Cord blood cytokine levels in neonates born to mothers with prolonged premature rupture of membranes and its relationship with morbidity and mortality

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ABSTRACT. The purpose of this study was to determine cord blood cytokine levels and their relationship with morbidity and mortality in neonates with prolonged, premature rupture of membranes (PPROM). Forty two premature neonates of 29-35 weeks gestational age with PPROM exceeding 24 hours were considered as the PPROM group and simultaneously, 41 premature neonates without PPROM were considered as the control group. All the neonates were admitted to the Neonatology Unit for further evaluation of subsequent complications such as early neonatal sepsis, pneumonia, intraventricular haemorrhage (IVH), respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC) and chronic lung disease (CLD). Cord blood and mothers’ blood samples were obtained during delivery in both groups and tested for IL-6, IL-8 and TNF-α levels. Twenty one percent of patients with PPROM had histological chorioamnionitis. The risk for developing early neonatal sepsis increased significantly in neonates whose mothers had histological chorioamnionitis (p < 0.05). There was a statistically significant relationship between PPROM and risk of developing NEC (p < 0.05); no significant increase was seen as regards early neonatal sepsis, IVH, RDS, pneumonia, or BPD. The mean IL-8 levels in cord blood and mothers’ serum were significantly higher in the PPROM group (p < 0.001, p< 0.005). In addition, IL-6 levels found in mothers’ serum were significantly higher than those found in the control group (p < 0.01). However, levels in cord blood were similar (p > 0.05). TNF-α levels were similar in both groups (p > 0.05). Neonates who developed NEC had higher IL-8 levels in their cord blood when compared to those without NEC (p < 0.05). In conclusion, the presence of PPROM increases the risk of chorioamnionitis. In addition, PPROM increases the risk of NEC, and patients who developed NEC had significantly higher cord blood IL-8 values. We may conclude that patients with PPROM and higher IL-8 levels in cord blood might be considered as at possible risk of NEC.

Keywords: prolonged premature rupture of membranes, cytokine, chorioamnionitis
women at similar gestational age without membrane rupture and their babies, were taken as the control group. Those women with a current or history of viral infection, or a urinary tract infection were excluded from the study. The Ethical Committee approved the study, and informed consent was obtained from the parents.

All mothers were evaluated with ultrasonography, and biophysical profiles by were prepared by obstetricians. Gestational age was calculated by mother’s last menstrual date, or, if not known, by ultrasonographic measures and the New Ballard scoring systems [4]. Oligohydramnios was determined by ultrasonographic examinations. PPROM was accepted as a prolonged rupture of membranes exceeding 24 hours. Clinical findings of chorioamnionitis were evaluated [5], and then placental remains from mothers with clinical chorioamnionitis were evaluated for histological chorioamnionitis.

All neonates with PPROM were admitted to the Newborn Intensive Care Unit and subsequent complications such as early neonatal sepsis, pneumonia, IVH, RDS, NEC, and chronic lung disease (CLD) were recorded. Diagnosis of early neonatal sepsis, pneumonia, IVH, RDS, NEC and CLD were based upon certain, defining criteria [6-8]. The Tollner scoring system was used to evaluate risk of early neonatal sepsis [9]. Cord blood and mothers’ blood samples were obtained during delivery in both groups. The blood samples were then centrifuged and serum samples were stored at -70°C. Both mothers’ and cord blood serum were tested for IL-6, IL-8 and TNF-α levels using a micro ELISA method (Pierce Endogen kit, Triturus, Grifols, Spain). The sensitivity of the tests for IL-6, IL-8 and TNF-α were < 1.0 pg/mL, < 2.0 pg/mL and < 1 pg/mL respectively. Specificity of the tests was 70-80% for IL-6, 72-78% for IL-8 and 75-89% for TNF-α. Internal quality control samples were studied in parallel with patients’ serum samples. Titrations of the control and PPROM groups were performed under the same conditions, on the same day.

Statistical analysis was performed with SPSS 10.0 Software. The Mann-Whitney U, dependent t-test, one way ANOVA and linear regression analysis were used to evaluate data.

RESULTS

There were 42 babies in the PPROM group and 41 babies in the control group. There were no statistical differences between age of mothers, gestational age and number of pregnancies in both groups (p > 0.05). Mean birth weight was 1 742 ± 539 g and 1 847 ± 448 g; and mean gestational age was 31.6 ± 2.0 weeks and 32 ± 1.5 weeks in the PPROM group and the control group, respectively

There were no statistical differences between age of mothers, gestational age and number of pregnancies in both groups (p > 0.05). Mean birth weight was 1742 ± 539 g and 1847 ± 448 g; and mean gestational age was 31.6 ± 2.0 weeks and 32 ± 1.5 weeks in the PPROM group and the control group, respectively (p > 0.05). Thirty-eight mothers in the PPROM group and seven mothers in the control group were treated with antibiotics.

The leukocyte count for both mothers and newborns in the PPROM Group was significantly higher than in the control group (1 4380 ± 4 098/mm³ in mothers and 15 180 ± 6 992/mm³ in newborns in the PPROM group and 11 759 ± 2 994/mm³ in mothers and 10 858 ± 3 556/mm³ in babies in the control group), (p < 0.05).

The placenta from 31 mothers in the PPROM group and 25 mothers in the control group were evaluated histopathologically. Six mothers (19%) from the PPROM group were diagnosed with histological chorioamnionitis, but none of the patients in the control group. Clinical chorioamnionitis was diagnosed in nine (21.4%) from PPROM group but in none of the patients in the control group. Histopathological and/or clinical chorioamnionitis was significantly more frequent in the PPROM Group (p < 0.05). IL-8 levels in cord blood and mothers’ serum were significantly higher compared to those in the control group (p < 0.01), though IL-6 levels in mothers’ serum were significantly higher than in the control group (32 days versus 13 days) (p < 0.05). Cumulative relative risk for neonatal complications in PPROM babies was 3.2. No significant increase was seen as regards early neonatal sepsis, IVH, RDS, pneumonia and CLD in the newborns with PPROM when compared to the control group (p > 0.05). However, the odds ratio was 8 for NEC, 3 for

| Cytokine levels in cord and mothers’ blood in PPROM and the control group |
|--------------------------|------------------------|------------------------|----------|
| Mother (pg/mL) | PPROM group Mean ± SD | Control group Mean ± SD | p        |
| IL-6  | 55.2 ± 159.6 | 40.2 ± 91.3 | < 0.01 |
| IL-8  | 269.7 ± 445 | 37 ± 155 | < 0.01 |
| TNF-α  | 15 ± 65.2 | 4.8 ± 1.6 | > 0.05 |
| Cord (pg/mL) |                      |                      |          |
| IL-6  | 135.9 ± 280.5 | 27.9 ± 44.9 | > 0.05 |
| IL-8  | 499.9 ± 597.1 | 22.3 ± 46.7 | < 0.001 |
| TNF-α  | 13.1 ± 38.6 | 5.1 ± 1.7 | > 0.05 |

Figure 1

Cord and mothers’ serum cytokine levels of PPROM and control group.
Cord blood cytokine levels in neonates born to mothers with prolonged premature rupture of membranes

and/or neonatal complications of their babies. They found significantly elevated IL-6 levels in those mothers whose babies had these complications. Similarly Pfeiffer et al. [17] claimed that mothers’ serum IL-6 levels may be of value in detecting fetal infections early; in their study, at a cut-off 11 pg/mL, IL-6 had a sensitivity of 81% and specificity of 76% for neonatal infections. Elevated IL-6 levels have been shown in preterm deliveries [10, 18, 19]. Tasci et al. [20] have studied cord blood IL-6 levels for predicting chorioamnionitis, funisitis and neonatal infection in term PROM, and reported that IL-6 levels were significantly higher in term PROM compared to healthy controls, and for predicting funisitis and positive newborn cord blood cultures, a cord blood IL-6 level of > 39 pg/mL had 100% sensitivity and 81% specificity. Fukuda et al. [21] studied cord levels of IL-6, IL-8 and TNF-α and showed no increase in preterm PROM compared to a control group. In the same study, amniotic fluid IL-6 levels had significantly increased in the preterm PROM group. Goeppert et al. [22] showed significantly increased cord blood IL-6 with preterm PROM or preterm labour. In that study, the increased levels of IL-6 were related to increased risk of periventricular leukomalacia, systemic inflammatory response syndrome and NEC in premature babies. Yoon et al. [23] found that cord IL-6 was significantly increased in premature babies compared to term babies, and in chorioamnionitis compared to healthy mothers. IL-6 has been shown to be released from placental endothelial cells during labour, but could be normal if there were a successful tocolysis [24, 25]. Similarly, Murtha et al. [11] showed that cord IL-6 levels increased in clinical and histological chorioamnionitis with preterm PROM, but were normal with preterm PROM lasting longer than 48 hours. In our study, although mothers’ serum IL-6 levels were higher in the PPROM group, cord IL-6 levels were similar in both groups. Smulian et al. [5] showed that cord blood IL-6 was a better predictor of early neonatal sepsis than clinical signs of chorioamnionitis, in preterm PROM. Similarly Kashlan et al. [26] concluded that elevated cord blood IL-6 was an important sign of sepsis syndrome in histological chorioamnionitis. In some studies, elevated cord blood IL-6 was associated with early neonatal sepsis and pneumonia [22, 27]. However, in the studies by Santana et al. [28] and Berner et al. [29], cord blood IL-8 levels were found to be the most sensitive indicator of early neonatal sepsis diagnosis. While TNF-α has been found to be increased in early neonatal sepsis in some studies [30], no difference was found in others [28]. In our study, we could find no difference in maternal and cord cytokine TNF-α levels in PPROM.

There are a few studies involving maternal IL-8 levels in preterm PROM. Pfeiffer et al. [17] showed a slight increase in IL-8 levels in mothers with preterm PROM. Stallmach et al. [31] showed elevated IL-8 levels in cord amniotic fluid, but only if there were chorioamnionitis, but there was no increase in maternal IL-8 levels. Uterine contractions and progressing labour have been linked with increased maternal IL-6 and IL-8 levels [32]. Von Minckwitz et al. [10] showed IL-6 and IL-8 significantly increased both in preterm labour and PPROM. However, Bahar et al. [12] did not detect an increase in maternal IL-6 and IL-8 levels in preterm labor. In our study, although cord blood IL-6 did not increase, maternal IL-6 levels increased in the PPROM group compared to the control

**DISCUSSION**

IL-6 and IL-8 are both well known cytokines secreted during inflammation. In preterm deliveries, von Minckwitz et al. [10] showed that IL-6 increased in mothers with preterm PROM compared to mothers without preterm PROM. Similarly, in preterm PROM with clinical or histological chorioamnionitis, Murtha et al. [11, 12] showed an elevation in maternal serum IL-6 levels. However, Bahar et al. [13] could not identify an increase in any of the cytokines IL-6, IL-8, or TNF-α in term or preterm labor; and chorioamnionitis has been detected in only 42.1% of preterm PROM mothers with high IL-6 levels by Hadzidaki et al. [14]. Shobokshi et al. [15] also showed that maternal IL-6 levels did not increase in preterm PROM and increased in only half of the patients with histological and/or chorioamnionitis. In the present study, we have detected increased maternal IL-6 levels in PPROM.

Lewis et al. [16] studied IL-6 levels in mothers with preterm PROM in order to detect potential infectious

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**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>PPROM group (n = 42)</th>
<th>Control group (n = 41)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early sepsis</td>
<td>3 (7.3)</td>
<td>1 (2.4)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>RDS</td>
<td>10 (23.8)</td>
<td>12.1 ± 2.08</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>7.4 ± 2.08</td>
<td>9.6 ± 29.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>NEC</td>
<td>8 (19)</td>
<td>4.6 ± 1.07</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IVH</td>
<td>4.6 ± 1.07</td>
<td>4.6 ± 1.07</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>CLD</td>
<td>4.6 ± 1.07</td>
<td>4.6 ± 1.07</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Number (%)</td>
<td>9 (21.4)</td>
<td>74 (18.6)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>p</td>
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</tbody>
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**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>NEC (+) 9 babies Mean ± SD</th>
<th>NEC (+) 74 babies Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>95.3 ± 221</td>
<td>42.0 ± 115</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IL-8</td>
<td>252.5 ± 499</td>
<td>142.8 ± 333</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>TNF-α</td>
<td>4.6 ± 1.07</td>
<td>10.6 ± 49.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Cord (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>129.5 ± 231</td>
<td>76.9 ± 206</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IL-8</td>
<td>655.1 ± 765</td>
<td>216.2 ± 425</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5.4 ± 2.08</td>
<td>9.6 ± 29.2</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

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and both cord and maternal serum IL-8 levels increased significantly in the PPROM group. We suggest that increase in IL-8 in cord blood might indicate fetal inflammation. We have not evaluated IL-6 and IL-8 in term labour, so we cannot comment about their effect in preterm labour.

Studies involving other cytokine, TNF-α, are mainly about its level in amniotic fluid [15, 33]. In a study by Von Minckwitz et al. [10], although IL-6 and IL-8 levels increased significantly, maternal TNF-α levels were not affected by preterm labour or preterm rupture of the membranes. Likewise, we could not find any increase in maternal TNF-α levels associated with with PPROM and chorioamnionitis.

There are only a few studies looking at cord blood TNF-α with PPROM. These studies mostly involve preterm PROM with chorioamnionitis [15, 26, 34]. Von Minckwitz et al. [10] could not find any significant increase in cord blood TNF-α levels in a preterm PROM group compared to a control group. While some studies showed cord blood TNF-α levels were not affected in preterm PROM with chorioamnionitis [26], some showed elevated levels [19, 35]. In term PROM, Zhang et al. [36] showed that maternal serum IL-6 and IL-8 levels and amniotic fluid IL-6, IL-8 and TNF-α levels were higher than the control patients. The levels of the cytokines were even higher in chorioamnionitis. In the present study, we could not show an increase in cord blood TNF-α with PPROM. In the present study, we have found increased levels of cord IL-8 in babies with NEC. To our knowledge, although elevated levels of cord blood IL-6 have been reported in babies who later develop NEC [22, 37] there is no other study showing elevated cord IL-8 levels in NEC patients. In conclusion, cord IL-8 levels increased significantly in PPROM and were found to be even higher in patients who later developed NEC. In addition, IL-8 levels and IL-6 levels in mothers’ serum were both elevated in the PPROM group, but TNF-α levels did not differ. Therefore, we may conclude that elevated cord blood IL-8 levels might be a predictor of NEC in premature babies.

REFERENCES


