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Umbilical cord blood endocan levels according to the delivery mode

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Summary

Purpose of investigation: The authors aimed to evaluate the endocan levels in the umbilical cord blood regarding the delivery mode. *Materials and Methods:* One hundred six women aged between 20 to 35 years, undergoing delivery at term were studied. Three groups were formed; 37 neonates born by spontaneous vaginal delivery (group 1), 34 neonates born by an elective cesarean section with the general anesthesia (group 2), and 35 neonates, born by an elective cesarean section with spinal anesthesia (group 3). In delivery, umbilical cord blood samples were collected and endocan levels were measured. *Results:* The endocan levels of cord blood (mean ± standard deviation, ng/ml) were found to be lower in group 2 (1.21 ± 0.46) compared to group 1 (1.52 ± 0.52) ($p = 0.011$). Cord blood endocan levels were not different in group 1 than those of group 3 ($p = 0.49$). *Conclusion:* It may be concluded that cord blood endocan levels are affected by the delivery mode.

Key words: Vaginal delivery; Cesarean section; Anesthesia; Neonate; Endocan.

Introduction

Endocan, a chondroitin-dermatan sulphate proteoglycan is called an endothelial cell-specific molecule-1 and has an important role in the control of cell proliferation [1]. It is synthesised by endothelial cells and regulated by tumor cell-derived factors, including vascular endothelial growth factor (VEGF) [2]. Anti-VEGF antibody was reported to be has an inhibitory effect on pregnancy development and placental angiogenesis in a rat model [3]. Also, various cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor alpha (TNF-α) play a role in the regulation of endocan synthesis [4]. IL-1β and TNF-α upregulate the endocan messenger RNA and increases the secretion of endocan by the endothelium. Endocan regulates leukocyte functions via inhibiting leukocyte function-associated antigen and intercellular adhesion molecule [5].

Angiogenesis is the formation of new vessels from pre-existing vessels and it plays an important role in the development and wound healing [6]. Zhang et *al.* [7] demonstrated that endocan is expressed in neogenically active tissue and cell types. They reported that endocan may be an important marker in angiogenesis and neogenesis. Scherpereel et *al.* [8] demonstrated higher serum endocan levels in septic shock patients compared to patients with sepsis. They suggested that endocan may be a marker to determine the severity of sepsis and a useful marker of endothelial cell activation. On the other hand, increased secretion of endocan has been observed in several malignant diseases such as hepatocellular carcinoma, bladder cancer, and clear cell renal cell carcinoma [9-11].

Both spontaneous vaginal and surgical delivery stress may cause the local inflammatory response in the fetus [12-14]. It has been shown that fetal well-being and maternal comfort are affected by the delivery mode, type of anesthesia, and different anaesthetic drugs [15-17]. The secretion of cytokines increases during perioperative period due to surgical stress and endocan secretion is regulated by cytokines [4, 12]. The present authors believe that cord blood endocan levels might be a marker to demonstrate fetal endothelial cell activation and neoangiogenesis. In this prospective observational trial, they aimed to evaluate the endocan levels in the umbilical cord blood regarding the delivery mode.

Materials and Methods

This prospective study was approved by the Ethics Committee of the Medical Faculty, Ataturk University and was performed over a ten-month period in two institutions (a university hospital and other state hospital). One hundred six women without antepartum complications, aged between 20 to 35 years, with full-term fetuses (≥ 37 weeks' gestation) in a vertex presentation, with no congenital malformation, Apgar score ≥7 at five minutes after birth were selected for this study. Written informed consent was obtained from all parents before participation. At first gestational week, amniotic fluid volume, presentation of fetus, and possible

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fetal malformation were detected via obstetric ultrasound. Smokers, alcohol consumers, mothers with complicated pregnancies (such as preeclampsia, placenta previa, oligohydramnios and intrauterine growth retardation), hypertension, diabetes mellitus, and multiple pregnancies were excluded from the study.

Patients undergoing elective cesarean section were informed about advantages and disadvantages of the anesthesia methods by an anesthetist. Spinal or general anesthesia was decided according to medical considerations and the patient's decision without randomization. The number of patients in each group was planned as 40 at the beginning of the study. Consecutive patients who preferred each anesthesia technique were included in this study. Before entering the operating room, all patients were premedicated with 0.02 mg/kg intravenous (iv) midazolam to provide synchronization and received 500-750 ml of Ringer lactate solution via iv cannula. Standard monitoring included non-invasive arterial pressure, electrocardiography, and pulse oximetry was established. Eventually, three groups were formed according to the delivery modes:

Group 1: Neonates, born by spontaneous vaginal delivery. All women had spontaneous contractions of adequate frequency. Induction of labour was done only with artificial rupture of fetal membranes when the cervix was three-cm dilated in all women. No other method was used for induction of labour such as syntocinon infusion. No analgesic method was used for pain relief such as opioids, nitrous oxide, and epidural analgesia. Continuous external fetal monitorisation and maternal blood pressure monitoring were applied.

Group 2: Neonates, born by an elective cesarean section with general anesthesia. General anesthesia was induced with iv propofol two mg/kg. Then iv rocuronium (0.6 mg/kg) was given to patients for facilitating endotracheal intubation. Anesthesia was maintained with nitrous oxide 50%, oxygen 50%, and sevoflurane 1-2%. After delivery of the baby, fentanyl one µg/kg was given by iv.

Group 3: Neonates, born by an elective cesarean section with spinal anesthesia. Ringer lactate solution (12 ml/kg) was given by iv in 15 minutes. After skin infiltration with 2% lidocain, 26-gauge Quincke's needle was inserted through the L_{2-3}/ L_{3-4} intervertebral space of sitting position patient. Once free flow of cerebrospinal fluid was obtained, hyperbaric bupivacaine 0.5%, nine mg (1.8 ml) was injected intrathecally. Then, the patient was positioned with a wedge under their right hip to prevent aortocaval compression. Oxygen was administered three to four L/minute with a face mask until delivery. In the case of hypotension (a 30% decrease in systolic blood pressure compared with preoperative values), ephedrine was administered. When the sensory block reached the T_4 dermatome, surgery was initiated.

All mothers had a normal complete blood count, negative for CRP and normal appearance of placenta. The operation indication was previous cesarean section in elective cesarean section groups in this study. All operations were performed between the hours of 08:00 and 12:00.

During delivery, the umbilical cord was doubly clamped and cord blood was drawn from the umbilical artery. Blood was collected into tubes and centrifuged at 3,000 g for ten minutes after coagulation. Then the obtained serum samples were kept at -80^{O}C until measurements were conducted.

Endocan levels in cord blood samples were detected via a sandwich-based enzyme-linked immunosorbent assay according to recommendations of the manufacturer. It was determined by the use of immunoassay kit and expressed in ng/ml. The detection limit of this measurement was 0.3 ng/ml. Results less than 0.3 ng/ml were accepted as '0'.

Table 1. — *Clinical characteristics of neonates and their mothers.*

	Group 1 (n=37)	Group 2 (n=34)	Group 3 (n=35)	*p* value
Maternal age (years)	29.82±3.43	28.27±3.73	28.86±3.73	> 0.05
Maternal BMI (kg/m^2)	24.05±1.47	24.21±1.47	23.66±1.51	> 0.05
Gestational age (weeks)	38.85±0.74	38.76±0.48	38.66±0.48	> 0.05
Gravidity	2.24±0.96	2.44±0.50	2.31±0.42	> 0.05
Birth weight (g)	3530±208	3512±168	3449±184	> 0.05
Apgar scores One minute	7.46±0.56	7.61±0.49	7.51±0.50	> 0.05
Apgar scores Five minutes	9.27±0.45	9.38±0.51	9.49±0.51	> 0.05
Duration of surgery (minutes)		49.12±3.79	42.57±2.54	= 0.0001
Duration of labour (hours)	8±1.3			

Group 1: spontaneous vaginal delivery, Group 2: general anaesthesia, Group 3: spinal anaesthesia. Data is given as mean ± standard deviation.

Sociodemographic information (age, body mass index, parity, the week of gestation), the duration of surgery, duration of labor, birth weight, Apgar scores at minutes one and five, and endocan levels in cord blood samples were recorded.

The authors tested for associations among cord blood endocan levels, delivery modes, and socio-demographic characteristics. The Kolmogorov-Smirnov test was used to assess the normal distribution of data. If data was not normally distributed, comparisons were determined using the Kruskal-Wallis and Mann-Whitney U-test. Comparisons were determined using the ANOVA test when data was normally distributed. SPSS software 12.0 was used for the statistical analysis. The data was calculated as mean ± standard deviation and $p < 0.05$ was considered significant. By performing a power calculation, when α error and β error were considered, respectively, as 0.05 and 0.04, with 85% power, the patient number for each group was determined as minimum 35. The common standard deviation within a group was assumed to be 0.50.

Results

Data from 14 neonates with Apgar scores less than 7 at five minutes after birth (three in group 1, six in group 2, and five in group 3) was excluded from the study. In the final analysis, data of 37 patients in the group 1, 34 patients in the group 2 and 35 patients in the group 3 were included.

There were no significant differences among the groups in terms of age, body mass index, parity, the week of gestation, birth weight, and Apgar scores (Table 1). The duration of surgery was significantly lower in group 3 (42.57 ± 2.54 minutes) compared with group 2 (49.12 ± 3.79 minutes) ($p < 0.0001$). There were no differences among patients in terms of duration of labor ($p > 0.05$). In group 3, hypotension developed in five patients just before the operation and was treated with iv ephedrine five mg. Hyper-

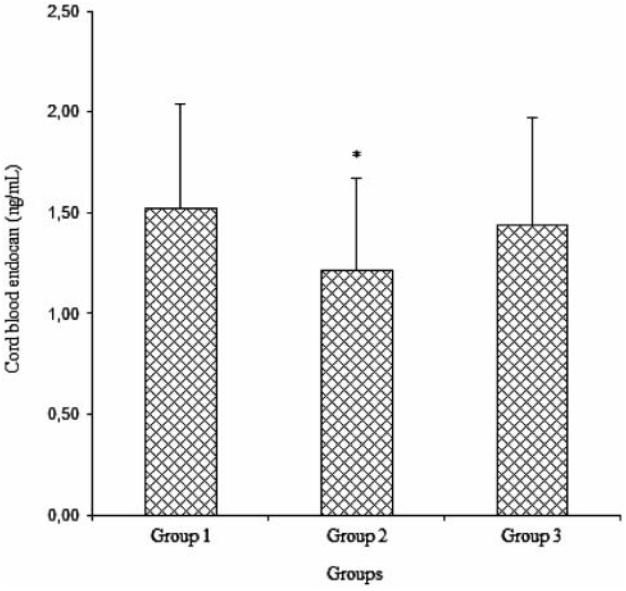

Figure 1. — Comparisons of cord blood endocan levels (ng/ml) according to the modes of delivery. Group 1: spontaneous vaginal delivery, Group 2: general anesthesia, Group 3: spinal anesthesia. *p = 0.011 compared to group 1.

tension (systolic/diastolic tension > 160/95 mmHg) and tachycardia (heartbeat of over 100 beats per minute) occurred in four patients of group 2 and this situation was corrected with hyperventilation.

Cord blood endocan levels in group 2 (1.21 ± 0.46 ng/ml) were found to be significantly lower than those of group 1 (1.52 ± 0.52 ng/ml, p = 0.011). Cord blood endocan levels in group 1 were similar to group 3 (1.44 ± 0.53 ng/ml, p = 0.49). Mean endocan levels were lower in group 2 compared with group 3, although the difference was not statistically significant (p = 0.064) (Figure 1). When all groups were evaluated together, no correlation was found between clinical characteristics and cord blood endocan levels (Spearman's rank correlation).

Discussion

This is the first study examining the association between endocan levels in cord blood and delivery modes. The authors found that cord blood endocan levels in healthy neonates were higher in spontaneously vaginally delivered, compared to cesarean section using general anesthesia. In neonates born by spontaneous vaginal delivery cord blood endocan levels were similar to those in neonates born by cesarean section with spinal anesthesia.

Endocan is secreted by endothelial cells and regulated by VEGF and various cytokines, especially IL-1β and TNF-α [4]. VEGF has an important role in fetal development and placental angiogenesis [3]. Zhang *et al.* [7] suggested that endocan may be a marker in the tissue damage, angiogenesis, oncogenesis, and the process of inflammatory reactions. Scherpereel *et al.* [8] found the relationship between endocan levels and severity of sepsis and endocan was reported as a useful marker of endothelial cell activation in their study. In another study [18] researchers found negative correlation between serum endocan levels and the development of acute lung injury in patients exposed to major trauma. Additionally, an increase in the content of endocan of endothelial cells has been detected in the lung, kidney, liver, colon, ovary, and brain tumours [19].

Surgical stress causes an increase in the levels of cytokines and this increase varies depending on severity of surgical injury [20]. The expression of endocan is regulated by cytokines [1]. The effects of different anaesthetic techniques on cytokine levels have been investigated in surgical patients [21-22]. Zura *et al.* [23] investigated the effect of spinal and general anesthesia on serum concentration of cytokines in patients undergoing transurethral resection of the prostate. They found low IL-2 levels on postoperative days 1 and 3 in general anesthesia group compared to spinal anesthesia group. It may be suggested that general anesthesia may induce cytokine release less than spinal anesthesia. In another study in contrast, the general anesthesia group had higher pro-inflammatory cytokines compared to the regional anesthesia group in patients undergoing laparoscopic cholecystectomy [24].

Researchers have reported variable levels of cytokines according to different delivery modes [25-27]. A study by Dermitzaki *et al.* [26] found no changes in maternal serum IL-6 and TNF-α concentrations between two different anesthetic techniques for cesarean section. Buyukkocak *et al.* [27] reported decreased acute phase protein levels in patients undergoing vaginal delivery with epidural analgesia compared to cesarean section and these alterations were not influenced by anaesthetic techniques (spinal, epidural, and general anesthesia). Consistent with these studies [26, 27], the present authors found similar cord blood endocan levels in patients undergoing elective cesarean section with general and spinal anesthesia. However, they observed higher cord blood endocan levels in patients undergoing spontaneous vaginal delivery compared to the patients undergoing elective cesarean section with general anesthesia. The use of different general anesthetic agents (propofol and thiopental) in studies may be a cause in obtaining these different results.

In the present institute, regional anesthesia is the most common method to provide anesthesia for cesarean section. However, general anesthesia is used in case of maternal refusal of regional techniques, failed regional attempts, and in the presence of contraindications to regional anesthesia such as coagulation disorders or spinal abnormalities. In this current study, randomization was not done and anesthesia technique was selected in consideration of the pa-

tient's request and medical condition. Because the present authors thought that selection of anesthesia technique without randomization may be more ethical. Also, they thought that the results of this study would not be affected by this situation.

After intravenous bolus administration of propofol which was used in the present study, it rapidly distributes to tissues (half-life of two to four minutes) and passes through the placenta. Propofol is known to suppress pro-inflammatory cytokine response in rats [28]. In the present study, lower endocan levels in neonates born by cesarean section with general anesthesia may be due to the anti-inflammatory effect of propofol. Conversely, spinal anesthesia may cause a reduction in maternal and uteroplacental blood flow/pressure due to maternal hypotension [29]. This situation may be a cause of endothelial activation and higher endocan levels in neonates born by cesarean section with spinal anesthesia than in general anesthesia. On the other hand, regional anesthesia was reported to be associated with a decrease in the stress-inducing hormones adrenaline, noradrenaline, and cortisol [30]. In this instance, the exact mechanisms for low cord blood endocan levels in spinal anesthesia group remains unclear.

The cause of a high level of cord blood endocan in spontaneous vaginal delivery group may be increasing stress caused by pain during uterine contractions. In this study, there was no CRP elevation in any of the patients. These results may show that elevation of endocan might be due to endothelial activation rather than acute inflammation. On the other hand, the present authors suggested that an increase of cord blood endocan levels may play a role in the beginning of uterine contractions in pregnant women. The current findings raise the interesting question of whether elevated cord blood endocan levels are useful for neonates.

Endocan is a new marker of endothelial cell activation and may play critical roles in many physiological and pathological events associated with endothelium [4]. Also, endocan is a biomarker of neoangiogenesis and some cellular activities such as migration, adhesion, and proliferation were regulated by endocan [2]. On the other hand, the range of cord blood endocan levels in spontaneous vaginal delivery group in the present study was 0.5 - 2.87 ng/ml, while the range of serum endocan levels was 0.48 - 1.21 ng/ml in healthy adults [31]. Whereas significantly increased levels of serum endocan were reported in patients with severe sepsis [8] and cancer, such as invasive bladder cancer [10] and hepatocellular carcinoma [9]. For these reasons, the present authors suggested that slightly higher levels of cord blood endocan may be beneficial for neoangiogenesis and neonatal development.

The present authors could not measure cytokines that may lead to elevation of endocan and this is a weakness of the present study.

Conclusion

Cord blood endocan levels are affected by the delivery mode. The present authors reported higher cord blood endocan levels in healthy neonates born by spontaneous vaginal delivery compared to the neonates born by cesarean section with general anesthesia. They suggested that slightly higher levels of cord blood endocan may be beneficial for neoangiogenesis of neonates. They also suggested that an increase in cord blood endocan levels may be a physiological process in the beginning of labor. Future studies are needed to identify the effect of the delivery mode on the endocan levels of fetal tissues such as placenta and amniotic fluid.

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