-34 weeks. Over a period of 9 months, 145 premature newborn infants were delivered in this hospital. Inclusion criteria were gestational age of less than 35 weeks and admission to the neonatal intensive care unit (NICU) or hospital nursery that permitted following of the patients for at least 72 hours. Exclusion criteria were premature infants with gestational ages over 34 weeks and meconial staining of amniotic fluid. At first, 93 neonates had inclusion criteria, then 4 patients were excluded from the study because of transfer to another hospital for surgical reasons in the first 3 days of life (3 cases), and inadequate blood sample (one case). The final study population was 89 patients. According to presence of PROM they were allocated to 2 groups: 45 newborn infants with PROM

and 44 neonates with intact membranes. Informed written consent was obtained from the parents, and the regional committee of ethics in medical research of the university approved this study. Interleukin-6 (IL-6) and C-reactive protein (CRP) levels were measured in cord plasma samples of the study population by enzyme linked immunoassay (ELISA) method. We performed sepsis workup for all neonates. The clinical course of these hospitalized neonates was closely monitored and recorded in detailed questionnaires by the neonatologists who were blind to cord blood sample results. Patients were evaluated for signs and symptoms of sepsis<sup>3,10</sup> including: 1. Temperature instability (hypothermia, hyperthermia, or >3 different recording with changes of more than 0.5°C in 24 hours). 2. Respiratory (grunting, intercostals retraction, tachypnea, cyanosis, apnea more than 20 seconds or >15 seconds associated with bradycardia, cyanosis or changes in color and tone). 3. Cardiovascular (bradycardia more than 5 times in 24 hours or persisting tachycardia more than 170 beats/min). 4. Poor perfusion with prolonged capillary refilling (>3 seconds) or poor skin color. 5. Hypotension requiring volume replacement or vasopressor infusion. 6. Neurologic symptoms including irritability, seizure, and lethargy. 7. Gastrointestinal (distended abdomen or feeding intolerance or hepatomegaly. Newborns with signs and symptoms of sepsis and positive blood culture in the first 72 hours of life were considered as early onset sepsis. Patients whose blood culture was negative but had at least 3 clinical signs of sepsis associated with laboratory findings (such as elevated immature to total neutrophil count, leukocytosis, leukopenia, or thrombocytopenia) were considered as clinical sepsis. We considered PROM when the delivery did not occur by 18 hours after rupture of the membranes. The presence of maternal fever ( $\geq 38^{\circ}\text{C}$ ) accompanied by the signs and symptoms of intrauterine infection such as foul smelling discharge, uterine tenderness, and maternal leukocytosis were considered as clinical signs of chorioamnionitis.<sup>1,3</sup> Gestational age was estimated by last menstrual period (LMP) and the estimated date with the first trimester ultra sonography. Immediately after the delivery, 3 ml cord blood samples were collected and centrifuged for separating plasma, which was stored at  $-70^{\circ}\text{C}$  until assayed. The IL-6 and CRP concentrations were measured by ELISA (Bender Med System, Vienna, Austria) and CRP ELISA IBL (IBL-Hamburg, Hamburg, Germany) were used for IL-6 and CRP measurement with a sensitivity of 1 pg/ml for IL-6, and 1  $\mu\text{g/ml}$  for CRP.

Statistical analysis was performed using SPSS 14 software. All values are expressed as the mean  $\pm$  standard deviation. Differences between groups and mean values of measured parameters were calculated using Chi-

Square, t-test, and Mann Whitney test. Differences were considered significant when  $p$ -value was less than 0.05. The Receiver Operating Characteristics (ROC) method was used in order to establish the optimal cut-off point. Then sensitivity, specificity, and positive and negative predictive value were calculated.

**Results.** In this study, 34.5% (50/145) of all neonates who were delivered prematurely had PROM, 38.6% (56/145) spontaneous premature labor, and 26.9% (39/145) obstetrically indicated premature labor. The demographic characteristics of the 89 premature neonates in both groups are shown in **Table 1**. The mean duration of membrane rupture in PROM group was  $108.90 \pm 245.80$  hours (18-350 hours). We diagnosed early onset sepsis with positive blood culture in 8 neonates (18%) in the PROM group, and 2 neonates (5%) in the control group ( $p=0.04$ ). Clinical sepsis was diagnosed in 9 patients (20%) of the PROM group, and 2 neonates (5%) in the control group ( $p=0.02$ ). The overall incidence of sepsis was more common in premature infants with PROM than infants with intact membranes (38% versus 10%,  $p=0.001$ ). There were clinical signs of chorioamnionitis in 9/45 (20%) mothers of the PROM group, and 3 of their neonates developed sepsis (2 positive blood cultures and one clinical sepsis). The mean IL-6 and CRP levels in the cord blood of newborns in both groups are presented in

**Table 2.** In the PROM group, the mean cord blood IL-6 level was  $414.28 \pm 336.93$  pg/ml in neonates who had early onset sepsis, whereas in neonates without sepsis it was  $40.44 \pm 49.97$  pg/ml ( $p=0.000$ ). In neonates with intact membranes, the mean IL-6 concentration was  $121.60 \pm 107.08$  in those with sepsis and  $19.97 \pm 15.85$  pg/ml in those with no sepsis ( $p=0.000$ ). Using a cut-off point of 20 pg/ml for cord blood IL-6, we obtained a sensitivity of 46% and specificity of 85%, for detecting early onset sepsis in the PROM group. In this study, cord IL-6 levels had a negative predictive value (NPV) of 39%, and a positive predictive value (PPV) of 88%.

**Discussion.** Every pregnancy complicated with PROM needs a risk-benefit analysis in which the complications of prematurity are balanced against the risks of expectant management for both mother and fetus. According to conflicting scientific data, managing PROM remains a significant problem in obstetrics and it is one of the controversial issues.<sup>1,4,11</sup> Once the diagnosis of PROM is made, it is very important that the gestational age be determined before therapy is started. When the gestational age is less than 28 weeks, and no signs of infection are present, expectant management is usual. In such cases, adverse effects of prematurity are far greater than the risk of chorioamnionitis.<sup>1,5,11</sup> When the gestational age is between 28-34 weeks, care must be

**Table 1** - Demographic characteristics of newborns.

Characteristics of newborns	Group		$P$ -value
	Intact membranes	Premature rupture of membranes	
Birth weight (gr)	$1528.40 \pm 253.89$	$1414.50 \pm 317.52$	0.07
Gestational age (wk)	$31.95 \pm 1.80$	$30.37 \pm 2.38$	0.001
Gender (male/female)	24/20	21/24	0.45
Multiple gestation/singleton	17/27	19/26	0.73
Positive history for maternal ante partum antibiotic therapy	14/44	33/45	0.000
Sepsis with positive blood culture	2/44	8/45	0.04
Clinical sepsis	2/44	9/45	0.02

**Table 2** - Mean levels of interleukin-6 (IL-6) and C-reactive protein (CRP) in cord plasma of neonates with premature rupture of membranes (PROM) and intact membranes.

Cord blood	Group	Mean	SD	Median	Min	Max	$P$ -value
IL-6	Intact membrane	33.35	51.56	17.00	2.40	278.40	0.000
	PROM	205.71	330.23	47.80	0.00	1458.40	
CRP	Intact membrane	1.69	2.44	1.10	0.00	13.7	0.41
	PROM	10.22	22.20	1.30	0.00	96.60	

individualized with risk of interference weighted against the risk of infection. For deciding between expectant management and termination of pregnancy, assessment should include fetal maturity, signs of fetal distress, and maternal chorioamnionitis.<sup>1,12</sup>

Interleukin-6 is a mediator of the host inflammatory response, and is one of the first cytokines to be secreted in response to bacterial infection.<sup>3,13,14</sup> Elevated concentrations of prostaglandins and cytokines have been found in the amniotic fluid of women with PROM and with positive fluid culture.<sup>13,15</sup> Intra amniotic infection or chorioamnionitis complicates 1-5% of term pregnancies and nearly 25% of patients with preterm delivery.<sup>14,16</sup> Microbial invasion of amniotic fluid usually is a result of prolonged rupture of chorioamniotic membranes. On occasion, intra amniotic infection occurs with apparently intact membranes.<sup>11,12</sup> Early and appropriate diagnosis of intra amniotic infection can reduce fetal and maternal complications.<sup>14,16</sup> Fetal plasma IL-6 concentration is significantly higher in the presence of inflammatory lesions in extra placental membranes and umbilical cord.<sup>13,15,17</sup> In this study, we found elevated IL-6 levels in cord blood samples of neonates with PROM who had developed early onset sepsis, as with other studies.<sup>5,8,9,14</sup>

There were clinical signs of chorioamnionitis in 20% of patients with PROM and only 3 of them developed early onset sepsis. Thus, elevated umbilical cord levels of IL-6 may have higher predictive value than clinical signs of chorioamnionitis for diagnosis of early onset sepsis. The histological examination of placenta, chorioamniotic membranes, and cord was not available in this study, and we could not evaluate the relationship between histological inflammatory findings with inflammatory cytokines levels in cord blood. Some studies showed elevated cord blood IL-6 levels in presence of histological chorioamnionitis, funisitis, or chorionic vasculitis.<sup>11,16</sup> In situations that it is not possible to perform histological examination of the placenta or chorioamniotic membranes in preterm labors with PROM, it is recommended to measure IL-6 levels in cord plasma to predict high risk neonates for sepsis. In PROM, clinical chorioamnionitis is one of the indications for termination of pregnancy and this condition carries an increased risk of sepsis in neonates with PROM. Neonatal sepsis is not always associate with clinical signs of chorioamnionitis in the mother.<sup>1-3,18</sup> It seems histological examination of cord and placenta or increased levels of IL-6 can predict neonatal sepsis better than clinical signs of chorioamnionitis, and further studies are warranted for the comparison of the predictive value between histological findings of inflammation and increased levels of IL-6 for early detection of neonatal sepsis.

In conclusion, the mean cord blood IL-6 level was higher in neonates with PROM. Increased cord blood IL-6 concentration in neonates who developed early onset sepsis can help us in close monitoring and sepsis work up of high-risk neonates and early detection of neonatal sepsis.

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## References

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