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Interleukin-6 and C-reactive protein levels in the amniotic fluid as indicators of preterm delivery in Turkish women

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Summary

Objective: The aim of this study was to determine the value of amniotic fluid interleukin-6 (IL-6) and C-reactive protein (CRP) levels in the prediction of preterm delivery in singleton pregnancies without any known risk factors for preterm delivery in Turkish women. *Materials and Methods:* Patients in the present perinatology department who underwent mid-trimester genetic amniocentesis due to evidence of increased risk of aneuploidy in their prenatal serum screening tests were included in the study. A sample of amniotic fluid from each patient was assessed for IL-6 and CRP. Concentrations of IL-6 and CRP in the amniotic fluid of preterm delivery and term delivery groups were compared. *Results:* Of 151 singleton pregnancies, 142 participants were included in the study. The participants were assigned to either the preterm or term delivery group based on pregnancy outcome. IL-6 levels in the amniotic fluid were significantly higher in the preterm delivery group, and there was a statistically significant negative correlation between IL-6 concentrations in the amniotic fluid and gestational age at delivery (correlation coefficient (CC): -18.5%, $p < 0.05$). A negative correlation was also detected between CRP levels in the amniotic fluid and gestational age at delivery, but the correlation was not statistically significant ($p = 0.068$). *Conclusion:* Measuring IL-6 in the amniotic fluid can identify women at risk of preterm delivery. Because it is not acceptable to perform amniocentesis for this screening, it is more convenient for patients in whom genetic amniocentesis is performed.

Key words: Preterm delivery; Interleukine-6; C-reactive protein; Midtrimester amniocentesis.

Introduction

Preterm birth, defined as delivery that occurs prior to 37 weeks of gestation, accounts for approximately 10% of all pregnancies [1]. Prematurity and prematurity-related complications are the major cause of infant mortality and morbidity [2, 3]. The survival rate of a premature infant depends primarily on birth weight and gestational age. The survival rate is greater than 90% after the 30th gestational week, but less than 10% before the 24th week [4]. Preterm parturition is a syndrome rather than a diagnosis, as it encompasses several different conditions. Preterm deliveries prior to 32 weeks of gestation frequently involve infections, infant morbidity, and higher long-term sequel risk, and they tend to recur in subsequent pregnancies.

Bacterial infection is one of the most important mechanisms related to preterm delivery [5,6]. It has been shown that infections are responsible for at least 35–40% of all preterm deliveries. Bacterial chorioamnionitis is the main cause of infection-related preterm labor. Amniotic fluid is supposed to be sterile, and bacteria are detected in less than 1% of women at term who are not in active labor. The presence of bacteria in the amniotic cavity is a pathologic condition known as microbial invasion of amniotic cavity (MIAC). Most MIAC cases are subclinical and cannot be diagnosed unless a microbial study of the amniotic fluid is performed. MIAC alone is not sufficient reason to promote preterm labor, as bacterial presence in the chorioamniotic membranes does not always induce a maternal and/or fetal inflammatory response [7].

An elevated level of interleukin-6 (IL-6) in the amniotic fluid is an indicator of an inflammatory process in the amniotic cavity and, in most cases, is related to bacterial infection [8-11]. IL-6 is released from either T-lymphocytes or macrophages, and it can act as both proinflammatory and anti-inflammatory cytokines. IL-6 is one of the most important cytokines that play a major role in acute phase response and fever pathogenesis. C-reactive protein (CRP) is a prototype for acute phase reactants. It is named C-reactive protein because it binds to the "capsule" antigen of pneumococci [12]. In fact, CRP binds to the phosphocholine on the surface of most bacteria, fungi, and parasites and causes complement activation to eliminate the circulating antigens [13]. High-sensitive CRP (hs-CRP) is a more accurate method of quantifying CRP that is sensitive to concentrations lower than 0.4 mg/L. Based on this information, the purpose in this study was to predict preterm delivery rates in the antenatal population without any known risk factors for preterm delivery by measuring IL-6 and CRP levels in the amniotic fluid.

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Materials and Methods

For this prospective study, amniotic fluid samples were collected from patients who underwent mid-trimester genetic amniocentesis in the perinatology department of the present tertiary care hospital between January and March 2011. Clinical data were approved by the Institutional Review Board of our hospital. All of the patients provided written informed consent in accordance with the Helsinki criteria.

All amniocentesis procedures were performed under transabdominal ultrasound guidance, and in all cases, detailed ultrasonography was performed to assess the fetal anatomy and determine the location of the placenta. The amniocentesis procedures were performed via a 21-gauge amniocentesis needle. The first one to two cc of amniotic fluid were discarded to avoid possible blood contamination; the following 18–20 cc were used for the genetic study; and the last two cc were used to determine IL-6 and CRP levels. The amniotic fluid samples were stored at -80°C until analysis.

The inclusion criteria were as follows: singleton pregnancy; age 18–45 years; mid-trimester genetic amniocentesis. Exclusion criteria included: known systemic disease or infection; use of antibiotics for any reason in the previous month; major fetal malformation detected in the ultrasound examination prior to amniocentesis; abnormal fetal karyotype; pregnancy loss during the first month after the amniocentesis (possibly procedure related); iatrogenic preterm delivery due to maternal or fetal indications such as preeclampsia, intrauterine growth restriction, gestational diabetes, or amniotic fluid disorders;

Pregnancy outcomes were obtained by accessing labor and delivery records or by contacting the patient if the delivery was not in the present hospital. IL-6 quantitative measurements were performed with an immunoassay system, and hs-CRP quantitative measurements were performed with a chemistry analyzer using a Tina-quant cardiac C-Reactive protein high-sensitivity kit.

Statistical analysis

Results are expressed as mean and standard deviation according to the distribution of data. Kolmogorov Smirnov's test was used to evaluate the normality of the distribution of the continuous data. The Mann–Whitney *U* test, Student's t test, and Spearman's rho correlation test were used according to the distribution of the variables (Mann-Whitney U test for continuous variables without normal distribution, and Student's t test for normal distributed variables) for the comparison and correlation of proportions. The diagnostic value of IL-6 and CRP levels for the prediction of preterm delivery was evaluated using receiver operating characteristic (ROC) curves. Sensitivity, specificity, and negative and positive predictive values were calculated for optimal cutoffs. The data were analyzed using SPSS 17 software.

Results

Amniocentesis was performed on 151 pregnant women with singleton gestations who met the inclusion criteria; the amniotic fluid samples were studied immediately after the procedure. Amniocentesis was performed when there were proper clinical indications, such as suspected fetal anomalies, family history of chromosomal abnormalities, advanced maternal age, and abnormal first or second trimester screening test. Nine patients were excluded from the study: two patients for intrauterine exitus, one patient for immune hydrops fetalis, three patients for undergoing a cesarean section due to a diagnosis of severe preeclampsia prior to 37 weeks of gestation, and three pregnancies were terminated due to diagnoses of Down syndrome according to amniocentesis. Thus, 142 patients' results were analyzed in this study.

Table 1. — *Demographic and clinical features of the subjects.*

Clinical characteristics	Preterm delivery N=15	Term delivery N=127	*p**
Maternal age (wks)	32.6 ± 6.1	31.1 ± 6.2	> 0.05
Gravidity	2.4 ± 1.2	2.1 ± 0.9	> 0.05

**p* Mann-Whitney *U* Test.

Table 2. — *IL-6 and CRP levels in the amniotic fluid in the preterm and term delivery groups.*

	Preterm delivery N=15 (10.6%) Mean±SD	Term delivery N=127 (89.4%) Mean±SD	*p*
CRP (mg/L)	0.15 ± 0.03	0.10 ± 0.009	0.527
IL-6 (pg/ml)	473 ± 346	313 ± 208	0.001[a]

[a]$p < 0.05$, Mann–Whitney *U* Test.

Table 3. — *Coordinates for sensitivity, specificity, PPV, and NPV to predict preterm delivery using IL-6 levels in the amniotic fluid.*

IL-6 threshold level (pg/ml)	Sensitivity %	Specificity %	NPV %	PPV %
228.5	93.3	52.8	98.5	18.9
360.5	80	72.4	96.8	25.5

The demographic and clinical characteristics of the subjects are shown in Table 1. The participants were 18–42 years of age (mean 31±6.25); 61.9% were over 30 years old. The authors found no correlation between maternal age and gestational age at delivery. Total gestational duration was 245–297 days according to last menstrual period or first trimester ultrasound imaging (mean gestational age 272±10 days). Fifteen patients (10.56%) who delivered prior to 259 days formed the preterm delivery group; 127 patients (89.44%) delivered at term. The preterm delivery rate in the present study was similar to that reported in the literature [14].

IL-6 levels in the amniotic fluid were significantly higher in the preterm delivery group than in the term delivery group. No correlation was found between CRP levels in the amniotic fluid and preterm delivery (Table 2).

There was a statistically significant negative correlation between IL-6 concentration in the amniotic fluid and gestational age at delivery (correlation coefficient (CC): -18.5%, $p < 0.05$). A negative correlation was detected between CRP levels in the amniotic fluid and gestational age at delivery, but the correlation was not statistically significant ($p = 0.068$).

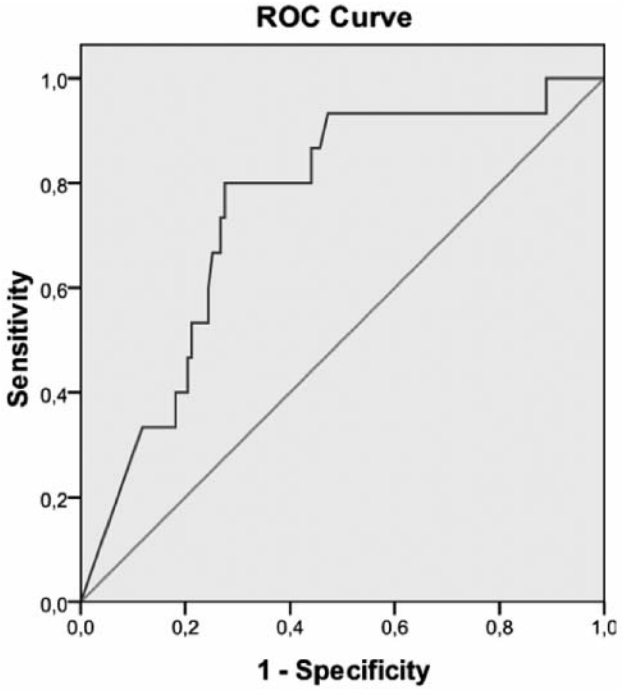

Figure 1. — ROC curve for negative correlation between IL-6 levels in the amniotic fluid and gestational age at delivery.

If a 360.5 pg/ml threshold was determined for IL-6 level in the amniotic fluid, preterm delivery would then predicted with 80% sensitivity and 72.4% specificity with 95% confidence interval (CI). Positive predictive value (PPV) and negative predictive value (NPV) would then be 96.8% and 25.5%, respectively. Preterm delivery would be predicted with 93% sensitivity and 52.8 specificity with 95% CI if the threshold level was determined at 228.5 pg/ml for IL-6 level in the amniotic fluid. PPV and NPV would then be 18.9% and 98.5%, respectively (Figure 1 and Table 3).

Discussion

Prematurity and prematurity-related neonatal complications are known to be the most important reasons for neonatal mortality and morbidity in the past decade. Various markers and methods that can be measured during the antenatal period have been put forward to prevent prematurity and prematurity-related neonatal complications. Inflammatory cytokines, especially IL-6, have become more of an issue recently, and recent studies have suggested that IL-6 is a promising marker for predicting preterm delivery [15, 16]. In these studies, not only were IL-6 concentrations in the amniotic fluid significantly higher in the preterm delivery group than in the term delivery group, but they also showed a negative correlation with gestational age. Cobo *et al.* stated the superiority of amniotic fluid IL-6 levels to predict microbial invasion of amniotic fluid as an inflammatory biomarker [17].

According to the present literature search, 32 studies in the literature associated IL-6 levels in the amniotic fluid with preterm delivery; however, only six of the studies investigated second-trimester singleton pregnancies [16, 18-21]. One of the most powerful and well-designed studies was conducted by Wenstrom *et al.* in 1998 [19]. In that study, the researchers determined a cutoff level of 250 pg/ml second trimester amniotic fluid IL-6 to predict preterm delivery. In the preset study, if the cutoff level was set at 228 pg/ml, the sensitivity of IL-6 for predicting preterm delivery was calculated as 93.3% (14 out of 15 preterm pregnancies). These data are also similar to the study published by Thomakos *et al.* in 2010 [16]. Cobo *et al.* reported 46% sensitivity and 93.8% specificity to predict preterm delivery, as well as 52.6% NPV and 92% PPV, when the cutoff level for amniotic fluid IL-6 level was determined to be 134 pg/ml [22]. Kim *et al.* showed significantly higher levels of amniotic fluid IL-6 levels in midtrimester amniocentesis for preterm delivery group and determined the cut-off value of 134.35 pg/ml with a sensitivity of 77.8% and a specificity of 61.1% for preterm delivery, slightly lower than the present cut-off value [21]. This may be due to higher overall amniotic fluid IL-6 levels in Turkish women. However, cytokine response to the inflammatory process may be different for each individual; some individuals may not show a cytokine response to inflammatory processes as others do.

There are fewer published studies regarding the relationship between CRP levels in the amniotic fluid and preterm delivery. The present research of the literature identified only two published studies on mid-trimester CRP levels in the amniotic fluid and preterm delivery [23, 24], only one of which was designed as a prospective study [23]. Özer *et al.* reported 92.9% sensitivity and 78.7% specificity with 99% NPV and 32.5% PPV for predicting preterm delivery if a 0.65-mg/L cutoff value was set for CRP concentration in the amniotic fluid. In addition, Ghezzi *et al.* reported that CRP concentrations in the amniotic fluid were found to be higher in the preterm delivery group [24]. However, several studies in the literature found no significant correlation between amniotic fluid CRP concentrations and preterm delivery, as in the present study [25]. These findings suggest that the inflammatory process of the fetomaternal unit is more important than the maternal systemic inflammatory response in the etiology of preterm delivery. Özer *et al.* suggested that the high-risk population for preterm delivery could be predicted by determining mid-trimester CRP levels in the amniotic fluid, and that proper precautions could take months before delivery. Other reports in the literature concluded that CRP is a commonly used laboratory parameter predicting inflammatory response, but measurement of CRP is very non-specific and therefore unreliable in predicting preterm labour [21, 23, 26].

Conclusion

The main purpose of the present study was to predict preterm delivery with a reasonable sensitivity and specificity

in the antenatal population without any risk factors for preterm delivery. For this purpose, the authors examined IL-6 and CRP levels in amniotic fluid. Determining IL-6 and CRP levels in amniotic fluid for genetic investigation is low in cost, and it is an easy procedure to perform; it increases the cost of the second trimester genetic amniocentesis procedure by only 2–3%. While some studies in the literature have shown that IL-6 and CRP levels in amniotic fluid are useful for predicting preterm delivery, other studies have indicated that these markers are useless for this purpose. In the present study, IL-6 levels in the amniotic fluid were significantly higher in the preterm delivery group. CRP levels in the amniotic fluid were also higher in the preterm delivery group, but the difference was not statistically significant. In addition, in the preterm delivery group, amniotic fluid IL-6 levels increased as gestational age at delivery decreased. According to the results of the present study, IL-6 measurement in the amniotic fluid seems to be an appropriate screening test to exclude preterm delivery rather than a predictor, because it has a higher sensitivity and NPV; amniotic fluid IL-6 study may be reasonable for patients in whom genetic amniocentesis is performed. In conclusion, further prospective studies are needed to detect a more solid relation between IL-6 levels in amniotic fluid and preterm delivery.

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