

Levels of IL-6 and IL-1 β in Umbilical Cord Blood in Relation to Mode of Birth

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OBJECTIVE: The present study aims to determine whether normal spontaneous vaginal delivery at term was associated with an inflammatory activation compared with scheduled cesarean section without previous onset of labor by detecting cord blood serum levels of interleukin-6 (IL-6) and interleukin-1 β (IL-1 β).

STUDY DESIGN: Eighty-eight patients were studied (vaginal delivery n=44 and scheduled cesarean n=44) with cord venous blood sampling after delivery of the fetus. IL-6 and IL-1 β levels were measured with commercial kits. All of the subjects had uncomplicated term singleton pregnancies.

RESULTS: Both study groups showed no significant difference in maternal age, gestational week, birth weight, body mass index ($p>0.05$). Cord blood IL-6 and IL-1 β levels were similar between spontaneous vaginal delivery and scheduled cesarean groups (3.1 ± 2.0 pg/mL versus 3.1 ± 2.9 pg/mL, $p=0.62$ and 2.0 ± 1.7 pg/mL versus 2.3 ± 1.8 pg/mL, $p=0.46$, respectively). Also maternal age, gestational week, birth weight and body mass index did not correlate with cord blood cytokine levels.

CONCLUSION: In healthy and term pregnancies, the exposition of spontaneous vaginal delivery and labor is not associated with activation of inflammatory mediators compared to cesarean. The data of the present study did not support the increasing ratio of newborn complications (respiratory distress syndrome, bronchopulmonary dysplasia, the need for prolonged oxygen therapy and neonatal intensive care unit, allergic diseases of infancy period) after cesarean delivery due to inadequate increasing levels of cytokines in newborns delivered by cesarean.

Key Words: Cytokines, Mode of birth, Cord blood, Inflammation

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Introduction

The mechanisms underlying the onset of spontaneous labor and delivery still remain unclear. The common pathway of labor is a complex process mediated by different physiological, biochemical, endocrinological, and immunological events. Inflammatory pathway mechanisms have been suggested to involve in the onset and progression of human labor and delivery in patients with preterm labor as well as sponta-

neous parturition at term in the presence or absence of evident infection.¹⁻⁴

Inflammatory activation at term labor was shown to be associated with a massive influx of immune cells into the gestational tissues characterized by an increased local synthesis of cytokines and chemokines.⁵ Cytokines, mainly the proinflammatory ones, interleukin-6 (IL-6) and interleukin-1 β (IL-1 β) have been suggested to play an important role during preterm and term parturition. These cytokines stimulate prostaglandin biosynthesis; thus, they can stimulate the myometrium contractility.⁶⁻⁸ Moreover, cytokines may alter the immunity of the newborn by means of the regulation of many biological pathways, so that cytokines present in maternal and fetal tissues may play an important role in the perinatal period.

Previous studies demonstrated controversial findings with regard to mode of delivery and inflammatory activation in maternal and fetal compartments.³⁻⁹ Current data concerning the relationship between term labor, systemic fetal immune response and mode of birth are still a matter of debate. The purpose of the present study was to assess the proinflammatory cytokine levels in cord blood depending on the mode of deliv-

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ery and labor. Particularly we aimed to investigate whether normal spontaneous delivery at term was associated with an inflammatory activation as compared to cesarean section before the onset of labor.

Material and Method

The design of the present study was approved by the Institutional Review Board of Zekai Tahir Burak Women's Health Care Education and Research Hospital where the study was conducted. Written informed consents were obtained from all participants.

A total of 88 pregnant women included in the study. Inclusion criteria were clinically healthy, term singleton pregnancies with a gestational age of 37+0 to 41+6 completed weeks. Gestational age was determined on the basis of menstrual history and early prenatal ultrasound (10-12 weeks of gestation). The participants were divided into two groups according to the mode of delivery: Group 1 (n=44) women with normal labor and spontaneous vaginal delivery (without the need of instrumental or vacuum extraction, spontaneous labor occurring without artificial aid by the use of pharmacological and surgical methods and without rupture of membranes) and group 2 (n=44) women with scheduled cesarean delivery. All of the women in scheduled cesarean group had pregnancies with a gestational age of 39+0 completed weeks. Cesarean sections were performed with spinal anesthesia and were chosen as a mode of delivery as the consequence of previous cesarean in the same mother and abnormal presentations. All pregnancies had uneventful follow-up and were without signs and symptoms of infection at birth as well as during the post-natal period. Women with a history of smoking, any kind of medication except for antianemic drugs, fetal congenital anomalies diagnosed in prenatal period were excluded as well as women with diabetic, hypertensive and allergic diseases.

Immediately after delivery umbilical cord venous blood (5 cc) was obtained. Sera were separated and kept frozen (-80°C) until analysis for IL-6 and IL-1 β within a maximum of 72 hours. Human cytokine assay ELISA kit (DRG International, Inc, USA) was used to determine the concentrations of two cytokines IL-6 and IL-1 β .

Statistical analysis was done by Statistical Package for Social Sciences (SPSS) 15 software (SPSS Inc., Chicago, IL, United States). While, continuous variables were shown as mean \pm standard deviation, otherwise, nominal data were expressed as number of cases and percentages. Characteristics of subjects among groups were compared by Student's *t* test. Differences regarding cytokine levels among groups were evaluated by Mann Whitney U test. Pearson's Chi-Square test was used for evaluation of the need for neonatal intensive care (NICU) unit. Associations between continuous variables were

specified by Spearman's rho correlation coefficient. A *p* value less than 0.05 was considered statistically significant.

Results

Maternal and neonatal characteristics of the study population are shown in Table 1. Mothers in the scheduled cesarean group were older but the difference was not significant. Gestational age, birth weight, APGAR scores and body mass index (BMI) were similar among groups (*p*>0.05). Cytokine levels also did not differ in relation to mode of delivery (*p*>0.05) (Table 2).

Table 1: Maternal and neonatal characteristics of the study groups

	Spontaneous vaginal delivery (n=44)	Cesarean delivery (n=44)	P*
Age (years)	25.8 \pm 4.5	28.2 \pm 4.4	0.05
Gestational age (days)	274 \pm 9	273 \pm 7	0.62
Birthweight (g)	3301 \pm 307	3393 \pm 466	0.28
APGAR at 5 min	9 (8-10)	10 (8-10)	0.20
BMI (kg/m ²)	28.7 \pm 4.6	30.1 \pm 3.6	0.11
NICU	3 (6.81)	5 (11.36)	0.26

Data are shown as mean \pm standard deviation or number (percentage) of individuals

BMI: Body mass index

NICU: Neonatal intensive care unit

*Calculated by Student T or Pearson Chi-Square tests

Table 2: Cytokine levels in umbilical cord blood with the mode of delivery

	Spontaneous vaginal delivery (n=44)	Cesarean delivery (n=44)	P*
IL-6 (pg/mL)	3.1 \pm 2.0	3.1 \pm 2.9	0.62
IL-1 β (pg/mL)	2.0 \pm 1.7	2.3 \pm 1.8	0.46

*Calculated by Mann Whitney U test

IL: Interleukin

NICU admission was needed for 8 newborns (3 from spontaneous delivery and 5 from cesarean group) because of temporary respiratory distress and tachypnea. These newborns discharged from hospital with their mothers after a short term follow up with oxygen therapy.

Umbilical cord cytokine levels were also found similar when the patients were divided into 2 groups based on gestational age as before and after 39 weeks, independently of the mode of delivery (*p*>0.05). Mean IL-6 and IL-1 β values were 2.9 pg/mL and 2.0 pg/mL for the women before 39 weeks of gestation (n=40); and 3.3 pg/ml and 2.2 pg/mL for the women after 39 weeks of gestation (n=44), respectively.

Associations between age, BMI, gestational age and cytokine levels were also detected in this study. Neither IL-6 nor IL-1 β showed significant positive correlation with age, BMI and gestational age. Table 3 and table 4 show the correlation analyses of the factors associated with cytokine levels.

Discussion

The onset of labor is suggested to be a combination of a series of biochemical and inflammatory changes in the uterus and the cervix that result from endocrine and paracrine signals coming from both the mother and the fetus. The initiation of labor involves the activation of genes involved in acute inflammatory response. It has recently been reported that human term labor is characterized by an acute inflammation gene expression signature in the fetal membranes even in the absence of evidence of clinical and histological chorioamnionitis.¹⁰⁻¹¹

Cytokines may alter the immunity of the newborn by means of the regulation of many biological pathways, so that cytokines present in maternal and fetal tissues may play an important role in the perinatal period. There is much evidence that labor and vaginal delivery have beneficial short and long term effects on neonatal respiratory adaptation to extra uterine life.¹² Cesarean section has been extensively associated with increased prevalence of atopic diseases compared with vaginal delivery.¹³ More recently cytokines are suggested to play an important interactive role both in the fetal neuroendocrine system, and surfactant metabolism as well as systemic hemodynamics following delivery.¹⁴ The purpose of the present

study was to assess the proinflammatory cytokine levels, IL-6 and IL-1 β , in cord venous blood depending on the mode of delivery and labor, thus to investigate whether normal spontaneous delivery at term was associated with an inflammatory activation as compared to cesarean section before the onset of labor.

IL-6 is located in the fetal and maternal tissues and possibly not only regulates the placental and fetal growth but also coordinates the biochemical, immunological and physiological changes necessary for the survival of the mother and the fetus.¹⁵ IL-1 β stimulates the production of prostaglandins by endometrial tissue; therefore it may be possibly responsible for the initiation of the labor.¹⁵⁻¹⁶

There are controversial data regarding cytokine levels compared between spontaneous vaginal delivery and cesarean section. Buonocore et al.¹⁶ detected IL-6 levels blood specimens taken from mothers undergoing vaginal delivery and cesarean and cord blood. They found that vaginal delivery group had higher IL-6 levels in maternal plasma and cord blood whereas the concentrations of IL-1 β were unchanged. Malamitsi-Puchner et al.⁸ also conducted a study on 88 women (52 women delivering vaginally and 26 women cesarean) to investigate whether cytokine concentrations in the mother, fetus and neonate depend on mode of delivery. They reported that IL-6 and IL-1 β levels were significantly higher in cases of vaginal delivery than in cases of elective cesarean as well as many other cytokines. They concluded that vaginal delivery promotes the production of various cytokines and

Table 3: Correlation of the IL-6 levels with clinical and demographic characteristics in patients with vaginal and cesarean delivery

	Spontaneous Vaginal Delivery (n=44)			Cesarean Delivery (n=44)		
	Rho	%	p	Rho	%	p
Age	0.173	17.3	0.26	0.195	19.5	0.20
Gestational age	-0.11	-11	0.47	0.283	28.3	0.05
BMI	-0.106	-10.6	0.49	0.084	8.4	0.59
IL-1 β	0.039	3.9	0.80	-0.052	-5.2	0.74

Rho: Spearman's Correlation Coefficient

BMI: Body mass index

Table 4: Correlation of the IL-1 β levels with clinical and demographic characteristics in patients with vaginal and cesarean delivery

	Spontaneous Vaginal Delivery (n=44)			Cesarean Delivery (n=44)		
	Rho	%	p	Rho	%	p
Age	-0.109	-10.9	0.48	-0.11	-11	0.48
Gestational age	0.12	12	0.22	0.057	5.7	0.71
BMI	0.052	5.2	0.73	0.266	26.6	0.08
IL-1 β	0.039	3.9	0.80	-0.052	-5.2	0.74

Rho: Spearman's Correlation Coefficient

BMI: body mass index

their receptors, which are implicated in neonatal immunity. Similarly, Zanardo et al.¹⁷ declared higher cortisol, noradrenalin, IL-6 and IL-1 β levels in the vaginal delivery group. They stated that reduced blood concentrations of neurohormonal and neuroendocrine markers may be responsible for altered postnatal adaptation and iatrogenic respiratory distress syndrome because of poor immune-neuroendocrine response at birth for newborns delivered by cesarean. Duncombe et al.³ also found increased IL-6 levels in women undergoing vaginal delivery than those of women undergoing cesarean, even in low risk laboring patients at term in the absence of evident infection. Schulpis et al.¹⁸ compared IL-6 levels in maternal sera before and after delivery in 26 women with normal labor and vaginal delivery and 30 women with scheduled cesarean. Their results showed that IL-6 levels were increased post delivery in vaginal delivery group and unaltered in the cesarean group. They also declared lower IL-6 levels from neonates born by cesarean section as compared to those born vaginally. On the contrary, Hata et al.¹⁹ investigated IL-6, IL-8 and tumor necrosis factor-alpha (TNF- α) levels in newborns delivered vaginally and by elective cesarean. Their results suggested that labor pains do not affect the concentrations of cytokines in cord blood. Cicarelli et al.⁹ compared proinflammatory markers in maternal serum and cord blood depending on mode of delivery. They found no differences for IL-1 β , IL-8 and TNF- α in cord blood samples or in maternal serum obtained in both spontaneous vaginal delivery and elective cesarean section. Similar data regarding no difference in interferon gamma (IFN- γ), IL-4 and IL-10 in cord blood between the two groups, either delivering vaginally or by elective cesarean section was found by Bakheit et al.²⁰ Our results are particularly in line with aforementioned studies^{9,19-20} and with those of Tutdibi et al.,⁴ who reported similar IL-6 and IL-1 β levels in cord blood obtained from 60 term newborns (35 cesarean and 25 vaginal delivery). They also evaluated several cytokine concentrations and their data suggested that in healthy, term neonates, the exposition of normal spontaneous delivery and labor is not associated with systemic activation of different inflammatory mediators compared with elective cesarean section except for transforming growth factor β 1 (TGF- β 1).

The present study showed similar IL-6 and IL-1 β levels in cord venous blood in the two study groups delivered vaginally or by scheduled cesarean. Controversial data in literature may be attributed to the design and sample size of the aforementioned studies. In addition, methodological differences in detectable capacity of cytokine assays could potentially contribute to the controversial study results. Relatively larger sample size and prospective design of the present study may provide advantages. It is also possible that our results differ from others^{3,8,16-18} because we only included patients without evidence of infection, inflammation or even early rupture of membranes before the onset of labor. The present study also

evaluated the correlation of cytokine levels with age, BMI and gestational age for the first time, to the best of our knowledge. Neither IL-6 nor IL-1 β showed significant positive correlation with age, BMI and gestational age. However, lack of histopathological examination of the placentas was a limitation of the present study, once inflammation or infection and vascular changes of the placentas are associated to different IL patterns in cord venous blood.

Consequently, in healthy and term pregnancies, the exposition of spontaneous vaginal delivery and labor is not found to be associated with activation of inflammatory mediators as compared to cesarean. The data of the present study also did not support the increasing ratio of newborn complications (respiratory distress syndrome, bronchopulmonary dysplasia, the need for prolonged oxygen therapy and NICU, allergic diseases of infancy period) after cesarean delivery due to inadequate increasing levels of cytokines in newborns delivered by cesarean section. Further studies with larger cohorts investigating cytokine profiles in maternal serum, fetal cord blood, and amniotic fluid are required to understand the different regulation of fetomaternal cytokine expression and the role of compartmentalization during the process of normal term labor and delivery.

Vajinal Doğum ve Sezaryende Umbilikal Kord Kanında Interlökin-6 ve İnterlökin 1 β Düzeylerinin Araştırılması

AMAÇ: Bu çalışmada termde normal spontan vajinal doğum ve enflamatuvar aktivasyon ilişkisinin, kord kanı interlökin-6 (IL-6) ve interlökin-1 β (IL-1 β) düzeyleri tespit edilip, sezaryen doğum yapan hastalarla karşılaştırılarak araştırılması amaçlanmıştır.

GEREÇ VE YÖNTEM: Çalışmaya 88 hasta (44 vajinal doğum, 44 sezaryen) dahil edilmiştir. Fetüsün doğumunu takiben umbilikal kord venöz kanı örneği alınarak IL-6 ve IL-1 β düzeyleri belirlenmiştir. Çalışmaya dahil edilen tüm hastalar komplikasyonsuz term gebelerden oluşmuştur.

BULGULAR: Gruplar arasında maternal yaş, doğumdaki gestasyonel hafta, doğum ağırlığı ve vücut kitle indeksi açısından fark yoktu ($p>0,05$). Spontan vajinal doğum ve planlı sezaryen doğum arasında kord kanı IL-6 ve IL-1 β düzeyleri arasında anlamlı fark bulunmadı (IL-6 vajinal doğum grubunda $3,1\pm 2,0$ pg/mL, sezaryen grubunda $3,1\pm 2,9$ pg/mL, $p=0,62$ ve IL-1 β vajinal doğum grubunda $2,0\pm 1,7$ pg/mL sezaryen grubunda $2,3\pm 1,8$ pg/mL, $p=0,46$). Ayrıca kord kanı sitokin düzeyleri ile maternal yaş, gestasyonel hafta, doğum ağırlığı ve vücut kitle indeksi arasında ilişki saptanmadı.

SONUÇ: Sağlıklı ve term gebeliklerde spontan vajinal doğum ve travay süreci sezaryen doğum ile karşılaştırıldığında enflamatuvar medyatörlerin aktivasyonu ile ilişkili değildir. Sezaryen doğumlardan sonra artmış bulunan yeni doğan komplikasyonları (respiratuar distres sendromu, bronkopulmoner displazi, artmış oksijen tedavisi ve yeni doğan yoğun bakım ünitesi ihti-

yacı, infantil allerjik hastalıklar) sezaryen doğumdaki yetersiz sitokin düzeylerine bağlı gibi görünmemektedir.

Anahtar Kelimeler: Sitokinler, Doğum şekli, Kord kanı, Enflamasyon

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