Comparison of serum granulocyte colony-stimulating factor levels between preterm and term births

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Abstract

Objective: Preterm birth (PTB) is the major obstetric problem in developed countries, accounting for the majority of neonatal mortality and morbidity. Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine that mediates the increase in leukocytes in pregnancy and may play a role in placentation. We aimed to investigate the differences of serum G-CSF levels between subsequent spontaneous PTB and term-delivered healthy pregnant women.

Material and Methods: Serum samples, collected from total of 600 singleton otherwise healthy pregnants at 24-28 weeks of gestation during a routine antenatal visit, were used to assess G-CSF levels; 40 of the total pregnants who delivered their infants spontaneously after preterm labor before 37 weeks of gestation were selected as the study group. Also, 120 pregnants were selected as a control group using a 1/3 ratio. Student's t-test, chi-square test, Mann-Whitney U-tests, and ROC curve analysis for prediction of PTB were used for the comparison of groups. $P<0.05$ was accepted as statistically significant.

Results: There was no significant difference in maternal serum G-CSF levels between the study and control groups ($p=0.28$) but maternal white blood cell (WBC) count was significantly different between them ($p=0.00$). In addition, G-CSF was insufficient in the prediction of PTB (AUC=0.419). In the preterm and term groups, no correlation was found between WBC and G-CSF ($p=0.165$ vs. $p=0.703$).

Conclusion: There were no differences in serum levels of G-CSF between term- and preterm-delivered pregnants. There was no predictive role for serum G-CSF in PTB. (J Turk Ger Gynecol Assoc 2014; 15: 208-11)

Key words: Preterm birth, granulocyte colony-stimulating factor, newborn infants

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Introduction

Preterm birth (PTB), or delivery before 37 weeks of gestation, is a major obstetric issue in developed countries, being responsible for most neonatal mortality and a considerable proportion of long-term neurological problems (1). Despite significant efforts to elucidate the molecular mechanisms that cause PTB, its etiology remains unclear (2). It has been proposed that there are certain proinflammatory mediators, including granulocyte colony-stimulating factor (G-CSF), that are involved in the mediation of events leading to PTB development (3-5). G-CSF is a hematopoietic cytokine affecting the proliferation, differentiation, and survival of neutrophil progenitors. G-CSF and its receptor (G-CSFR), which mediates its activity, are expressed at the maternal-fetal interface (6), G-CSF being produced by the placenta (7) and G-CSFR being present on trophoblast cells. G-CSF is believed to have an important role in pregnancy outcomes through the regulation of placentation (6).

Increased levels of G-CSF have been found in preterm infant serum in comparison with infants who are born at term (3). According to Seremak-Mrozikiewicz et al. (4), with a small number of cases, G-CSF levels in amniotic fluid were found not to differ between full-term and preterm pregnancies, although a relationship between maternal serum and amniotic fluid G-CSF levels and infection in utero was demonstrated. Plasma G-CSF level samples from 24 to 28 weeks of pregnancy were found to be a predictor of spontaneous PTB for deliveries earlier than 32 weeks of pregnancy (5). It is unclear whether increased G-CSF at 24-28 weeks is indicative of an acute influence or a cumulative influence of inflammation in the development of PTB or whether G-CSF makes a contribution as an indicator of disease progression. Another study (8) showed an association of PTB with high serum G-CSF levels at the beginning of the second trimester, implying that PTB may be the result of events starting early in pregnancy, if not before.

In this study, we planned to investigate differences in plasma granulocyte colony-stimulating factor between consecutive spontaneous preterm births and births that were delivered at term in healthy pregnant women, who were screened routinely at 24-28 weeks of gestation.

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Material and Methods

This study was conducted between January 2011 and July 2011 in our perinatology clinic. Ethics approval was obtained from our institutional review board. Informed consent was given by all study participants. The study was designed as a prospective case-control study. PTB was defined as a delivery that occurs before the 37th week of gestation (9, 10). Inclusion criteria in the study group were singleton pregnancies with a diagnosis of preterm labor leading to preterm birth at or before 37 weeks of gestation. Women who had preterm premature membrane rupture (P-PROM), clinical signs of intrauterine infection (uterine tenderness, fetal or maternal tachycardia, maternal fever, etc.), or bacterial infection in the cervical/vaginal culture were not included in the study or control groups. Also, women who had a history of maternal cardiac disease, cervical incompetence, cervical cerclage or conization, fetal anomaly, or vaginal bleeding were excluded from the study and control groups.

A total of 600 singleton pregnant women were screened at 24-28 weeks of gestation and routinely followed up at the outpatient clinic until delivery. Demographic values of all pregnant women were recorded. Forty pregnant women delivered their infants spontaneously after preterm labor before 37 weeks of gestation. Also, 120 pregnant women were selected as a control group among these 560 women via a simple random sampling method, who delivered their infants after 37 weeks of gestation. All participants had 24-28-week plasma samples available for granulocyte colony-stimulating factor analysis.

For both groups, maternal blood samples were collected at 24-28 weeks of gestation during a routine prenatal screening program in the outpatient clinic. Venous blood samples from patients were collected into sterile, silicone-coated tubes, centrifuged, and stored at -70°C. Maternal serum G-CSF levels were measured with the G-CSF Human Enzyme-Linked Immunosorbent Assay (ELISA) kit (Novex®; Invitrogen, Los Angeles, USA).

The Statistical Package for the Social Sciences 14.0 (SPSS Inc.; Chicago, IL, USA) statistical package was used for the statistical analysis. Descriptive statistical methods (mean, standard deviation), as well as a comparison of quantitative data, Mann-Whitney U-test, chi-square test, and Student's t-test, were used for the determination of differences between the two groups. Statistically significant levels for the tests were set at a p value <0.05. Spearman's rank correlation coefficient rho was used for the correlation study.

Results

The characteristics of 160 women and the comparison of the two groups are shown in Table 1. The difference of the mean age of the women between groups was not statistically significant (p=0.8). There were no significant differences in maternal serum G-CSF levels between groups (p=0.28).

Smoking status was different between cases of PTB and full-term pregnancies, but a statistically significant difference was not found (p=0.284).

ROC curve analysis was performed for the prediction of PTB, but unfortunately, the area under the curve was 0.419, and G-CSF was found to be insufficient for the prediction of PTB. Therefore, there was no need to calculate the sensitivity and specificity of this marker for PTB birth (Figure 1).

As expected, there was a significant difference between the study and control groups in the requirement for neonatal intensive care. We observed ratios of 47.5% versus 0% in the study and control groups, respectively (p=0.03).

In the preterm and term groups, no correlation was found between WBC and G-CSF (p=0.165 vs. p=0.703) (Table 2).

Table 1. Characteristics of values in the overall population and comparison between the two

	Preterm (n=40)	Term (n=120)	p value
Age (years)	27.7±5.9	27.4±4.8	0.821[a]
Height (cm)	161.2±4.26	161.4±6.13	0.866[a]
Weight (kg)	70.8±9.48	67.37±10.68	0.128[a]
Birth weight (gr)	2065±883	3590±408	0.00[b]*
Birth week (weeks)	32±3.98	39.5±1.38	0.00[b]*
G-CSF	25.43±1.42	25.77±1.39	0.28[a]
WBC	13.2±5.65	9.8±2.4	0.00[b]*

[a]Independent-sample t-test, [b]Mann-Whitney U-test

Values as mean±standard deviation (SD) or median±interquartile range (IQR)

*Significant if p<0.05

G-CSF: granulocyte colony-stimulating factor; WBC: white blood cell

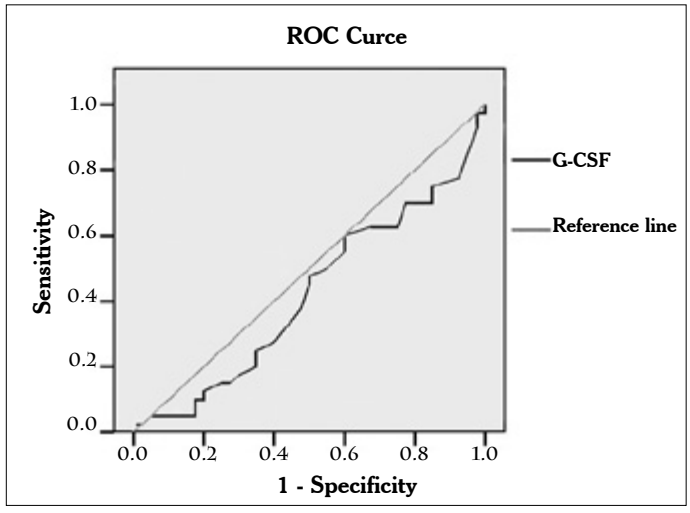

Figure 1. ROC curve analysis for G-CSF

Table 2. Correlation in both groups for WBC and G-CSF

	rho	p
Preterm	0.224	0.165
Term	-0.62	0.703

Spearman's correlation coefficient used.

G-CSF: granulocyte colony-stimulating factor; WBC: white blood cell

Discussion

Preterm labor still represents one of the major obstetric problems in developed countries and leads to poor results for the pregnancy and for the newborn's outcome. For reducing the proportion of preterm births and for their prediction, our efforts seem to be insufficient, even though, according to the literature, many studies on this topic have been conducted previously. G-CSF is a marker associated with inflammatory processes (11). Recent studies suggest that the majority of early spontaneous preterm births is associated with, and probably caused by, bacterial infection within the chorion-decidual interface (12). This infection causes an inflammatory response modulated by various cytokines, one of which is G-CSF (13), and elevated levels have also been observed in association with many other types of inflammatory processes.

We proposed to evaluate any differences in G-CSF levels between term- and preterm-delivered pregnant women. In the literature, some studies, such as Goldenberg et al., observed higher serum levels of G-CSF in early preterm-delivered patients (5), although other studies could not find any difference in serum levels of G-CSF between preterm- and term-delivered women (4). In our study, we observed no significant differences in serum levels of G-CSF at 24-28 weeks of pregnancy between term- and preterm-delivered women. It is well known that infection may cause an increase in maternal serum and amniotic fluid G-CSF values. In a literature search, the existence of G-CSF was described during clinically diagnosed intra-amniotic infection in amniotic fluid (14-17), and its relationship to labor was reported as either increased serum levels of G-CSF or no difference between term- and preterm-delivered patients (4, 5). In our study, any participant with any sign of infection was excluded in order to eliminate a bias in G-CSF levels. We considered an insufficiency in our study to be that G-CSF levels were evaluated only in maternal serum, not in amniotic fluid or in umbilical cord blood. It may have provided different results if a detailed examination of G-CSF levels was performed in body fluids, making it easier to determine any relationship between infection and serum G-CSF levels leading to preterm birth.

White blood cell count is increased in pregnancy (18). Leukocytosis, occurring during pregnancy, is due to the physiologic stress induced by the pregnant state (19). A normal WBC value for third trimester is between 5.6 to $16.9 \times 10^3/mm^3$ (20). In our study, white blood cell count was significantly higher in preterm-delivered women compared to term-delivered participants, although no significant correlation was found between the two parameters in either the term- or preterm-delivered groups, even though we excluded patients who had any sign of infection. These differences were not considered clinically significant, because it was in the normal range for the pregnancy state.

A weakness of our study was the limited number of subjects fulfilling the inclusion criteria.

In conclusion, we found no differences in serum levels of G-CSF between term- and preterm-delivered pregnant women. Previous studies on this issue in the literature are conflicting. All of the findings above suggest that a predictive role of serum G-CSF in PTB is questionable. Therefore, we consider that to reach a definitive conclusion, further studies should be performed to evaluate the conflicting results on the relationship between G-CSF levels and preterm and term labor.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Institutional Review Board.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - Ç.K., M.U.; Design - Ç.K., M.U.; Supervision - M.U., B.S.Ü; Resource - Ç.K.; Materials - Ç.K., B.S.Ü.; Data Collection&/or Processing - Ç.K., İ.A., P.K.; Analysis&/or Interpretation - B.S.Ü., Y.Y.; Literature Search - P.K., K.T.; Writing - B.S.Ü., Y.Y.; Critical Reviews - M.U., İ.A., K.T.*

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