The Relationship Between Fetal Nutritional Status and HPA Axis in Neonates

Yenidoğanlarda Fetal Nütrisyonel Durum ve HPA Ekseni İlişkisi

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ABSTRACT

Objective: The aim of this study is to evaluate the possible relationship between fetal nutritional status and hypothalamic pituitary adrenal axis in neonates.

Methods: The prospective observational study was conducted on singleton term appropriate for gestational age and small for gestational age infants during a 1-year period. All of the neonates' cord-blood cortisol and adrenocroticotropic hormone (ACTH) levels were measured, and those identified as suffering from fetal malnutrition (FM) were placed into the study group, while the cord-blood cortisol and ACTH levels of well-nourished term newborns comprised the control group.

Results: A total of 588 term newborns were included in the study. The body weight, height and head circumference values of the study group were found to be lower in the study group compared to the control group (p=0.001). The cortisol levels were found to be increased by 22.6% in the study group and by 13.9% in the control group. No difference was found between the cord blood cortisol levels. No difference was observed between the study group and control group in terms of the cord blood ACTH levels.

Conclusion: The study results were not found association between the cord blood cortisol and/or ACTH values and FM. Cord blood cortisol and ACTH values do not seem to be appropriate laboratory tests in terms of supporting the diagnosis in neonates with FM.

Keywords: Adrenocorticotropic hormone, nutrition assessment, cord blood, cortisol, fetal malnutrition

ÖZ

Amaç: Bu çalışmanın amacı yenidoğanlarda hipotalamik hipofis adrenal aksının fetal beslenme durumu ile ilişkisini araştırmaktır.

Gereç ve Yöntemler: İleriye dönük gözlemsel çalışma, 1 yıllık bir süre boyunca gestasyonel yaş ile uyumlu term tekiz bebekler ile gestasyonel yaşa göre küçük term tekiz bebekler ile yapıldı. Yenidoğanların tümünün kord kanı kortizol ve adrenokortikotropik hormon (ACTH) düzeyleri ölçüldü ve fetal malnütrisyon olarak tanımlanan yenidoğanlar çalışma grubuna alındı, iyi beslenmiş term yenidoğanların kord kanı kortizol ve ACTH düzeyleri kontrol grubunu oluşturdu.

Bulgular: Çalışmaya toplam 588 dönem yenidoğan dahil edildi. Çalışma grubunun vücut ağırlığı, boy ve baş çevresi değerleri kontrol grubuna göre daha düşük bulundu (p=0,001). Çalışma grubunda kortizol düzeyleri %22,6, kontrol grubunda %13,9 artmış idi. Kord kanı kortizol düzeyleri arasında fark bulunmadı. Çalışma grubu ile kontrol grubu arasında kord kanı ACTH düzeyleri açısından fark saptanmadı.

Sonuç: Çalışma sonuçları kord kanı kortizol ve/veya ACTH değerleri ile fetal malnütrisyon arasında ilişki bulunmadı. Kord kanı kortizol ve ACTH değerleri, fetal malnütrisyonlu yenidoğanlarda tanıyı destekleme açısından uygun laboratuvar testleri gibi görünmemektedir.

Anahtar kelimeler: Adrenokortikotrop hormon, beslenme değerlendirmesi, kordon kanı, kortizol, fetal malnütrisyon

INTRODUCTION

Fetal malnutrition (FM) is the clinical picture where adequate subcutaneous adipose and muscle tissue cannot develop or is lost. It may occur during any gestational week (1-4). Production of placental corticotropin releasing hormone (CRH) is related with the duration of pregnancy. Therefore, maternal serum CRH levels increase incrementally and make a peak at the time of delivery. As labor approaches, CRH/adrenocroticotropic hormone (ACTH)/cortisol fluctuation stimulates fetal adrenal cortex, adrenal 3β-hydroxysteroid dehydrogenase (3β-HSD) activity increases and the ratio of placental 11β-HSD2/11βHSD1 relatively decreases (5). In humans, reduction in placental 11β-HSD2 mRNA production and increase in fetoplacental cortisol levels lead to an increase in placental prostaglandin (PGE2 and PGF2α) synthesis and this leads to contractility in the uterus and excess CRH production. Increased fetal adrenal cortisol production and reduced placental cortisol clearance are synchronized with maturation of critical organs (lung, liver, intestines, kidney, and brain). Consequently, term delivery can be regarded as a mechanism escape from intrauterine hypercortisolemia setting (5).

According to fetal origin hypothesis, intrauterine negative environmental factors play a role in the fetal hypothalamo-pituitary-adrenal (HPA) axis in development of fetal tissues and may lead to organ dysfunction and diseases in the advanced stages of life (6-8). In studies conducted with experimental animals, it has been found that inadequate maternal nutrition increases the plasma cortisol levels both in the mother and in the fetus with growth and developmental retardation.

In this study, it was aimed to evaluate the relation of the cord blood cortisol and ACTH levels with FM in neonates diagnosed with FM based on the hypothesis that negative intrauterine conditions may lead to FM and thus fetal HPA axis may be affected.

MATERIALS AND METHODS

Study Design

The prospective observational study was conducted between May 1st 2012 and June 1st 2013. The single, term (37-42 completed gestational weeks), living appropriate for gestational age (AGA) or small for gestational age (SGA) babies born by cesarean section or normal vaginal delivery between the 37th and the 42nd gestational weeks according to ultrasonographic measurements and last menstruation date who were found to have only infants with FM constituted the study group.

Study exclusion criteria consisted of multiple pregnancy, prematurity (<37 completed gestational week), mothers being given antenatal steroid for threatened preterm labour previously, being postterm (>42 completed gestational week), being large for gestational age (LGA) (birth weight >90th percentile by gestational week), having died in the delivery room or receiving postnatal resuscitation, presence of major congenital anomaly and presence of cyanotic or acyanotic congenital heart disease and emergency cesarean section, chronic drug usage or precence of the maternal chronic disease, obviously birth stress and asphyxia.

The control group composed of the healthy, term, single, AGA neonates who had FM score of >24.

The history of gradivity (G), parity (P), abortus (A), curettage (C), the number of pregnancies and history of preterm delivery, smoke and alcohol habits, drug usage or chronic disease related with previous pregnancies were recorded. Ethics committee approval was obtained from the hospital's ethics committee. Oral or written informed consent was obtained from all parents.

Study Protocol

The gestational age was identified 12-24 hours after delivery with the date of the last menstrual period and Dubowitz scoring system, independent of the Clinical Assessment of Nutritional Status Score (CANSCORE). The weight, height and head circumference of the newborns were measured by the neonatology nurse who worked in the delivery room. Afterwards, the newborns were classified as SGA, AGA and LGA babies using the Lubchenco intrauterine growth curves.

The CANSCORE scale described by Metcoff was used in the study (1). According to this scale the babies with a score of <24 were considered to have malnutrition (1). The CANSCORE assessment was performed in the first 12-24 hours of life. According to the CANSCORE method, the newborns were divided into two groups as the group with FM (study) and the well nutrition group (control). FM was evaluated by CANSCORE after the classified as SGA, AGA and LGA babies using the Lubchenco intrauterine growth curves.

The cord blood cortisol and ACTH levels of all newborns included in the study were studied in the laboratory and recorded. Body mass index (BMI) of mothers was calculated for BMI: weight/(height)^2 formula.

Laboratory Measurements

Approximately two cubic centimeters of blood sample was obtained from the cord blood in all newborns included in the study at the time of delivery and placed in hemogram tubes with ethylenediaminetetraacetic acid . These samples were centrifuged at 4000 rpm for 15 minutes. The serum samples separated were placed in Eppendorf tubes on which the names and protocol numbers were noted and frozen at -80 degrees in refrigerator. The serum samples, saved for approximately two months until all analysis samples were completed, were studied by chemiluminescent immunoassay method at 37 °C as microgram/dL with the kit with the lot number L2KCO2 325 for cortisol value and as picogram/mL with the kit with the lot number L2KAC2 245 for ACTH value using a chemiluminescent immunometric assay on an Immulite 2500 analyzer (Siemens Immulite 2500, Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). The birth weights of the newborns were measured with a digital weighing scale (SoehnleSilver Sense, Nassau, Germany), the heights were measured with an infantometer (Seca 416, Seca, Hamburg, Germany) and the head circumferences were measured with a head circumference-meter (Dekka) in the delivery room and recorded on the birth cards. Afterwards, the newborns were classified as SGA (below the 10th percentile), AGA (10-90th percentile) and LGA (above the 90th percentile) babies using the Lubchenco intrauterine growth curves. The first and fifth minute APGAR scores of all newborns were recorded.

Statistical Analysis

Statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 statistical software (Utah, USA) and independent t-test was used in comparison of two groups for variables which showed a normal distribution, Mann-Whitney U test was used in comparison of two groups for variables which did not show a normal distribution and chi-square and Fisher's exact test were used for comparison of qualitative data in addition to descriptive statistical methods. Logarithmic transformation was used because of the distributions of the variables of cortisol and ACTH. The areas under the receiver operating characteristic (ROC) curve were calculated for the differential diagnosis of FM. A p value of <0.05 was considered significant.

Results

Total 588 neonates (318 study group, 270 control group) were included in the study. FM was 174 (54.72%) of the AGA neonates and 144 (45.28%) were SGA neonates with identified with CANSCORE. No difference was observed between the groups in terms of gestational week and gender distribution. The mean weight, height and head circumference and CANSCORE values were found to be lower in the FM group compared to the control group (p<0.05) (Table 1). There was no difference between the, gestational age, gender, mean 1st and 5th Apgar scores, cortisol and ACTH values between the control and FM groups (p>0.05).

History of pregnancy and factors relating to the mother between the groups are shown in Table 2. No differences were observed in parity and maternal age, parity, smoking habit and maternal alcohol intake between the FM and control groups.

Delivery, amniotic fluid and placenta characteristics are presented in Table 3. No difference was found between the groups in terms of gravidity, parity, abortus and number of previous deliveries (p>0.05) and presence of amniotic fluid and placenta anomaly (p=0.907).

There was no correlation was found between the CANSCORE and cortisol values (r=-0.085, p=0.403) and between the CANSCORE and ACTH values (r=0.013, p=0.901) (Table 4). In the differential diagnosis of FM, the areas under the ROC curve for the CANSCORE and for the variables of ACTH and cortisol were measured. Accordingly, the CANSCORE area was found to be higher compared to cortisol and ACTH (p=0.001) (Figure 1).

Table 1: General characteristics of study and control groups

	Fetal malnutrition (n=318) mean ± SD	Control group (n=270) mean ± SD	p
Gestational age (week)	38.42±1.12	38.53±0.82	0.71
Male	180 (56.6%)	162 (60.0%)	0.73
Female	138 (43.4%)	108 (40.0%)	
Birth weight (g)	2962.64±321.4	3351.56±318.6	0.0001
Birth height (cm)	48.26±1.92	49.58±2.01	0.001
Head circumference (cm)	33.58±1.25	34.28±1.3	0.008
APGAR score			
1-minute	8.58±1.18	8.56±1.17	0.46
5-minutes	9.75±0.75	9.78±0.71	0.58
CANSCORE	21.25±1.65	29.91±2.87	0.001
Cortisol (mcg/dL)	17.29±12	15.93±11.52	0.57
ACTH (pg/mL)	91.33±105.14	90.24±73.7	0.95

CANSCORE: Clinical Assessment of Nutritional Status Score, SD: Standard deviation, ACTH: Adrenocroticotropic hormone

Table 2: History of pregnancy and factors relating to the mother

		Fetal malnutrition (n=318) mean ± SD	Control group (n=270) mean ± SD	p
Maternal age (years)		27.09±4.69	26.68±6.13	0.75
Parity		1.75±0.98	2.02±1.11	0.25
Maternal weight gain (kg)		10.3±2.74	14.14±3.79	0.0001
Maternal body mass index		21.3±0.74	18.74±0.59	0.93
Maternal smoking	(+)	72 (22.60%)	42 (15.55%)	0.37
Maternal alcohol intake	(+)	18 (5.6%)	12 (4.4%)	0.78

SD: Standard deviation

Table 3: Delivery, amniotic fluid and placenta characteristics

		Fetal malnutrition (n=318) n (%)		Control group (n=270) n (%)		p
Meconium stained delivery	(+)	36	11.32%	12	2.22%	0.08
Delivery mode	NSD	192	60.38%	156	57.78%	
	CSA	126	39.62%	114	42.22%	0.79
Birth weight by gestational week	AGA	174	54.72%	270	100.00%	
	SGA	144	45.28%	0	0.00%	0.001
Appearance of the amniotic fluid	Clear	288	90.57%	258	95.56%	
	Meconium stained	30	9.43%	6	2.22%	
	Bloody	0	0.00%	6	2.22%	0.19
Placental anomaly	(+)	6	1.89%	6	2.22%	0.90

NSD: Normal spontaneous delivery, CSA: Cesarean section, AGA: Appropriate for gestational age, SGA: Small for gestational age

Table 4: Correlation analysis in the fetal malnutrution group (n=318)

		Fetal malnutrution
Mother age	R^2	0.156
	p	0.175
Parity	R^2	–0.125
	p	0.218
Weight gain of mother (kg)	R^2	–0.168
	p	0.549
Cortisol (mcg/dL)	R^2	0.165
	p	0.130
CANSCORE	R^2	0.32
	p	0.001
ACTH (pg/mL)	R^2	0.129
	p	p=0.17

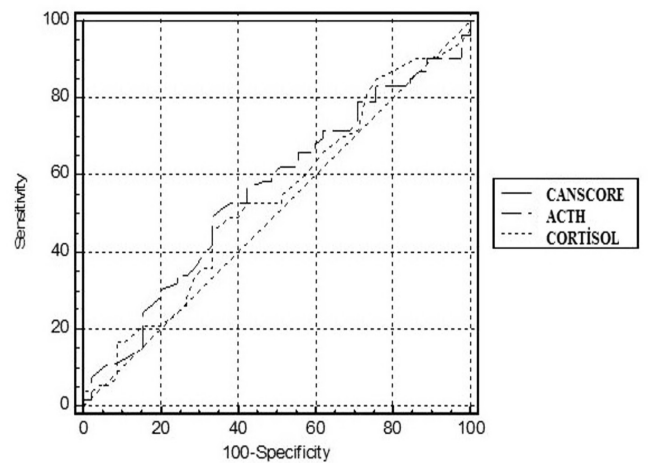CANSCORE: Clinical Assessment of Nutritional Status Score, ACTH: Adrenocroticotropic hormone

Figure 1: Comparison of the CANSCORE, cortisol and ACTH values on the ROC curve

CANSCORE: Clinical Assessment of Nutritional Status Score, ACTH: Adrenocroticotropic hormone, ROC: Receiver operating characteristic

Discussion

The clinical findings of FM vary depending on the gestational week and duration of malnutrition (1). In the studies of Georgieff and Sasanow and Metcoff (9,10), it was reported that the height and head circumference were within the normal limits, while the body weight was substantially low in newborns affected in the late period of pregnancy. In our study, the weight, height and head circumference values were found to be lower in the study group compared to the control group.

Previous studies reported that the rates of FM identified with CANSCORE were 4-8.3% in AGA babies and 23.3-59.9% in SGA babies (11,12). In the study of Kushwaha et al. the AGA rate was found to be 20% in the FM group and the SGA rate was found to be 80% (13). In our study, the AGA rate was found to be 54.72% and the SGA rate was found to be 45.28% in the malnutrition group. These relatively higher rates might be related with genetic factors, maternal factors and placental factors.

In the study of Belkacemiet al. conducted with experimental mice, it was shown that increased maternal glucocorticoids induced by maternal malnutrition and stress caused intrauterine growth retardation in the fetus (14). In the study conducted by Lesage et al. the cortisol, ACTH and catecholamine levels were measured in the baseline and in stressful conditions in male experimental mice aged 4 months who had malnutrition throughout the perinatal life and in the control group based on the hypothesis 'perinatal malnutrition determines sympathoadrenal and HPA axis response to limited stress in adult male experimental mice (15). It was concluded that increased basal corticosterone value created a higher corticosterone effect in the target cells and this regulated the negative feedback mechanism on the HPA and sympathoadrenal axis with the effect of reduced corticosterone binding globulin (CBG) and increased hippocampal mineralocorticoid receptors in mice with perinatal malnutrition.

In the study conducted by Ducsay and Myers, the effect of hypoxia on steroid synthesis, the effect of nitric oxide (NO) on steroid synthesis and the increase in endothelial NO synthase (eNOS) were investigated (16). Accordingly, it was shown that NO played a key role in protecting normal cortisol levels and eNOS activation resulted in a decrease in transcription of the critical proteins which regulate cortisol synthesis (*CYP11A1* and *CYP17*) in hypoxic fetus. This hypothesis was found to be compatible with reduction observed in cortisol synthesis and CYP expression despite high basal ACTH level in the adrenal cortexes in sheep fetuses that have remained hypoxic for a long-term in the intrauterine period. However, it was found that large increases in ACTH (approximately 10-20-fold higher than the basal level) overcame the inhibitory effect of NO under conditions of a second stress and resulted in substantially increased cortisol synthesis compared to the control group.

In line with this study, it was found that marked increase did not occur in cortisol levels with respect to the ACTH level which increased as a response to stress in our study. Although human studies related with this issue are inadequate, one of the reasons for increased cortisol level in neonates with FM in our study might be suppression of local steroid synthesis by NO related with endothelial NOS induced by stress. Response of the fetus with such a mechanism to protect fetal growth in conditions of stress appears to be compatible with the local inhibitor effect of NO.

In the study conducted by Rakers et al. in which the effect of prenatal maternal stress on the HPA axis and fetal cortisol level in the early and late period of pregnancy was evaluated, blood cortisol levels were measured in pregnant mice by exposing them to regular stress in the early and late period of pregnancy (17). Blood cortisol levels were also measured in the same mice following administration of betamethasone which is a synthetic glucocorticoid and is used to induce fetal lung maturation in clinical practice. In both groups, it was found that stress and betamethasone given in the early phase of pregnancy caused significantly higher cortisol levels compared to the control group.

In the study of McNeil et al., it was found that fetal organs exhibited different timing in glucocorticoid receptor and 11bHSD synthesis (18). It was proposed that especially the liver was more sensitive to cortisol in the late phase of pregnancy, exposure to glucocorticoid naturally caused differences in growth and glucocorticoid sensitivity in tissues and this occurred in three different phases of gestation. Stjernholm et al. reported in their study that cortisol was higher in the vaginal delivery group at the onset of labor as compared to the cesarean section preoperative group (19). There were also significant differences between postpartum and postoperative cortisol levels of vaginal delivery group as compared to cesarean section group. In 2015, in a study called "Stress-related Hormone Response of the Neonates", Su et al. reported that prenatal maternal stres may negatively affect neonatal outcome and cord plasma ACTH and cortisol levels (20). Control group's mean ACTH level was 19.75±7.15 pg/mL and mean cortisol

level was 252.80±22.86 ng/mL. In our study, vaginal or cesarean delivery modes were not separated for ACTH and cortisol levels but we did not show any difference between the ACTH and/or cortisol levels of groups.

Deodhar et al. were done a prospective observational study on 601 newborns (21). In this study, maternal risk factors such as <18 or >35 years, pre-pregnancy weight <40 kg, height <145 cm, pregnancy-induced hypertension and bad obstetric factors were found to have significant impact on FM. In our study, there was no correlation was found between the CANSCORE and cortisol values and/or ACTH values.

CONCLUSION

Finally, our study results were not found association between the cord blood cortisol and/or ACTH values and FM. Cord blood cortisol and ACTH values do not seem to be appropriate laboratory tests in terms of supporting the diagnosis in newborns with FM.

Ethics Committee Approval: It was taken.

Informed Consent: It was taken.

Authorship Contributions

Surgical and Medical Practices: E.D., Ö.S., Concept: E.D., Ö.S., Design: E.D., Ö.S., E.C., Data Collection or Processing: E.C., Ş.T.N., S.H., Analysis or Interpretation: E.C., Literature Search: E.D., S.H., Ö.S., Writing: E.D., Ö.S., E.C., Ş.T.N., S.H.

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