The Effect of Anaesthetic Techniques on Maternal and Cord Blood Brain-Derived Neurotrophic Factor Levels

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Objective: Brain-derived neurotrophic factor (BDNF), a member of neurotrophins, plays a critical role in neuronal tissue. In this study, the effects of spinal or general anaesthesia on cord and maternal peripheral blood BDNF and malondialdehyde (MDA) levels were investigated in patients undergoing elective caesarean section.

Methods: Eighty patients with term pregnancy were included. General anaesthesia was induced with intravenous (IV) propofol 2 mg kg⁻¹ in the general anaesthesia group (n=36). In the spinal anaesthesia group (n=35), hyperbaric bupivacaine 0.5%, 9 mg (1.8 mL) was injected intrathecally. Maternal blood samples were taken immediately after positioning the patient on the operating table (T1), before clamping the umbilical cord (T2) and 24 hours after the first sample was obtained (T3). Cord blood samples were drawn from the umbilical artery (T4).

Results: Maternal BDNF levels (pg mL⁻¹) measured at T2 time point were higher in the general anaesthesia group compared to the spinal anaesthesia group (p<0.001). Cord blood BDNF levels were higher in the general anaesthesia group compared to the spinal anaesthesia group (p<0.001). In both groups, cord blood BDNF levels were significantly lower compared to the maternal blood samples collected at any time point (p<0.001, for all). There was a negative association between both maternal and cord blood BDNF levels with maternal MDA and cord blood MDA levels, respectively (r=−0.379, p<0.001; r=−0.375, p=0.001, respectively).

Conclusion: The anaesthetic technique may have an influence on maternal peripheral and cord blood BDNF levels.

Keywords: Anaesthetic technique, brain-derived neurotrophic factor, maternal peripheral blood, cord blood

Introduction

Nerve growth factor, brain-derived neurotrophic factor (BDNF) and neurotrophin 3 are known as neurotrophins. Neurotrophins have protective and anti-apoptotic activities in neurons, and they prevent cell death in the peripheral nervous system (1). It was reported that neurotrophins can reduce axonal degeneration in the spinal cord in a rat model created by traumatic axonal injury (2). Thus, it is clear that neurotrophins play a crucial role for neuronal tissue (1).

In healthy pregnancies, the BDNF levels in umbilical cord blood were found to be significantly lower than in maternal peripheral blood (3). A positive correlation was reported between serum and cortical BDNF levels (4). Kodomari et al. (5) suggested that BDNF passes the utero-placental barrier, reaches the foetal brain and might contribute to foetal development. On the other hand, factors causing a stress response in the central nervous system (such as hypotension, surgical stress and depression) regulate the expression of neurotrophin (6). Van den Hove et al. (7) reported an approximate 50% decrease in brain cell proliferation and a decrease in BDNF protein content in both the olfactory bulbs and hippocampus in rats exposed to prenatal stress.
Malondialdehyde (MDA) is a lipid peroxidation product, and tissue MDA levels increase in case of oxidative stress (8). Surgical stress leads to a systemic immuno-endocrine response and a stimulation of the sympathetic nervous system (9). Studies (10, 11) showed that neuraxial blocks are related with decreased stress response to surgical trauma, and they are better alternatives in comparison with general anaesthesia in regard to oxidative stress. Also, stress causes changes in neurotrophin expression in the central nervous system (6). On the other hand, it was reported that anaesthetic drugs influence BDNF plasma kinetics in healthy males undergoing elective minor surgery (12, 13).

We hypothesised that spinal anaesthesia affects maternal peripheral blood BDNF levels. For this purpose, we conducted a prospective study that compared the effects of spinal and general anaesthesia on maternal peripheral and cord blood BDNF and MDA levels in patients undergoing elective caesarean section. The primary outcome was the change in maternal peripheral blood BDNF levels measured pre-, intra- and postoperatively. The secondary outcome was to determine a difference in cord blood BDNF levels between the two groups. Another secondary outcome was to detect an association between BDNF and MDA levels.

We selected only elective caesarean section procedures to provide minimal perioperative stress response and eliminate other factors that can alter the levels of BDNF, such as foetal distress and emergency caesarean section.

**Methods**

Ethical approval for this study (the protocol number: B.30.2.ATA.01.00/34) was provided by the Ethical Committee of Ataturk University, Medical Faculty, Erzurum, Turkey. Prior to enrolment, all parents gave written informed consent following information about the study. This study was conducted at the Anesthesia Department of Ataturk University (mean annual delivery rate: 3,000 deliveries, mean percentage of caesarean: 51%) and Nenehatun Hospital (mean annual delivery rate: 6,000 deliveries, mean percentage of caesarean: 30%), Erzurum, Turkey. Eighty patients aged 20-35 years with term pregnancy who underwent elective caesarean section were included in this study. Smokers, alcohol consumers, mothers with complicated pregnancies (such as preeclampsia, placenta previa, oligohydramnios and intrauterine growth retardation), hypertension, diabetes mellitus, depression, body mass index ≥30 kg m⁻², multiple pregnancies and who used assisted reproductive technologies to become pregnant, were excluded from the study.

Spinal or general anaesthesia was selected according to medical considerations and the patient's preference. There was no randomisation. Forty consecutive patients in each group who preferred each anaesthesia method were included. All caesarean sections were performed between 8.00 and 12.00 a.m. Before entering the operating room, all patients received 500-750 mL of Ringer lactate solution via intravenous (IV) cannula, and none of the patients were pre-medicated. Standard monitoring included non-invasive arterial pressure, electrocardiography and pulse oximetry was established for all patients after providing 15° left lateral tilt positions on the operating table. A total of 80 women with full-term foetuses (≥237 weeks of gestation) in a vertex presentation, and no congenital malformation, were selected for this study. Eventually, two groups were formed according to the used anaesthetic techniques.

**In the general anaesthesia group:** General anaesthesia was induced with IV propofol 2 mg kg⁻¹ following pre-oxygenation (100% oxygen for 3 minutes). Then, IV rocuronium (0.6 mg kg⁻¹) was given to patients for facilitating endotracheal intubation. Tracheal intubation was performed, and anaesthesia was maintained with nitrous oxide 50%, oxygen 50% and sevoflurane 1%-2% at end-tidal carbon dioxide between 30 and 35 mmHg (12). Caesarean section was performed with Pfannenstiel skin incision, and a low transverse uterine incision. After delivery and umbilical cord clamping, midazolam 2 mg and fentanyl 2 µg kg⁻¹ were given intravenously and 5 units oxytocin by IV injection (followed by 20 units in 1000 mL Ringer's lactate solution over 8 hours), and an antibiotic (ampicillin 1 gr IV, if the patient was not allergic to the drug) was administered. The uterus was sutured without exteriorisation, and the parietal peritoneum was sutured with a single suture. Neuromuscular block was reserved with neostigmine (0.04 mg kg⁻¹, IV) and atropine (0.02 mg kg⁻¹, IV). On admission to the post-anaesthesia care unit of all patients, postoperative analgesia for the first 24 hours was provided using intravenous tramadol via patient controlled analgesia (PCA) pump with the following settings: loading tramadol dose 50 mg IV, optional bolus dose 25 mg, lockout interval 15 minutes, 4-hour limit of 300 mg. Ondansetron (4 mg, IV) was used to treat postoperative nausea and vomiting.

**In spinal anaesthesia group:** After skin infiltration with 2% lidocaine, a 26-gauge Quincke needle was inserted through the L₂₋₃/L₃₋₄ intervertebral space of the patient in sitting position. Once the free flow of cerebrospinal fluid was obtained, hyperbaric bupivacaine 0.5%, 9 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 3-4 L minute⁻¹ with a face mask until delivery (12). Cold and pin-prick perception were used to assess the level of sensory block. When the sensory block reached to T₅ dermatoïme, surgery was initiated. Spinal anaesthesia induced hypotension (a 30% decrease in systolic blood pressure compared with preoperative values) was treated by uterine displacement and rapid infusion of fluid and colloid, and ephedrine application was planned in case of persistent hypotension. General anaesthesia protocol was planned for patients with three unsuccessful attempts to reach to spinal space. Also, if adequate surgical anaesthesia was not achieved after 15 minutes, spinal anaesthetic technique was consid-
erred as failure, and general anaesthesia protocol was planned for these patients. Intraoperative pain was assessed using a verbal rating scale (0=no pain, 10=the worst pain). If discomfort was observed during surgery, IV thiopenthal with a dose of 100 mg was administered in these patients. Block failure was defined for these patients. The same surgery procedure, oxytocin and postoperative analgesic regimens as the general anaesthesia group were applied to the patients from the spinal anaesthesia group.

Maternal blood samples for BDNF and MDA levels were taken immediately after positioning the patient on the operating table (T₀), before clamping the umbilical cord (T₁) and 24 hours after the first sample was obtained (T₂). During delivery, the umbilical cord was double clamped, and cord blood was drawn from the umbilical artery (T₃). Blood samples were collected into tubes and centrifuged at 3,000 g for 10 minutes. Then, the obtained serum samples were kept at −80°C until measurements were conducted. BDNF levels were detected via a sandwich-based enzyme-linked immuno-sorbent assay according to the manufacturer’s instructions (Quantikine® Human BDNF Immunoassay, R&D Systems, Minneapolis, USA) and expressed as pg mL⁻¹. Serum MDA levels were determined according to the method described by Ohkawa et al. (14) and expressed as µmol mL⁻¹.

All mothers had a normal complete blood count, negative for c-reactive protein and normal appearance of placenta. The operation indications were previous or repeat caesarean section for patients selected for this study. Socio-demographic information (age, body mass index, parity, the week of gestation), the duration of surgery, birth weight, Apgar scores at minute 1 and 5, cord blood pH, and BDNF and MDA levels in blood samples were recorded.

There are no studies in the literature that compare the changes in maternal BDNF levels during caesarean delivery where different anaesthetic techniques have been used. In the study of Bienertova-Vasku et al. (3), the BDNF levels in maternal peripheral blood were compared between preeclampsia cases and healthy controls. Since they only collected blood samples during the 2-hour prepartum period, we could not compute the sample size according to their study. Hence, a power analysis for this study was calculated based on the work of Vutskits et al. (12). In their study, peripheral blood BDNF levels (mean±SD) were 631±337 pg mL⁻¹ at baseline and 549±512 pg mL⁻¹ at 15 minutes after anaesthesia induction with propofol. In this current study, the primary outcome was the detection of a difference in BDNF levels at the T₂ time point between the groups. Russ Lenth’s Power and Sample Size Calculation was used for power analysis (15). When α error and β error were considered, respectively, as 0.05 and 0.04, with 85% power, the minimum patient number for each group was determined to be 35. We decided to enrol 40 patients per group to allow dropout.

Statistical analysis

We tested for associations among cord blood BDNF and MDA levels, maternal peripheral blood BDNF and MDA levels, anaesthetic techniques and socio-demographic characteristics. The Kolmogorov-Smirnov test was used to assess the normal distribution of data. If data were not normally distributed, comparisons were determined using the Mann-Whitney U test. Comparisons were determined using the Independent T test when data were normally distributed. Confidence interval was 95%. Chi-squared test was used for categorical data. Comparisons of BDNF and MDA values within each group were conducted using the unpaired t-test. Spearman’s correlation coefficient was used to estimate correlations. Statistical Package for the Social Sciences software 12.0 (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis. The data were calculated as mean±standard deviation, median (minimum–maximum) or n, and p<0.05 was significant.

Results

A flow chart of the study is shown in Figure 1. There were no significant differences between the groups in terms of socio-demographic, obstetric and operative characteristics (Table 1). Two patients were excluded due to three unsuccessful attempts to reach the spinal space. No patient required intraoperative analgesic support during surgery in the spinal anaesthesia group. No severe hypotension and bradycardia occurred among the patients in our study. APGAR scores were more than 7 at 5 minutes, and umbilical artery pH was not lower than 7 in all newborns. There was no requirement of endotracheal intubation and mechanical ventilation for all newborns. Oxygen therapy was needed in 5 newborns (2 in the spinal anaesthesia group and 3 in the general anaesthesia group), and these newborns were monitored in the neonatal intensive care unit only in order to observe them.

![Figure 1. Flow chart showing enrollment of study subjects](image-url)
Maternal serum and cord blood BDNF levels (pg mL⁻¹) are presented in Table 2. In the general anaesthesia group, maternal serum BDNF levels were significantly higher in the blood sample collected at T2 time point compared to the blood samples collected at T1 and T3 time points (p=0.017, p=0.002; respectively). In both the groups, cord blood BDNF levels were significantly lower compared with the maternal blood samples collected at any time point (p<0.001, for all). In comparisons within the group; there were no significant changes in the maternal serum BDNF levels at T1 time point and T3 time point in the spinal anaesthesia study group (p=0.909). However, maternal serum BDNF levels on the first postoperative day were similar to preoperative values in both groups (p=0.285 for the general anaesthesia group, p=0.909 for the spinal anaesthesia group) (Table 2). Maternal and cord blood MDA levels are presented in Table 3. There was a negative association between both maternal and cord blood BDNF levels with maternal MDA and cord blood MDA levels, respectively (r=-0.379, p<0.001; r=-0.375, p=0.001, respectively) (Figures 2 and 3). There was no correlation between BDNF levels and socio-demographic, obstetric and operative characteristics.

Discussion

In this current study, the effects of anaesthetic technique on maternal and cord blood BDNF levels were investigated in patients undergoing elective caesarean section. Maternal BDNF levels measured before clamping the umbilical cord were found to be lower in the spinal anaesthesia group than in the general anaesthesia group. Maternal serum BDNF levels were significantly higher in the blood sample collected at T2 time point compared to the blood samples collected at T1 and T3 time points in the general anaesthesia group. Conversely, maternal serum BDNF levels on the first postoperative day were similar to preoperative values in both groups.

<table>
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<tr>
<th>Table 1. Clinical characteristics of neonates and their mothers</th>
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<td>General anaesthesia group</td>
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<td>Maternal age (years)</td>
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<td>Maternal BMI (kg m⁻²)</td>
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<td>Gestational age (weeks)</td>
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<tr>
<td>Gravidity</td>
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<td>Birth weight (g)</td>
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<td>Apgar scores 1 minute</td>
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<td>Apgar scores 5 minutes</td>
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<td>Duration of surgery (minutes)</td>
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<td>Gender of the foetus (male/female)</td>
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<td>Cord blood pH</td>
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†Independent T test (mean±SD); *Mann-Whitney U-test median (minimum–maximum); ††Chi-squared test (n); BMI: body mass index

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<th>Table 2. Brain-derived neurotrophic factor levels (pg mL⁻¹) in maternal peripheral and cord blood samples</th>
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<td>General anaesthesia group</td>
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<td>T3</td>
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<td>Cord blood</td>
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Values are median (minimum–maximum). Statistical test: Mann-Whitney U test. T1, 2, 3: The results of maternal peripheral blood samples; T1: after positioning the patient on the operating table; T2: before clamping the umbilical cord; T3: 24 hours after the first sample (T1) was obtained.

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<th>Table 3. Malondialdehyde levels (μmol mL⁻¹) in maternal peripheral and cord blood samples</th>
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<td>General anaesthesia group</td>
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<td>Cord blood</td>
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Values are median (minimum–maximum). Statistical test: Mann-Whitney U test. T1, 2, 3: The results of maternal peripheral blood samples; T1: after positioning the patient on the operating table; T2: before clamping the umbilical cord; T3: 24 hours after the first sample (T1) was obtained.

Figure 2. Association of maternal BDNF with maternal MDA levels; (r=-0.379, p<0.001)
BDNF: brain-derived neurotrophic factor; MDA: malondialdehyde
in the general anaesthesia group. Also, cord blood BDNF levels were higher in patients using the general anaesthesia technique compared with patients using the spinal anaesthesia technique. Moreover, we found a negative association between both maternal and cord plasma BDNF with maternal MDA and cord MDA, respectively. This current study reported that anaesthetic method had an influence on maternal and cord blood BDNF levels.

The BDNF, which is a member of neurotrophins, plays critical roles in neuronal tissue, such as suppressing of apoptosis, providing nerve growth, reducing axonal degeneration and inhibiting of cortical cell death (1). Neurodegenerative diseases lead to decreased BDNF expression in the brain (16). It was reported that acute and chronic stresses reduce brain BDNF levels (17). Also, depression was found to be associated with low serum BDNF levels (18). Antidepressants may increase serum BDNF levels in depressed patients (19). Anaesthesia and surgery create stress response, including a number of hormonal changes initiated by neuronal activation of the hypothalamic–pituitary–adrenal axis (9).

General anaesthesia provides a safe control of the airways and the ventilation, but it carries risks of pulmonary aspiration, failed intubation, neonatal depression, maternal awareness and uterine atony. Conversely, spinal anaesthesia allows patients to remain conscious during the entire procedure (20). Ozer et al. (13) investigated the effect of general anaesthesia on BDNF levels in patients undergoing inguinoscrotal surgery. They found lower BDNF levels in the general anaesthesia group compared to the spinal anaesthesia group in the periods after anaesthesia induction, and before transferring the patient to service from recovery room. They observed increased serum BDNF levels in the period before the end of surgery compared to the period after anaesthesia induction in the general anaesthesia group. Also, there was no significant difference between the serum BDNF levels in the spinal anaesthesia group. In contrast to their results, we reported lower maternal BDNF levels measured before clamping the umbilical cord in the spinal anaesthesia group compared with the general anaesthesia group. Also, we found that maternal BDNF levels during the intraoperative period were significantly higher compared to the preoperative and postoperative periods in the patients who received general anaesthesia. In contrast to the general anaesthesia group, maternal BDNF levels in the intraoperative period were lower than preoperative and postoperative values in spinal anaesthesia group. These differences may be due to the differences in the clinical characteristics of the patients selected for this current study. While this study was performed in patients with term pregnancy, the above study (13) was performed in male patients undergoing inguinoscrotal surgery. Also, blood collection periods were different in two studies. In the above study, blood samples were collected before anaesthesia induction, after anaesthesia induction (but immediately before surgical incision), at end of the surgery and during transfer from recovery room to the service. In this current study, maternal blood samples for BDNF were taken immediately after positioning the patient on the operating table, before clamping the umbilical cord and 24 hours after the first sample was obtained.

On the other hand, surgical stress leads to a systemic immune-endocrine response with a stimulation of the sympathetic nervous system (9) and BDNF levels decrease in case of increased oxidative stress (17). Stress caused by intraoperative awareness may lead to lower BDNF levels in patients who received spinal anaesthesia. Actually, Murakami et al. (17) reported that acute and chronic stresses reduce BDNF mRNA expression in the rat hippocampus. Also, depression was found to be associated with low serum BDNF levels (18).

We didn’t compare the groups in terms of maternal hemodynamic parameters during surgery. Purtuloglu et al. (21) compared the effects of general and spinal anaesthesia on maternal hemodynamic parameters in patients undergoing elective caesarean section. They reported similar arterial mean blood pressure and heart rate values in both groups during the first 30 minutes of surgery. Actually, arterial mean blood pressure and heart rate values were lower in the spinal anaesthesia group, but these differences were not statistically significant in their study. In this current study, maternal blood samples were taken within the first 30 minutes of surgery and 24 hours after surgery. Also, intravenous fluid loading and maternal positioning were applied to patients in order to minimise the risk of maternal hypotension. Therefore, we did not observe severe maternal hypotension in all patients. Even so, the cause of lower cord blood BDNF levels in the spinal anaesthesia group may be a result of the transient changes in maternal and utero-placental blood flow/pressure caused spinal anaesthesia-induced maternal hypotension.
In our study, BDNF levels at 24 hours after surgery were observed to be close to the baseline levels in both groups. This status may result from the termination of the effect of general and local anaesthetics. We thought that BDNF levels increase during the late postoperative period. Contrary to our results, Ozer et al. (13) found that serum BDNF levels measured from recovery to transfer to ward were lower than the basal value in both general and spinal anaesthesia groups. Also, Vutskits et al. (12) reported significant differences in plasma BDNF levels before anaesthesia induction with propofol and 24 hours after surgery. These conflicting results may have been caused by differences between the clinical trial designs and patient populations. While our study population includes women with term pregnancy undergoing elective caesarean section, the abovementioned studies (12, 13) were conducted in male patients undergoing elective minor surgery. Ozer et al. (13) didn’t measure BDNF levels 24 hours after surgery. On the other hand, studies in healthy human adults showed that BDNF concentration in the peripheral blood was lower in males than in females (22, 23).

It was shown that BDNF penetrates through the utero-placental barrier and regulates foetal brain development (4). Malamitsi-Puchner et al. (24) showed that serum levels of BDNF are higher in the mother than in the infant. In a recent study, Flöck et al. (25) reported lower umbilical cord BDNF levels than maternal serum concentrations. Also, they reported that the mode of delivery and gestational ages at delivery are determinants of circulating BDNF in the mother and newborn. Similar to their results (24, 25), we found lower cord blood BDNF levels compared to maternal BDNF levels in this current study.

Malondialdehyde, which is a marker of lipid peroxidation, plays an important role during pregnancy due to an increased cellular activity caused by the growing foetus (8). Oxidative stress may cause down-regulation of neurotrophic factors (17). Dhobale et al. (26) found a negative relation between both maternal and cord serum BDNF levels with maternal MDA and cord MDA levels. In this current study, a negative correlation was also found between both maternal and cord serum BDNF levels with maternal MDA and cord MDA levels.

Propofol has a similar structure to anti-inflammatory drugs, and it rapidly distributes to tissues (half-life of 2–4 minutes) and passes through the placenta. It is used for the induction and maintenance of anaesthesia with rapid onset and rapid recovery (27). Propofol has also protective effects against poly-microbial sepsis via attenuating the pro-inflammatory cytokine response (28). Moreover, Corcoran et al. (29) found that propofol has a protective effect on tissue lipid peroxidation and injury via decreasing MDA production and systemic inflammatory response in patients with impaired myocardial function. The increase in cord blood BDNF levels that we observed in patients using the general anaesthetic technique may be due to the anti-inflammatory effect of propofol. Our results support the relieving effect of propofol on oxidative stress. Unlike our results, Vutskits et al. (12) found a decrease in BDNF levels in the propofol group compared to the thiopental-isoflurane group. These inconsistent results may be due to the collection of blood samples at different times. They measured BDNF levels in the early period of propofol anaesthesia before starting surgery. We collected cord blood samples after the baby was delivered approximately 5 minutes after anaesthesia.

In our institute, regional anaesthesia is commonly preferred for caesarean section (30). However, general anaesthesia when necessary, e.g. when the mother refuses regional techniques, in case of failed regional attempts and in the presence of contraindications to regional anaesthesia such as coagulation disorders or spinal abnormalities. Because we thought that selecting of anaesthetic technique without randomisation may be more ethical, patients in our study were not randomised. Also, we postulated that the results of our study are not affected by this situation.

In the current study, we first evaluated the changes of maternal and cord BDNF levels during caesarean delivery where different anaesthetic techniques have been used. There are some drawbacks to our study. First, we did not compare maternal hemodynamic parameters. Second, it would be interesting to evaluate maternal blood samples at different sampling times such as after cord clamping, 2 hours after operation and 6 hours after operation. There are no data in the literature evaluating long-term significance of altered maternal and cord blood BDNF concentrations caused by anaesthetic technique. It is unknown how this change affects mother’s or newborn’s outcome. It may be interesting to look at the significance of cord blood BDNF levels in relation to neurological and physical developments.

**Conclusion**

We first evaluated the effect of the anaesthetic technique on the BDNF levels in maternal peripheral and cord blood. Maternal BDNF levels measured before the umbilical cord clamping were found to be lower in the spinal anaesthesia group than in the general anaesthesia group. Also, cord blood BDNF levels were higher in patients in who the general anaesthesia technique was applied compared to patients in who the spinal anaesthesia technique was applied. A negative association was observed between both maternal and cord plasma BDNF with maternal MDA and cord MDA, respectively. In summary, our data showed that the aesthetic technique may have an influence on maternal peripheral and cord blood BDNF levels. General anaesthesia induced with propofol and maintained with N₂O-sevoflurane leads to higher BDNF levels in maternal and cord blood than spinal anaesthesia utilising bupivacaine. There is a significant negative correlation between the serum BDNF levels and MDA levels that reflect the stress response. Further studies need to investigate the effects of various regional anaesthetic techniques on maternal and cord blood BDNF levels.
Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ataturk University School of Medicine (B.30.2.ATA.01.00/34).

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

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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