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Stress Response Assessment between First and Second Elective Caesarean Sections by Comparing Cortisol Levels

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Abstract

Objectives. The aim of this study was to compare the stress response produced during elective CS for the first and second time. For that goal, cortisol blood levels before, during and after childbirth were measured. **Materials and Methods.** We performed this prospective observational study during the period of September 2020 to September 2021. Blood samples were taken from all participants at three different stages. A statistical analysis was performed to compare the CS1 (first elective Caesarean) and CS2 (second elective Caesarean) groups. **Results.** At every stage, the levels of cortisol were statistically higher in the CS1 group than in the CS2 group. Therefore, CS2 generates a significantly less stressful response than CS1. Between stages, in CS2 cortisol was lowered at a faster rate than in CS1, meaning the stress response initiated was present for a longer time period in the CS1 group. **Conclusion**. A second elective caesarean section is a safe procedure that does not place an unnecessary burden upon the mother. This is an important fact that practitioners can rely upon while designing the ideal management of a pregnant woman for the stressful environment of birth.

Key Words: Cortisol ▪ Stress Response ▪ Caesarean Section ▪ Pregnancy ▪ Childbirth.

Introduction

Stress is a stimulus, originating either from the environment or from the organism itself, that produces a response from the receptor organism. The response that serves as an answer to the stimuli is called the stress response (1). All living organisms face physiological stress, and nature has adapted a series of systems with the sole purpose of achieving the desired homeostasis. Two basic feedback systems provide a fast positive impact, the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenocortical (HPA) axis. The SNS elevates blood pressure and heart rate, and stimulates hepatic glucogenolysis. The glucocorticoids produced from the HPA axis restrict the burden and time allocated by the organism to the stress response. Lengthening the time allocated to the stress response may prove harmful and be a cause of loss of stress control (2). Interestingly, it has been found that the HPA axis, as a source of stressful stimuli, holds an important place in the development of the embryo and the safe resolution of the pregnancy (3, 4).

Pregnacy requires a careful balance of the endocrine and immune systems in order for the human body to adapt to the new conditions (5). The fetus lies in an increasingly stressful environment at the end of pregnancy, foretelling the beginning of labor (6). These stress signals produce a response from the fetal HPA axis in the release of dehydroepiandrosterone sulfate (DHEAS) and cortisol (CORT) by the adrenal glands of the fetus (7). The high levels of estrogen in the blood are created from the conversion of DHEAS. The low progesterone levels, in conjunction with the high estrogen, reconstruct the cellular tissue of the

cervix, resulting in the stages of dilation. After the birth of the newborn, the levels of the corticotropin releasing hormone (CRH) return to the values before the pregnancy (8).

The correlation between prenatal maternal stress and maternal endogenous cortisol is well known (9, 10). The normal development of the fetus is dependent upon the cortisol hormone (11). The mother's cortisol levels may increase up to 2 to 4 times (4), which is necessary for the neural development of the newborn (12). On the other hand, excess maternal cortisol exposure may prove harmful to the development of the child's brain (13, 14). The effectiveness of the HPA axis is known to change during caesarean section surgery. The stress that the mother is subjected to has been recorded to decrease with the administered analgesia. Cortisol levels have been calculated to be lower in mothers undergoing caesarean delivery compared to vaginal delivery, a phenomenon worth investigating (8, 15).

The HPA axis of the pregnant woman shows the state of hypercortisolism. Pregnancy increases levels of cortisol, the adrenocorticotropic hormone (ACTH), the corticotropin-releasing hormone (CRH) and corticosteroid-binding globulin (CBG). Research suggests that the placenta secretes CRH and ACTH, increasing cortisol levels in the blood. Placental CRH is believed to be unaffected by negative feedback. In fact, the cortisol level of the fetus enhances placental CRH production. The highest CRH levels in the maternal blood are detected during the third trimester of pregnancy. Childbirth is the time point after which CRH returns to the pre-pregnancy values. Apart from this, circulating estrogens have a positive effect on CBG production by the liver. More free cortisol is bound, thus negative feedback is lessened, cortisol synthesis is increased, and the clearance rate of cortisol decreases (16).

The published literature suggests that caesarean section (CS) causes a reduced stress response in the newborn, taking into account the lower levels of cortisol in the umbilical blood (17). Knowing that there is a distinct correlation between the HPA axis of the newborn and the stressful environment of birth, it is well worth performing studies that assess the relationship between stress occurring at birth and the response of the fetus's mother (16, 18). The precise mechanisms that cause the phenomenon described here to take place are worth exploring further. For that goal, cortisol blood levels before, during and after childbirth were measured. Cortisol acts as the mediator for the "fight or flight" response in stressfull stimuli, promoting hormonal adaptations to these stimuli (19).

The aim of this study was to compare the stress response produced during elective CS for the first and second times.

Materials and Methods

We performed this prospective observational study during the period from September 2020 to September 2021 at the "Helena Venizelou" General and Maternity Hospital in Athens.

Demographic Characteristics

The women that were included in this study fulfilled a specific set of characteristics. Inclusion criteria were as follows: Aged between 20 and 44 years old; Singleton pregnancies with consent for elective CS given before going into labor; Labour did not start spontaneously; CS performed during the hours 9:00–14:00; Obstetric history (current or past) free of complications; CS performed at term. Exclusion criteria were as follows: Aged younger than 20 years old or older than 44 years old; Multifetal pregnancy; Labor started before 37 weeks or after 40 weeks; Medical history with obstetric complications in the current or past pregnancy (pregnancy hypertension, preeclampsia, gestational disorders, intrauterine growth restriction (IUGR), oligohydramnios or hydramnios, gestational/mellitus diabetes, corticosteroid treatment, autoimmune diseases, hepatic insufficiency); Conception using assisted reproduction techniques (ART).

The sample size of this study (N=40) was divided into two groups according to the number of CSs that they had undergone, CS1 for the group of

women that were undergoing elective CS for the first time (N=20) and CS2 for the group of women that were undergoing CS for the second time. All recorded CSs were performed under epidural anesthesia and did not present intraoperative complications.

Blood Sample Analysis

Blood samples were taken from all participants. Samples were collected from the median antebrachial vein of the women at three different stages: Stage I: 120 minutes before childbirth; Stage II: 120 minutes after childbirth; Stage III: 48 hours after childbirth. Ethylenediaminetetraacetic acid (EDTA) test tubes were used to collect the blood samples. The test tubes were left to clot for 30 minutes at room temperature after collection. The samples were centrifuged in a refrigerated centrifuge at 4000 rev/min for 10 minutes in order to remove the clots. The blood serum was then transported to 0.5mL aliquots and stored at -80 °C.

Standard competitive enzyme-linked immunosorbent assay (ELISA) was employed for the quantification of blood serum levels of cortisol. Commercial kits were used, according to the manufacturer's instructions (cortisol parameter assay kit, provided by the R&D Systems Inc., Minneapolis, USA). 96-well microtiter plates were used for the analysis. A microplate reader (Varsamax, Molecular Devices, Sunnyvale, CA, USA) at 450 nm was used for color formation measurement. Calculations were made using SoftMax Pro software (Molecular Devices). Average values were recorded after duplicate analysis of samples (19).

Ethics Statement

The Bioethics Committee of the hospital approved the study protocol and the consent form (document number: 137/12-05-2020) in accordance with the Helsinki Declaration. Oral instructions about the process and goals of the study were given to all women whose participation was approved. All women included in the study signed a written consent form.

Statistical Analysis

Statistical analysis was performed to compare the CS1 and CS2 groups regarding the levels of cortisol. In addition, data from the patients' clinical records were compared, that is, maternal age, gestational age and birth weight. Data were expressed as mean±SD and the Shapiro-Wilks test examined the normal distribution of the parameters. Homogeneity between groups was performed using the independent samples t-test. We used the two-way Mixed ANOVA model using as factors "caesarean delivery" (between groups) and "time" (within the group) for analysis of the biochemical markers. Since there was statistically significant interaction between these factors, we used univariate analysis, e.g. comparison between groups for each stage separately and comparison of stages for each group separately, making the appropriate adjustment of P-values based on the Bonferroni correction. More specifically, a one factor Repeated Measures ANOVA model was used for comparison of the different stage measurements of biochemical markers for each group, and the independent samples t-test was used for the between groups comparison at each stage separately, making all the necessary adjustments of P-values. Sensitivity analysis concerning the baseline-balance between the two groups was performed using the analysis of covariance model (ANCOVA) considering the absolute change from Stage I to Stage II and Stage III respectively as dependent variables, the group (caesarean delivery 1 vs. caesarean delivery 2) as a factor and the Stage I value as a covariate. All tests are two-sided, and statistical significance was set at $P<0.05$. All analyses were carried out using the statistical package SPSS vr 21.00 (IBM Corporation, Somers, NY, USA).

Results

40 women participated in the prospective study presented. Twenty were primigravida that delivered via caesarean section, and twenty underwent caesarean section for the second time. There was no statistically significant difference between

the maternal ages (P=0.331) and the babies' birth weights (P=0.280) in the two groups, whereas there was a difference in the gestational age (P=0.014), but due to the small and similar standard deviations in the two groups, in effect, the difference in the gestational age was 0.5 weeks, or 3.5 days (Table 1).

There was statistically significant interaction between "caesarean delivery" and "time" factors F(2.76)=7.6 , P=0.001. There were statistical significantly differences between "caesarean deliveries" at Stage I t(38)=4.8 , P<0.0005 , Stage II t(38)=9.2 , P<0.0005 and Stage III t(38)=4.8 , P<0.0005 respectively (Table 2, 3).

There was a statistically significant difference between stage measurements for the "caesarean delivery 1" group (P<0.0005). Pairwise comparisons presented a statistically significant difference between Stage I and Stage II (P<0.0005) Stage III (P<0.0005) respectively and Stage II and Stage III (P<0.0005).

There was a statistically significant difference between stage measurements for the "caesarean delivery 2"" group (P<0.0005). Pairwise comparisons presented a statistically significant difference between Stage I and Stage II (P<0.0005) and Stage III (P<0.0005), respectively, and Stage II and Stage III (P<0.0005) (Figure 1).

The absolute change in cortisol levels from Stage I to Stage II was statistically significantly lower for "caesarean delivery 2" than "caesarean delivery 1" -F(1.37)=45.2 ; P<0.0005. The absolute changein cortisol levels from Stage I to Stage III was statistically significantly lower for "caesarean delivery 2" than "caesarean delivery 1" F(1.37)=16.7; P<0.0005 (Figure 2).

Table 1. Demographic Characteristics

Characteristics	Caesarean delivery 1	Caesarean delivery 2	Mean difference (95% CI)	P-value*
Age	28.65±4.68	30.20±5.25	-1.55 (-4.73–1.63)	0.331
Gestation period	38.29±0.61	37.81±0.58	0.49 (0.10–0.87)	0.014
Birth weight	3159.50±382.82	3030.25±362.62	129.25 (-109.44–367.94)	0.280

*Independent samples t-test.

Table 2. Comparison of Cortisol between Groups during the Observation Period

Group	Stage I	Stage II	Stage III	P-value (wg)
	($\bar{x}$±SD)			
Caesarean delivery 1	225.6±11.3	127.9±9.8*	105.1±7.1*,†	<0.0005
Caesarean delivery 2	210.6±8.3	102.4±7.7*	94.1±7.4*,†	<0.0005
P-value (bg)	<0.0005	<0.0005	<0.0005	-

P-value (wg)_within groups; P-value (bg)=between groups; *P<0.0005 vs Stage I; †P<0.0005 vs Stage II; Analyses (wg) were performed using the one factor Repeated Measures ANOVA model and the Bonferroni test; Analyses (bg) were performed using the Independent samples t-test.

Table 3. Maca Stage Comparison

Group	Maca* Stage I to II	Maca* Stage I to III
Caesarean delivery 1 (mean±SE†)	-91.1±2.3	-112.6±1.9
Caesarean delivery 2 (mean±SE†)	-114.8±2.3	-124.4±1.9
P-valuebg‡	<0.0005	<0.0005

*Mean absolute change adjusted from Stage I: †Standard Error; ‡Analysis of Covariance model (ANCOVA model).

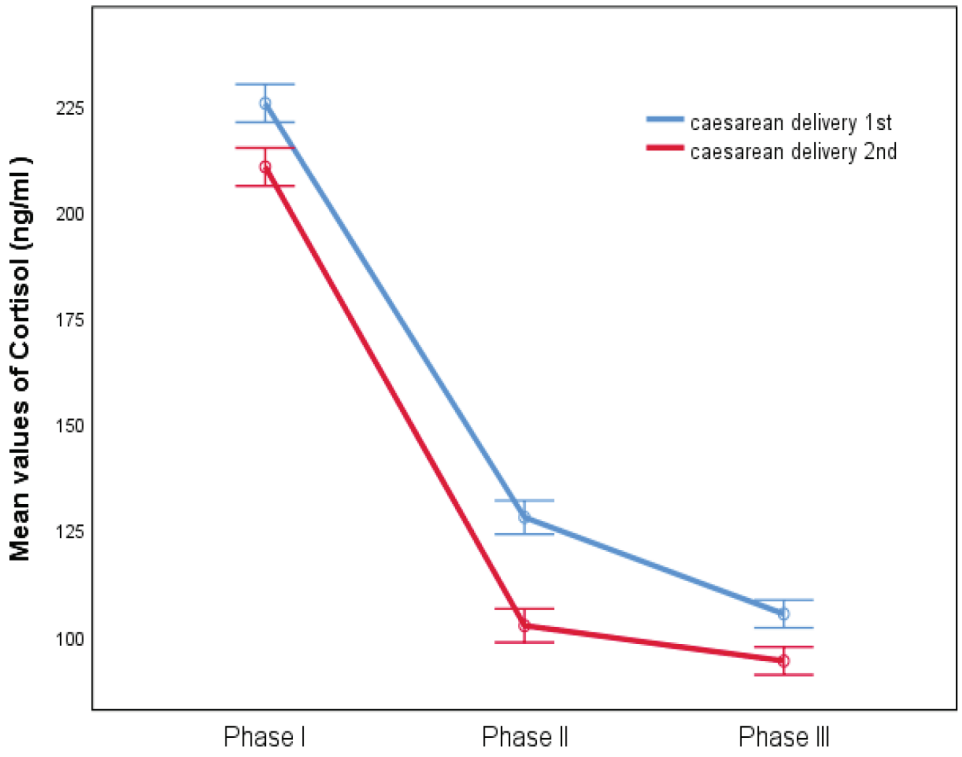

Figure 1. Mean values of Cortisol levels between groups during the observation period.

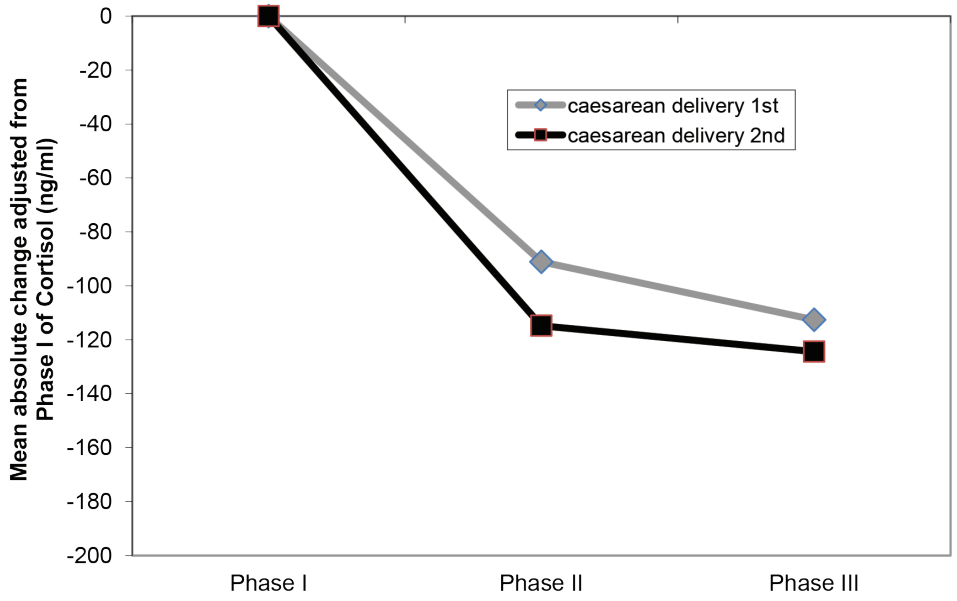

Figure 2. Absolute change in Cortisol levels adjusted from Stage I between groups during the observation period.

Discussion

In this prospective study, we attempted to assess the stress response created by caesarean sections for the first and second time. This attempt was performed by comparing the levels of cortisol between two groups of women. To our knowledge, this is the first study attempting to correlate the cortisol levels in these two groups. As such, our results could serve as a point of reference for future studies. First, we examined whether cortisol fluctuated in the same way in both groups. The results of the study presented statistically significant interaction between the two groups at every stage, pointing to the fact that cortisone levels do not change in the same way in the two groups. At every stage the levels of cortisol were statistically higher in the CS1 group than in the CS2 group, showing that CS2 generates a significantly less stressful response than CS1.

Next, we made a pairwise comparison between stages to determine the variations in the rhythm of cortisol in the two groups. We detected that cortisol levels were statistically lower between stages, from Stage I to Stages II and III, as well as that in CS2 cortisol decreased at a faster rate than in CS1. The conclusion drawn is that the initiated stress response is present for a longer time period in the CS1 group.

From the above findings, it may be concluded that a second caesarean section is a safe procedure that does not place an unnecessary burden on the mother. This is an important fact that practitioners can rely upon while designing the ideal management of a pregnant woman for the stressful environment of birth. All women participating had to adhere to the set of criteria mentioned above, however a limitation of the study was that the measurements of cortisol were taken from different women. It is worth considering whether pregnant women with different biocharacteristics could affect the fluctuation rate of cortisol.

A credible indication exists in the method of analgesia. A considerable number of women are reluctant to be subjected to the laborious condition of childbirth without anesthesia. Findings suggest that the benefits of the advancements in analgesia procedures have a positive effect on stress management and the mental health of the prospective mother (15, 20), as well as postpartum depression, although evidence is conflicting at best (21). An organized study with the goal of cataloguing the experiences and mental states of women undergoing a second caesarean section could yield intriguing results.

Limitations of Study

A major limitation of the study is its small sample size and the lack of sample size estimation.

In addition, recruitment was performed using convenience sampling of the patients attending the hospital without randomization. Lastly, our study, being the first of its kind, has no direct comparison to draw from. More studies in this area of expertise could yield useful data.

Conclusion

The conclusions derived from this study could also be of benefit to practitioners when performing follow-up of high-risk pregnancies. It is currently unknown whether the cortisol rate behaves the same way in the management of high-risk pregnancies. This study indicates this finding, but research specifically constructed to target this goal could prove invaluable.

What Is Already Known On this Topic:
Stress is a stimulus, originating either from the environment or from the organism itself, that produces a response from the receptor organism. The published literature suggests that caesarean sections (CS) cause a reduced stress response in the newborn, taking into account the lower levels of cortisol in the umbilical blood.

What This Study Adds:
The aim of this study was to compare the stress response produced during elective CS for the first and second times. For that goal, cortisol blood levels were measured before, during and after childbirth. The analysis showed that it may be concluded that a second caesarean section is a safe procedure that does not place an unnecessary burden on the mother. This is an important fact that practitioners can rely upon while designing the ideal management of a pregnant woman for the stressful environment of birth.

Author Contributions: Conception and Design: NK; Methodology: NK; Acquisition,analysis and interpretation of data: DG and CB; Drafting the article: DG, PKK and AS; Revising it critically for important intellectual content: NK, FA and ID; Approved final version of the manuscript: NK and AM; All authors have read and agreed to the published version of the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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