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N terminal-pro brain natriuretic peptide in fetal umbilical cord meconium-stained amniotic fluid: a prospective case control study

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Summary

Purpose of investigation: To evaluate the value of N terminal-pro brain natriuretic peptide (NTpBNP) levels in fetuses with meconium-stained amniotic fluid (MSAF). *Materials and Methods:* In this case control study, of 36 fetuses, 19 had MSAF and 17 had normal, as controls. The blood samples were taken from the fetal umbilical cord just after birth to measure NTpBNP levels. *Results:* Mean NTpBNP values were 1.01 ± 0.49 ng/ml in the patient group and 1.70 ± 0.93 ng/ml in the control group. The difference between the groups was statistically significant ($p = 0.01$) and power was 78% at 95% confidence interval. *Conclusion:* Serum NTpBNP levels were decreased in the study group. The result suggests that NTpBNP may be a valuable marker for fetuses with MSAF.

Key words: Fetal acidosis; Fetal hypoxia; Meconium-stained amniotic fluid; N-terminal-pro brain natriuretic peptide; Umbilical cord.

Introduction

The incidence of meconium-stained amniotic fluid (MSAF) is 7% to 22% in term, and 40% in post-term deliveries [1]. MSAF is a risk factor for chorioamnionitis, meconium aspiration syndrome (MAS), neonatal hypoxic-ischemic encephalopathy, neonatal sepsis, and cerebral palsy. The most severe consequence of MSAF predisposes to meconium aspiration and further MAS, which occurs in approximately 3% to 5% of all neonates, and with a case fatality rate of 5%-40% in otherwise healthy term or post-term infants [2, 3]. Fortunately, most neonates with MSAF do not have evidence of hypoxia or metabolic acidosis at delivery, but obstetricians must be aware of complications of MSAF and have knowledge of its management [3].

A prohormone called pre-pro brain natriuretic hormone (pre-pro BNP) is cleaved into the biologically active brain natriuretic peptide (BNP) and the inactive N-terminal-pro-brain natriuretic peptide (NTpBNP) which is released into the circulation [4]. BNP is a 32-amino acid ring structure peptide that has a short half-life of 20 minutes and is unstable at room temperature. NTpBNP has a longer half-life (60 to 120 minutes) than BNP, and is stable under a range of storage conditions [5]. The main source of BNP is the cardiac ventricles, and it is released into circulation in response to increased end diastolic pressure, volume increases, volume loading, pressure loading, and ventricular stress [5, 6]. BNP acts as a vasodilator and has diuretic and natriuretic properties [7]. The net effect of BNP is a reduction of intravascular volume, ventricular preload, and afterload [5]. Myocardial stretch is a key factor in the secretion of BNP [8]. Additionally, recent studies report that pro-inflammatory cytokines and sympathetic activity have been identified as triggers for BNP secretion [9]. Transcription of the BNP gene has been described in the lung and it has been suggested that capillary leakage by itself could locally increase BNP levels [10]. Furthermore, BNP levels are often elevated in the intensive care unit (ICU) patients even without cardiac dysfunction [10]. Many factors such as sepsis and hypoxemia are related to the elevation of BNP in patients in the ICU [4]. BNP and NTpBNP levels are elevated in children with heart disease and intrauterine fetal anemia [11, 12]. In the literature, there is no knowledge regarding whether the NTpBNP levels are affected in the fetuses with MSAF. Therefore, the present authors designed a study that NTpBNP would be a useful diagnostic peptide in fetuses with MSAF and it could be used in daily clinical practice.

Materials and Methods

The study was designed as a case-control study with 38 participants (20 patients in the study group and 18 in the control group), and conducted between January and March 2011 in the obstetric unit of the Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey. The study was approved by the ethics committee of the Zekai Tahir Burak Women's Health Edu-

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Table 1. — *Clinical characteristics of the two groups.*

Variables	Study (n=19)	Control (n=17)	*p*
Age (years)	28.26 ± 7.22	27.94 ± 6.15	0.60*
BMI (kg/m^2)	30.59 ± 4.59	30.01 ± 4.25	0.82*
Gestation (weeks)	39.53 ± 1.26	39.49 ± 1.14	0.95*
Labor induction	8 (42%)	7 (41%)	1**
Cesarean section	5 (26%)	4(23%)	1**
Birth weight (g)	3254±476	3377±465	0.33*

*Student's *t*-test with mean ± SD; **Pearson Chi Square;
BMI: body mass index; SD: standard deviation.

Table 2. — *Comparison of the two groups for mean NTpBNP values, 1st- and 5th-minute Apgar scores and umbilical artery blood gas analysis.*

Variables	Study (n=19)	Control (n=17)	p
NTpBNP (ng/ml)	1.01 ± 0.49	1.70 ± 0.93	0.01 *
Apgar 1st minute	7 (6-9)	9 (8-10)	0.34**
Apgar 5th minute	7 (6-9)	9 (7-10)	0.34**
pH	7.25±.08	7.28 ± 0.05	0.39 *
pO_2 (mmHg)	22.44 ± 5.59	23.15 ± 6.82	0.70 *
pCO_2 (mmHg)	46.39 ± 8.15	48.64 ± 10.15	0.52 *
BE	-7.69 ± 4.39	-6.68 ± 2.16	0.43 *

*Student's *t*-test with mean ± SD; **Mann Whitney U test with range;
SD: standard deviation; NTp BNP: N-terminal-pro-brain natriuretic peptide;
BE: base excess.

cation and Research Hospital and was performed in accordance with the Helsinki Declaration with written informed consent obtained from all participants.

Data were collected and analyzed independently for each subject. Inclusion criteria were gestational age > 36 weeks, single gestation, and vertex presentation. Exclusion criteria were pregnancies with systemic diseases (preeclampsia, diabetes mellitus, and thyroid disease, etc.), previous cesarean section, preterm labor, fetus with intrauterine growth restriction, or congenital fetal anomalies. Mode of delivery and the appearance of the amniotic fluid following spontaneous rupture of membranes or artificially ruptured membranes were recorded. Fetuses with thick MSAF were enrolled in the study group. Meconium was considered as "thick" if it had a pea-soup quality with visually identified particulate matter. Fetuses with clear amniotic fluid were enrolled in the control group. Neonatal outcome variables included birth weight, Apgar scores at one and five minutes, arterial umbilical cord blood analysis, presence of fetal metabolic acidosis, and admission to the neonatal intensive care unit (NICU). Fetal metabolic acidosis was defined as pH < 7.0 and BE ≤ -12.

Immediately after birth two fetal umbilical artery blood samples were taken from the fetal umbilical cord. Then a two-ml blood sample was immediately sent to the laboratory for blood gas analysis. Eight ml of blood samples for NTpBNP evaluation were placed into a tube. This was immediately centrifuged at 4,000 rpm for ten minutes. After centrifugation the serum was frozen and stored at -80°C until assayed. Frozen serum was thawed before analysis. NTpBNP levels were measured using a ELx808 absorbance microplate reader and ELx50 microplate strip washer with a human NTpBNP ELISA kit from EIAab & USCNLIFE. In this immunoassay, the coefficient of variability was 3–7% and the minimum detection limit was 0.15 ng/ml. Fetal blood gas analysis was performed with an automatic blood gas analyzer.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences Software, version 15.5, and power analysis with the OpenEpi.com web site in February, 2014. Student's *t*-test, Mann-Whitney U-test, and Pearson Chi Square test were used for statistical analysis. Parametric test results are presented as mean ± standard deviation; non-parametric test results are presented as median with quartile. A *p*-value of less than 0.05 was considered statistically significant.

Results

There were no statistically significant differences between the groups according to characteristics of maternal age, body mass index (MBI), gestational age, or fetal birth weight (Table 1).

There was one extreme value of NTpBNP (9.08 ng/ml) in the patient group and one (8.66 ng/ml) in the control group. These data were removed from statistical analyses. The mean NTpBNP value was 1.01 ± 0.49 ng/ml in the patient group, and 1.70 ± 0.93 ng/ml in the control group, respectively. The difference between groups was statistically significant (p = 0.01) and power was 78%. Labor induction with oxytocin was similar in patient and control groups [eight (42%) and seven (41%), respectively, p = 1]. Cesarean section rates did not differ between the two groups [five (26%) in the patient group, and four (23%) in the control group, p = 1]. First- and fifth-minute Apgar scores and umbilical blood gas analyses were similar in the two groups (Table 2). There were no cases of fetal acidosis, meconium aspiration syndrome, and admission to NICU in the two groups.

Discussion

In this study, the authors detected that the NTpBNP levels in the fetuses with MSAF were significantly lower compared to the fetuses with clear amniotic fluid. Studies demonstrated that Natriuretic peptide was elevated in pulmonary and cardiac disorder [4, 8, 9]. To the present authors' knowledge, this is the first reported study with decreased NTpBNP levels in fetuses.

BNP and NTpBNP levels are elevated in children with heart disease, which leads to an increase in ventricular pressure and volume loading [5]. Merz *et al.* reported that fetuses with increased volume load due to anemia have increased NTpBNP levels, and the increase is highest in hydropic fetuses. The degree of anemia is also well-correlated with the increased NTpBNP level. After fetal intrauterine transfusion (IUT) to address fetal anemia, the NTpBNP level decreases and this decrease correlates with an increased hemoglobin level [12]. In addition, it is reported that pro-inflammatory cytokines and sympathetic activity are triggers for BNP secretion [9, 10]. Furthermore, transcription of the BNP gene has been described in the lung and it has been suggested that capillary leakage by itself could lo-

cally increase BNP [4]. BNP levels are often elevated in ICU patients in whom cardiac function is normal [4].

Meconium is a source of pro-inflammatory cytokines, such as tumor necrosis factor alfa (TNFα) or interleukins (IL-1, IL-6, and IL-8). In the case of meconium aspiration, if the distal pulmonary is reached, pulmonary surfactant is inactivated and this triggers the development of inflammation, pulmonary edema, vasoconstriction, and broncho-constriction. Inflammation plays an essential role in the pathogenesis of MAS. The substances in meconium may injure the lung tissue directly or indirectly by activating inflammation and result in the development of acute respiratory distress syndrome (ARDS), which may lead to primary pulmonary hypertension of neonate (PPHN) [13]. In adult patients, ARDS is associated with pulmonary hypertension and right ventricular dysfunction, resulting in increased BNP levels [4]. BNP levels are significantly higher in neonates with a diagnosis of PPHN [14-16]. BNP is a marker of ventricular wall stress secondary to myocardial dysfunction or PPHN. These biomarkers may in the future help to predict mortality and outcome following a perinatal hypoxic-ischaemic insult [16].

The exact etiology of meconium passage into the amniotic fluid is unknown; therefore there are many mechanisms postulated to explain the passage of meconium. Meconium passage into the amniotic fluid may be a response to fetal stress, such as hypoxia, or infection. Darkhaneh *et al.* reported that infants with MSAF have higher absolute nucleated red blood cell counts than infants with clear amniotic fluid. Increased nucleated red blood cell count (NRBC) is related to a hypoxic situation [17]. In addition, fetuses with MSAF have elevated erythropoietin levels at birth, regardless of gestational age [18]. These findings suggest that neonates with MSAF suffer from fetal hypoxia in the uterus before delivery, and pregnancy with MSAF is a high risk circumstance for fetal hypoxia. Dollberg *et al.* reported that NRBC counts were elevated in neonates with MSAF [19]. These findings support the hypothesis that fetuses with MSAF exposure experience hypoxic conditions in the intrauterine prenatal period. In the term infant, the most common mechanism of hypoxia is caused by circulatory problems, such as placental abruption, placental artery clotting, inflammatory processes, or uterine contraction during labor. These problems cause diminished exchange of oxygen and carbon dioxide [16]. In the occurrence of such hypoxic conditions, the fetal heart rate changes, there is an increase in arterial blood pressure, and a redistribution of cardiac output to supply adequate blood to vital organs (brain, coronary, and adrenal glands) at the beginning of hypoxia [20]. Hypoxia stimulates fetal chemoreflex, which triggers the vagal nerve and results in bradycardia. In the case of hypoxia, the fetal heart declines its rate to reduce myocardial work and oxygen requirements during hypoxia [21]. In addition, hypoxia that is associated with a decrease in myocardial performance has a direct depressant effect on myocardial function [22]. Additionally a correlation exists between fetal hypoxia and increased intestinal peristalsis. Hypoxia stimulates the vagal nerve, which then triggers intestinal peristalsis and anal sphincter relaxation, resulting in meconium passage into the amniotic fluid as soon as the fetus can compensate for hypoxia [1, 23]. This would probably explain why most fetuses with MSAF during delivery are in good condition at birth. The presence of meconium in the amniotic fluid is more likely associated with fetal hypoxia than fetal acidosis [1, 24]. The present authors may explain this condition in that fetal hypoxia causes activation of the vagal reflex, which decreases fetal heart beat and relaxes the anal sphincter, resulting in meconium passage. Decreased fetal heart beat may result in decreased heart wall stretch, leading to decreased NTpBNP release into fetal circulation. This may explain why NTpBNP levels were lower in the patient group than the control group in this research.

Conclusion

The results of this study suggest that the serum NTpBNP levels were decreased in the fetuses with MSAF, and NTpBNP may be a useful cardiac biomarker for fetuses with MSAF. Due to small number of the study population the authors could not draw definitive conclusions. Further studies with larger populations are needed to evaluate the value of NTpBNP in fetuses with MSAF.

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