Maternal serum proinflammatory cytokines in preterm labor with intact membranes: neonatal outcome and histological associations

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ABSTRACT. Our aim was to compare maternal serum concentrations of interleukin(IL)-1α IL-1β, IL-6 and IL-8 in pregnancies complicated by preterm labor (PTL), with the levels in healthy controls at comparable gestational age, and to determine if these assays have any value in the prediction of early-onset neonatal infection or histological chorioamnionitis. The study population consisted of 65 women with new-onset PTL, and 31 healthy controls. Maternal serum concentrations of IL-6 (8.40 versus 3.30 pg/mL; p = 0.002) and IL-1β (2.20 versus 0.50 pg/mL; p = 0.003) were significantly higher in patients with PTL as compared to healthy pregnant women. The IL-1β concentration (13.60 versus 1.20 pg/mL; p = 0.02) was significantly higher in the serum of mothers whose babies developed early-onset infections, than in mothers of newborns that were healthy. However, its predictive value, and the value of the other cytokines studied, was poor. In addition, IL-1β levels (28.79 versus 5.19 pg/mL; p = 0.001) were significantly higher in patients with histological chorionamnionitis, than in those without the condition,. The cut-off value of ≥ 14 pg/mL predicted inflammatory changes with a sensitivity of 80%, specificity of 86%, PPV of 80% and NPV of 86%. IL-1β seems to be of moderate value in the prediction of histological chorioamnionitis.

Keywords: cytokine, histological chorioamnionitis, infection, maternal serum, preterm labor

Despite advances in perinatal care, preterm labor (PTL) is still the leading cause of perinatal morbidity and mortality [1]. It complicates 5–10% of all deliveries [1]. Although different mechanisms may be responsible for preterm delivery, intrauterine infection and the secondary production of various inflammatory mediators are believed to play the main role in the pathogenesis in most cases of preterm labor. Recent investigations have determined that up to 26% of patients with preterm labor and intact membranes have a subclinical, intra-amniotic infection [2, 3].

Currently, there are no reliable clinical markers to indicate adequately subclinical infection in cases of preterm labor. Identification of such a marker would improve our ability to manage this significant perinatal problem. Numerous studies have demonstrated that subclinical intra-amniotic infection can be detected by measuring various mediators in amniotic fluid. However, obtaining amniotic fluid requires amniocentesis which is, unfortunately, an invasive technique. Therefore, alternative methods to assess the microbial status of the intrauterine environment, indirectly and non-invasively, are proposed. Analysis of maternal serum is a much simpler approach, which offers the possibility of obtaining biological material in a minimally invasive way.

Interleukins are peptidic substances released in response to various inflammatory processes [2]. Over the years, several proinflammatory cytokines in the maternal circulation, amniotic cavity and fetus, have been tested for their use in the diagnosing of intra-amniotic and neonatal infections. Some studies point out that the maternal compartment differs from the fetal compartment and that the inflammatory responses in the fetal compartment are not necessarily reflected in maternal serum. [3, 4]. However, the diagnostic value of maternal serum interleukins is not completely understood, especially in pregnancies with preterm labor and intact membranes.

The aim of this study was:

1. To evaluate and compare maternal serum concentrations of interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) in pregnancies complicated by threatened preterm labor (PTL), with the concentrations in healthy controls at comparable gestational age.

2. To determine if maternal serum concentrations of these cytokines have any value in the prediction of early-onset neonatal infection or histological chorioamnionitis.

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METHODS AND MATERIALS

A total of 96 women with singleton pregnancies and intact amniotic membranes, between 24 and 36 weeks of pregnancy, were enrolled after providing written, informed consent. The study was approved by the University Human Subject Review Committee. Gestational age was based on the last menstrual period and confirmed by early second trimester ultrasonographic examination. Sixty five women with the diagnosis of new-onset PTL made up the study group. None of these women showed clinical signs of infection or of any other maternal or fetal complications. PTL was defined as the occurrence of uterine contractions at least every ten minutes, documented, despite hydration, for one hour on an external tocodynamometer, and associated with changes in cervical effacement and dilatation. In these cases, tocolysis with the use of magnesium sulphate and/or betamimetics was performed. The control group consisted of 31 healthy women at preterm, with uncomplicated gestation.

In the study group, a detailed analysis of cytokine concentrations was carried out, and the results were compared with the presence/absence of early-onset neonatal infection or histological chorioamnionitis. Fetal outcome was evaluated by a neonatologist unaware of maternal serum cytokine results, according to the clinical signs of the neonate and its microbial status. Blood cultures were taken from all 65 infants. Neonatal congenital infection was assumed if it occurred within 48 hours of delivery, based on the maternal history and the presence of three or more of the following categories of clinical signs: skin color (pallor, jaundice, cyanosis); respiratory (apnea, tachypnea >60/min, grunting, nasal flaring, intercostal or sternal retractions, need for high ventilator settings or oxygen); cardiovascular (brady/tachycardia, poor peripheral perfusion, hypotension); neurological (hypotonia, irritability, lethargy, seizures); gastrointestinal (abdominal distension, green or bloody residuals, vomiting; temperature instability), and/or positive blood culture. Chest X-rays and laboratory tests routinely performed in the management of infection included CRP levels, white blood cell count (WBC), platelet count, and the immature to total neutrophil ratio, contributed to the diagnosis. Neonatal sepsis was recognized as proven when it was microbiologically confirmed. In other cases, it was defined as suspected (clinical) sepsis [5-7].

All infected newborns received antibiotics after delivery; two died due to neonatal sepsis. For the diagnosis of histological chorioamnionitis, microscopic analysis of the placenta was performed using more than 10 polymorphonuclear leukocytes in 10, non-adjacent microscopic fields from the extraplacental membranes, chorionic plate or umbilical cord blood vessels, and examined at × 400 magnification [8].

Cytokine levels were measured using an ELISA kit (Biotrak; Amersham Pharmacia Biotech, Buckinghamshire, England). The sensitivities of the kits were less than 1 pg/ml for IL-1 beta and IL-6, and less than 2 pg/ml for IL-1 alpha and IL-8. The WBC count was determined automatically with Celldyn 1700 and Celldyn 3500 instruments (Abbott Laboratories, IL, USA); the percentage of band cells was determined by microscopic examination, and C-reactive protein (CRP) was measured quantitatively by immunoturbidometry with the Olympus AU 560 system (Olympus Diagnostica, Hamburg, Germany). The cervicovaginal fluid was cultured for aerobic and anaerobic bacteria.

Statistical analysis was performed with the Statistica 5.5 software and MedCalc 7.2 software. The Shapiro-Wilk test was used to check the distributions of the features analyzed. Differences between the groups were assayed using the Chi-squared test for discrete variables and by the Mann-Whitney U-test for continuous variables. P-values less than 0.05 were considered significant. For all cytokines studied, a receiver operating characteristic (ROC) curve analysis was used to establish the cut-off values that optimized the prediction of neonatal infection or histological chorioamnionitis. Sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) were then calculated. Also, areas under the ROC curves (AUC) were evaluated for all study tests. Additionally likelihood ratios (LR) were calculated and values greater than 5.00 were accepted as useful.

RESULTS

The clinical characteristics of the study and control groups are shown in *table 1*.

Thirty (46.16%) women from the study group delivered prematurely; 35 of them (53.84%), after successful tocolytic treatment, delivered near term (> 36 weeks). All

Table 1
Clinical characteristics of the study and control groups

	Study group N = 65	Control group N = 31	Significance
Maternal age (years) (mean ± SD)	26.5 ± 6.4	26.7 ± 4.9	NS
Primiparous (N. %)	35 (53.8)	16 (51.6)	NS
Multiparous (N. %)	30 (46.2)	15 (48.4)	NS
Preterm delivery in anamnesis (N. %)	14 (21.5)	4 (12.9)	NS
Gestational age at sampling (weeks) (mean ± SD)	30.5 ± 3.0	28.0 ± 2.7	NS
Gestational age at delivery (weeks) (mean ± SD)	35.2 ± 4.3	38.9 ± 1.4	$p < 0.001$
Cervical dilatation (cm) (mean ± SD)	1.8 ± 1.8	0.4 ± 0.6	$p < 0.001$
Cervical length (cm) (mean ± SD)	1.5 ± 0.6	1.8 ± 0.3	$p < 0.02$
Birth weight (g) (mean ± SD)	2651 ± 962	3510 ± 456	$p < 0.001$
5 min. Apgar score (points) (mean ± SD)	8.7 ± 1.8	9.7 ± 0.6	$p < 0.001$

women from the control group delivered at term. Maternal serum concentrations of IL-6 and IL-1β were significantly higher in patients with PTL, when compared to healthy women. The levels of IL-1α and IL-8 were comparable between the groups *(table 2)*.

The pregnancies complicated by PTL were subject to particularly careful analysis. In none of these cases were clinical signs of infection found. However, in these women laboratory indices suggesting the presence of subclinical intrauterine infection were investigated by evaluating WBC count (> 15.0 G/L) [9], the band cells percentage (> 10%) [10], CRP concentration (> 10 mg/L) [11] and by cervico-vaginal secretion culture. The frequency of occurrence is shown in *table 3*. In 11 positive cultures of cervico-vaginal secretion, the following microbes were found: *Candida albicans* (5), *Bacteroides capillosus* (2), *Streptococcus B* (2), *Actinomyces naeslundii* and *Prevotella* (1), *Actinomyces naeslundii* with *Peptostreptococcus* (1). Although in 44.61% (n=29) of cases complicated by PTL, at least one laboratory parameter suggesting the presence of subclinical intra-amniotic infection was observed, the differences in maternal serum proinflammatory cytokines concentrations between the patients with and without positive laboratory indices of infection were not significant.

Out of 65 patients enrolled in the study group, 15 (23.08%) of their neonates developed early-onset infection. Neonatal blood cultures were positive only in five (33.3%) of the infected neonates: *Streptococcus agalactiae* in 3 cases and *Enterococcus foecalis* in 2 cases. In the remaining 10 (66.7%) neonates from the study group, suspected sepsis was diagnosed. Maternal serum concentrations of IL-1β were significantly higher in women who delivered newborns with signs of infection than in those who delivered healthy newborns (13.60 verus 1.20 pg/mL; $p < 0.02$). The levels of other cytokines evaluated were comparable *(table 4)*. To determine the critical values that could predict neonatal, congenital infection, ROC curves were generated for all cytokines studied. The prognostic cut-off values for these markers are shown in *table 5*. ROC curve analysis revealed that the predictive performance of all cytokines studied for predicting early-onset neonatal infection was of limited clinical value *(tables 5, 6)*.

The placentas were examined in 48 (73.85%) patients out of the study group. Inflammatory changes were found in 20 of them. In this subgroup, maternal serum concentrations of IL-1β were significantly higher than in the subgroup without histological chorioamnionitis (28.79 versus 5.19 pg/mL; $p = 0.001$) (*table 7*). The cut-off value of IL-1β ≥ 14 pg/mL predicted inflammatory changes of the placenta with a sensitivity of 80%, specificity of 86%, positive predictive value of 80%, negative predictive value of 86% and likelihood ratio of 5.62 *(table 8)*. ROC curve analysis also revealed that the diagnostic performance of IL-1β was superior to that of other study markers *(table 9)*.

DISCUSSION

In our study, maternal serum IL-6, and additionally IL-1β concentrations in patients with preterm labor and intact

Table 2
Comparison of maternal serum cytokines between the study and control groups

	Study group (n = 65) Median	Control group (n = 31) Median	P-value
IL-1 alpha (pg/mL)	2.75	1.75	0.12
IL-1 beta (pg/mL)	2.20	0.50	0.003
IL-6 (pg/mL)	8.40	3.30	0.002
IL-8 (pg/mL)	4.29	3.90	0.73

Table 3
Results of laboratory indices suggesting the presence of infection at the onset of preterm labor

	Preterm labor (study group) N = 65	
	N	%
White blood cells > 15.0 G/L	8	12.31
Band forms > 10%	25	38.46
CRP > 10 mg/L	20	37.77
Positive cervico-vaginal secretion culture	11	16.92

Table 4
Maternal serum proinflammatory cytokines in preterm labor in relation to congenital infection of the newborn

	Healthy newborn (n = 50) Median	Congenital infection (n = 15) Median	P-value
IL-1 alpha (pg/mL)	2.85	2.75	0.81
IL-1 beta (pg/mL)	1.20	13.60	0.02
IL-6 (pg/mL)	7.35	16.50	0.19
IL-8 (pg/mL)	4.36	3.90	0.96

Table 5
The prognostic value of evaluation of maternal serum proinflammatory cytokines in preterm labor for prediction of congenital infection of the newborn

Concentration	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR
IL-1 alpha ≥ 20 pg/mL	33	78	31	80	1.52
IL-1 beta ≥ 14 pg/mL	40	84	43	82	2.50
IL-6 ≥ 40 pg/mL	27	90	44	80	2.67
IL-8 ≥ 5 pg/mL	40	54	21	75	0.87

PPV – positive predictive value; NPV – negative predictive value; LR – likelihood ratio.

Table 6
Evaluation of areas under the ROC curve for maternal serum proinflammatory cytokines in preterm labor, for the prediction of congenital infection of the newborn

	IL-1 alpha	IL-1 beta	IL-6	IL-8
AUC	0.52	0.69	0.62	0.51
95%CI	0.39-0.68	0.56-0.80	0.49-0.73	0.38-0.63
SE	0.086	0.083	0.086	0.086

AUC – area under curve; CI – confidence interval; SE – standard error.

Table 7
Maternal serum proinflammatory cytokines in preterm labor in relation to the presence of inflammatory changes in the placenta

	Lack of changes (n = 28) Median	Inflammatory changes (n = 0) Median	P-value
IL-1 alpha (pg/mL)	5.07	57.12	0.18
IL-1 beta (pg/mL)	5.19	28.79	0.001
IL-6 (pg/mL)	18.11	17.41	0.54
IL-8 (pg/mL)	5.31	2.17	0.45

Table 8
The prognostic value of evaluation of maternal serum proinflammatory cytokines in preterm labor for prediction of inflammatory changes in the placenta

Concentration	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR
IL-1 alpha ≥ 20 pg/mL	62	77	65	71	2.77
IL-1 beta ≥ 14 pg/mL	80	86	80	86	5.62
IL-6 ≥ 40 pg/mL	42	83	65	65	2.83
IL-8 ≥ 5 pg/mL	33	52	33	52	0.62

PPV – positive predictive value; NPV – negative predictive value; LR – likelihood ratio.

Table 9
Evaluation of areas under the ROC curve for maternal serum proinflammatory cytokines in preterm labor, for prediction of inflammatory changes in the placenta

	IL-1 alpha	IL-1 beta	IL-6	IL-8
AUC	0.65	0.88	0.59	0.41
95%CI	0.41-0.81	0.69-0.98	0.34-0.76	0.20-0.63
SE	0.114	0.077	0.120	0.119

AUC – area under curve; CI – confidence interval; SE – standard error.

membranes were significantly higher when compared to healthy women at comparable gestational age. This is in agreement with the reports of Turhan *et al*. [12] who found significantly higher IL-6 levels in a study group of 82 patients in preterm labor than in healthy controls. Alvarez-de-la-Rosa *et al*. [2] also noted that the concentration of maternal serum interleukins 1, 6 and 8 in women at 26-37 weeks who were in preterm labor, in comparison to those who were not in labor, was elevated although not significantly. Minckwitz *et al*. [13] found significantly increased serum levels of IL-6 and IL-8 in patients with preterm labor or preterm rupture of the membranes when compared to the control group. However, in their study interleukin-1β did not correlate with clinical outcome.

In most of the recent studies, maternal serum proinflammatory cytokine levels were mainly evaluated as regards neonatal outcome in cases of amniorrhexis. Pfeiffer *et al*. [14] showed that determination of IL-6 in maternal serum can contribute significantly to an earlier detection of fetal infection in patients with PROM. At a cut-off of 11 pg/mL, IL-6 reached a sensitivity of 81% and a specificity of 76% in the prediction of neonatal infection. In their study, IL-8 results showed a less significant correlation with fetal outcome. In the study of Lewis *et al*. [15], the presence of IL-6 in the maternal plasma, obtained prior to delivery in patients with preterm premature rupture of membranes between 24 and 35 weeks' gestation, predicted neonatal complications. Hatzidaki *et al*. [5] concluded that IL-6 concentrations in maternal blood, taken during delivery, are a very sensitive, reliable, and early marker of neonatal sepsis and can provide an accurate indication of whether a neonate will develop early sepsis, thus, offering the opportunity for prompt diagnosis and aggressive therapeutic intervention. In their study, receiver-operating characteristic analysis revealed that using a cut-off concentration of 81 pg/ml for maternal serum IL-6, resulted in sensitivity of 90%, specificity 97.4%, positive predictive value 94.7%, and negative predictive value 94.9% for neonatal sepsis. Recently, Sorokin *et al*. [16] observed that elevated maternal serum IL-6 concentration is risk factor for preterm birth < 32 weeks and subsequent development of neonatal intraventricular hemorrhage. Conversely, Bahar *et al*. [1] stated that maternal serum levels of IL-6 and IL-8 were not increased in preterm labor compared to normal control women. They concluded that there is doubt regarding the usefulness of maternal serum measurement of these cytokines for the detection of early fetal infection in preterm labor.

Our study revealed that, only the IL-1β concentrations were significantly higher in the serum of mothers whose babies developed early-onset infection than in mothers whose newborns were born healthy. However, its predictive values, as well as the values of the other cytokines studied, were poor.

Only a few previous studies have evaluated the relationship between maternal serum cytokines to histological chorioamnionitis. Salafia *et al*. [4] evaluated concentrations of IL-1β and IL-6 in maternal serum from 32 consecutive patients at 20-36 weeks were experiencing progressive labor and tocolytic failure, and observed that serum levels of these cytokines were not associated with the presence or severity of histological evidence of acute placental inflammation. Nowak *et al.* [17] observed that maternal serum levels of IL-6 and IL-8 during both term and preterm labor were elevated in comparison to levels found in pregnant women who were not in labor. However, the elevated levels of these cytokines were not connected to any histological chorioamnionitis. Wenstrom *et al*. [18] considered that women with subclinical inflammation might be identified by analysis of the amniotic fluid following amniocentesis and, unfortunately, cannot be identified by maternal serum Il-6 levels before the procedure. These studies point out that the maternal compartment differs from the fetal compartment and the inflammatory responses in the fetal compartment are not necessarily reflected in maternal serum [1]. On the other hand, Greig *et al*. [19] stated that women in preterm labor and who delivered preterm with evidence of histological chorioamnionitis had significantly higher serum concentrations of interleukin-6 than did those in preterm labor who delivered in the absence of chorioamnionitis. In their study, the cut-off value of IL-6 > 6 pg/mL predicted placental inflammatory changes, with a sensitivity of 100%, specificity of 67%, PPV 88% and NPV of 100%. Previously, the same group of researchers [20] reported that maternal serum interleukin-6 concentrations are elevated in patients with preterm, premature rupture of membranes with histological chorioamnionitis. They suggested that evaluation of this cytokine in maternal serum may enable physicians to diagnose infection before the onset of clinical symptoms. Recently, Murtha *et al*. [21] reported that maternal serum IL-6 appears to be a biomarker for the identification of women with preterm, premature rupture of membranes likely to develop funisitis. Maeda *et al*. [22] also evaluated whether maternal serum IL-6 and IL-8 levels are useful for the diagnosis of histological chorioamnionitis. They examined blood samples and placentas from 29 women who delivered preterm between 22 and 34 weeks of gestation and found that only IL-6 determinations are useful for the prediction of placental changes. Oleszczuk *et al*. [23] found elevated IL-6 levels in maternal serum from pregnant women with signs of preterm labor and with laboratory markers of infection. They concluded that this cytokine may be the marker of preterm labor caused by infection. Shimoya *et al*. [24], who examined serum from 22 mothers with chorioamnionitis and 81 mothers without chorioamnionitis at term delivery, reported elevated concentrations of IL-8, but no increase in the concentration of IL-1α, IL-1β or IL-6. They concluded that measurement of maternal IL-8 is useful for rapid, prenatal screening for histological chorioamnionitis at term.

In our study, only maternal serum levels of IL-1β were significantly higher in patients with histological chorionamnionitis than in those without the condition. The cut-off value of this cytokine, ≥ 14 pg/mL, seemed to be a good predictor for the prognosis of inflammatory changes in the placenta, with a sensitivity of 80%, specificity of 86%, PPV of 80%, NPV of 86% and LR 5.60. The AUC was also the greatest for IL-1β, which confirms this marker as the best predictor of histological chorioamnionitis. We identified IL-1β as being a more sensitive marker of histological chorioamnionitis than other cytokines studied, including IL-6. This discrepancy, between ours and others' findings, could be explained by the fact that our samples were taken early, immediately after establishing the diagnosis of new-onset, preterm labor, none of the patients having clinical signs of chorioamnionitis. It is known that the increase in serum IL-1β concentrations occurs earlier, before that of IL-6 in response to infection [25], and that IL-1 β stimulates the production of other cytokine mediators [26-29].

In conclusion, our observations suggest that the evaluation of maternal serum proinflammatory cytokines, obtained shortly after establishing the diagnosis of new-onset, preterm labor, is of limited value. Only IL-1β seems to be moderately valuable in the prediction of histological chorioamnionitis. However, this observation needs further investigation.

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