CEOG Clinical and Experimental Obstetrics & Gynecology

Obstetric factors associated with salivary cortisol levels of healthy full-term infants immediately after birth

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Summary

Purpose: To evaluate the contribution of demographic and obstetric factors to birth stress as measured by salivary cortisol levels in healthy full-term infants. *Materials and Methods:* Ninety-three newborn infants were born vaginally after uncomplicated pregnancies in Japan. An observational study was conducted in 2009. Saliva was collected at one minute after checking Apgar scores and determined using a commercial high-sensitivity enzyme immunoassay kit. *Results:* In multiple linear regression analysis, length of second-stage labor [β (95% confidence interval) = 0.041 (0.022–0.059), standardized β = 0.452] and bleeding immediately after birth [β (95% confidence interval) = 0.006 (0.001–0.010), standardized β = 0.239] were mutually and independently associated with infant salivary cortisol levels. *Conclusion:* The length of second-stage labor and third-stage bleeding may be causative factors or factors reflecting the cause of infant stress. This finding also provides support for the use of salivary cortisol levels in evaluating infant stress.

Key words: Salivary cortisol; Stress; Spontaneous birth; Length of second-stage labor; Bleeding at third-stage; Healthy full-term infant.

Introduction

Birth is one of the most stressful life events for both mother and infant. It has been well known that cortisol plays a multisystem role in preparing the fetus to make a successful transition to extrauterine life [1]. Maternal and fetal/ neonatal birth stress was previously examined by measuring the umbilical cord vein or artery cortisol levels. Cortisol levels are increased after the onset of spontaneous labor [2]. The umbilical cord cortisol levels in infants delivered instrumentally have also shown to be significantly higher compared with the levels in those delivered spontaneously [2–7]. Those studies have made clear that the mode of delivery is the most important factor affecting newborn infant's stress after birth. Miller *et al.* [4] also determined that the factors affecting infant cord arterial cortisol levels were epidural anesthesia use, length of second-stage labor, and baseline fetal heart rate of infants during delivery. The length of second-stage labor is influenced by epidural anesthesia use [8, 9]. Moreover, maternal pregnancy complication, gestational age, fetal growth, and fetal complications are thought to influence fetal production of cortisol [1, 2, 10, 11]. Therefore, the present authors evaluated healthy full-term infants to minimize the influence of various confounding factors.

Measuring salivary cortisol levels is recognized as a noninvasive and practical method to determine the neonatal immediate stress response to both physical and environmental stimuli because salivary cortisol levels show a close correlation with plasma cortisol levels [10, 12]. Few studies have recently used infant salivary cortisol levels as a marker of neonatal stress [13–16].

The aim of this study was to evaluate the contribution of demographic and obstetric factors on birth stress measured by salivary cortisol levels in healthy full-term infants who were delivered vaginally. The authors hope that their finding provides basic evidence for measuring salivary cortisol levels to reduce infant stress immediately after birth.

Materials and Methods

This study was an observational study that was part of a larger study [17]. The study protocol was approved by the Ethics Review Committee of the Nagoya University School of Medicine, Nagoya, Japan (565).

The authors evaluated 147 consecutive infants spontaneously delivered at two maternity hospitals in Aichi Prefecture, Japan, from January to October 2009. The parents provided written informed consent to participate and for the use of personal information concerning pregnancy and delivery in the medical records and laboratory data during the admission of the mothers in the hospital. Eligibility criteria included: 1) singleton pregnancy, 2) no medications

Revised manuscript accepted for publication February 16, 2017

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during labor, 3) spontaneous vaginal delivery without any medical interventions, 4) full-term pregnancy, 5) uncomplicated pregnancy and delivery, and 6) bleeding < 1,000 ml during third-stage labor. Thus, 98 women were eligible. In addition, salivary cortisol levels one minute after birth were obtained from 93 healthy full-term infants included in the final analysis.

After checking the one-min Apgar scores, saliva specimens were collected from newborns using the Sorbette without any stimulation. All newborns' mouths were checked gently for any contaminating components before saliva collection. The saliva was frozen after collection at −20 °C at the hospitals and stored at −83 °C until analysis. Salivary cortisol concentrations were determined by using a commercial high-sensitivity enzyme immunoassay kit following the manufacturer's protocol as the present authors have previously published [17]. Demographic, pregnancy, and delivery data of both mothers and infants were collected from the medical records during hospitalization.

Statistical analysis was performed by using SPSS version 22.0. Descriptive statistics were used to summarize the demographic factors. All continuous variables were shown as mean ± standard deviation (SD), and categorical data were presented as frequency and percentage. The correlation of salivary cortisol levels with other study variables was determined by using Spearman rank-correlation coefficient.

Multiple regression analysis was performed by using the forced entry method to estimate the contribution of study variables to salivary cortisol levels. The dependent variable was salivary cortisol level. The independent variables included significantly associated factors with salivary cortisol levels by Spearman rank-correlation analysis ($p < 0.05$). A p-value < 0.05 was considered statistically significant.

Results

Salivary cortisol samples were obtained from 93 infants one minute after birth. The mean maternal age was 30.4 ± 4.8 (range: 19.0–40.0) years, and 55 women (59.1%) were multiparous. The mean length of first-stage labor was 8.6 ± 7.7 (range: 0.8–47.6) hours, and that of second- and third-stage labor was 37.5 ± 45.0 (range: 2.0–230) minutes, and 5.6 ± 2.3 (range: 3.0–16.0) minutes, respectively. The mean amount of bleeding immediately after birth was 199.0 ± 166.4 (range: 5.0–800.0) ml. The mean gestational age of infants was 277.9 ± 6.8 (range: 261–292) days, and the mean birth weight was 3,025 ± 339 (range: 2334–4098) grams. The mean Apgar scores at one and five minutes were 8.9 ± 0.5 (range: 7.0–10) and 9.4 ± 0.5 (range: 9–10), respectively. The umbilical cord pH value was 7.3 ± 0.1 (range: 7.2–7.4). The mean infant salivary cortisol level at one minute was 6.9 ± 4.3 (range: 0.5–29.5) μg/dl. Other participant characteristics are shown in Table 1.

Table 2 presents the association of obstetric factors with infant's salivary cortisol level. Spearman correlation coefficients (ρ) indicated that positive associations were found between salivary cortisol levels and the length of first-stage labor ($\rho = 0.33$, $p = 0.001$), length of second-stage labor ($\rho = 0.38$, $p < 0.001$), bleeding immediately after birth ($\rho = 0.25$, p = 0.017), and gestational age ($\rho = 0.22$, $p = 0.038$). Only the number of previous live births was significantly and inversely associated with salivary cortisol levels ($\rho = -0.37$, $p < 0.001$).

Multiple regression analysis of factors related to infant salivary cortisol levels are shown in Table 3. The length of second-stage labor (β [95% confidence interval] = 0.041 [0.059–0.452]; standardized β = −0.452, $p < 0.001$), and bleeding immediately after birth (β [95% confidence interval] = 0.006 [0.001–0.010]; standardized β = −0.239, $p <$ 0.001) were mutually and independently associated with infant salivary cortisol levels (F = 8.803, $R^2 = 0.0339$, adjusted $R^2 = 0.300$).

Discussion

The present study is the first report elucidating that the length of second-stage labor and bleeding immediately after birth were associated with increased infant salivary cortisol levels independent of gestational age, parity, and length of first-stage labor.

To the best of the present authors' knowledge, few studies have examined the association between obstetric factors and umbilical cord blood cortisol level. Miller *et al.* [4] studied the relevant factors in cord arterial cortisol levels in 92 primiparous women who delivered their infants vaginally at 36–42 weeks. In multiple regression analysis, cord arterial cortisol levels were significantly and negatively associated with combined spinal/epidural use and positively associated with the length of second-stage labor and fetal heart rate baseline at the start of second-stage labor. Cord arterial cortisol levels also correlated negatively with cord arterial pH levels. Chiba *et al.* [7] evaluated the association of maternal/neonatal factors with cortisol levels obtained from the umbilical vein before placental delivery among 23 normal singleton vaginal deliveries. The cortisol level in first babies was higher than that in second babies. After multiple regression analysis, the negative and significant association of cortisol levels with the number of parity was shown. In the present study, the same negative and significant association was observed by using univariate analysis, but it attenuated after performing multivariate analysis. However, this multivariate analysis model included the length of second-stage labor as an independent variable, whereas it was not performed in the multivariate model by Chiba *et al.* [7]. The length of the first- and second-stage labor in multiparous women generally tends to be shorter than that in primiparous women. The association of cortisol level with parity may be partly explained by the association with the length of second-stage labor. Sano *et al.* [18] recently examined the association of cortisol levels

Table 1. *Characteristics of mothers and infants (n = 93).*

	Mean	SD	Range
Maternal age (years)	30.4	4.8	19.0–40.0
Gravid status (%)			
Primigravida	38	(40.9)	
Multigravida	55	(59.1)	
Parity (%)			
First child	38	(40.9)	
Second child	39	(41.9)	
Third child or more	16	(17.2)	
Pre-pregnancy BMI (kg/m^2)	20.6	3.2	15.4–35.2
Weight gain during this pregnancy (kg)	10.1	3.6	0.0–21.7
Amount of glycated hemoglobin at third trimester (g/dl)	10.8	1.1	7.8–14.4
Systolic blood pressure at 36 weeks (mmHg)	113.3	10.2	89.0–134.0
Diastolic pressure at 36 weeks (mmHg)	68.1	7.8	48.0–85.0
Onset of delivery (%)			
Onset of labor	71	(76.3)	
PROM	22	(23.7)	
Length of first-stage labor (hours)	8.6	7.7	0.8–47.6
Length of second-stage labor (min)	37.5	45.0	2.0–230.0
Length of third-stage labor (min)	5.6	2.3	3.0–16.0
Bleeding immediately after birth (ml)	199.0	166.4	5.0–800.0
Placental weight (g)	579	96	390–844
Gestational age (d)	277.9	6.8	261–292
Birth weight (g)	3025	339	2334–4098
Birth height (cm)	49.1	1.9	45.0–55
Apgar score at 1 min	8.9	0.5	7–10
Apgar score at 5 min	9.4	0.5	9–10
Umbilical cord pH at birth	7.3	0.1	7.2–7.4
Salivary cortisol at 1 min after birth (μg/dl)	6.9	4.3	0.5–29.5
Boys/girls	45/48		

Table 2. — *Association of obstetric factors with infant's salivary cortisol level.*

Salivary cortisol	Spearman correlation	*p*-value
Maternal age	0.06	0.58
Number of previous live births	−0.37	**0.001**
Pre-pregnancy BMI	−0.02	0.84
Weight gain during pregnancy	−0.02	0.85
Glycated hemoglobin	0.08	0.84
Systolic blood pressure	−0.09	0.38
Diastolic blood pressure	0.02	0.87
Length of first-stage labor	0.33	**0.01**
Length of second-stage labor	0.38	**0.00**
Length of third-stage labor	0.13	0.22
Bleeding immediately after birth	0.25	**0.02**
Gestational age	0.22	**0.04**
Birth weight	−0.03	0.77
Birth height	−0.14	0.17
Apgar score 1 min	0.08	0.42
Umbilical cord pH	−0.13	0.21
Placental weight	0.08	0.84
Boys/girls*	7.40(5.33)/6.38(2.94)	0.48

** mean (SD). Student-t test: F = 2.354, df = 91; 95% CI: −7.32 to 2.79; p = 0.249. BMI: body mass index*

Table 3. — *Multiple regression analysis of factors related to infant's salivary cortisol level one minute after birth.*

Salivary cortisol	β	95% CI	Standardized β	*p*-value
Number of previous live births	−0.211	−1.306–0.884	−0.041	0.70
Length of first-stage labor	0.015	−0.089–190.0	0.029	0.77
Length of second-stage labor	0.041	0.022–59.0	0.452	**0.00**
Bleeding immediately after birth	0.006	0.001–100.1	0.239	**0.01**
Gestational age	0.044	−0.062–0.150	0.074	0.41

$F = 8.803$. $R^2 = 0.339$. Adjusted $R^2 = 0.300$
95% CI indicates 95% confidence interval, forced entry method.

with delivery duration among 33 umbilical blood samples, and demonstrated that the duration of the second-stage of delivery particularly showed a stronger positive correlation with cortisol levels. Based on the aforementioned studies using umbilical cord blood and the present study using saliva, the length of second-stage labor is suggested to be the most important determinant of infant cortisol levels immediately after delivery.

Interestingly, the present authors found a significant and positive association between infant salivary cortisol levels and maternal bleeding immediately after birth independent of the length of second-stage labor. Altman *et al.* [19] systematically reviewed four studies examining postpartum hemorrhage and showed a significant association between postpartum hemorrhage and prolonged second-stage labor, with an odds ratio of 2.8. In the present study, a marginally significant difference was found between the length of second-stage labor and bleeding immediately after birth ($\rho = 0.19$, $p = 0.07$) although the authors excluded mothers with > 1,000 ml blood loss immediately after birth. Although all of the mothers were administered an intramuscular injection of oxytocin immediately after delivering the placenta, eight of 93 mothers had > 500 ml blood loss during third-stage labor. The present finding suggests the contribution of some factors related to maternal bleeding other than prolonged second-stage labor. Possible causes for postpartum hemorrhage are failed uterus contraction after delivery, retained placenta, inverted or ruptured uterus, and cervical, vaginal, or perineal lacerations. If these complications occurred at a minimal degree before birth, the fetus might be subjected to some kinds of stress.

The unitary mechanism regarding the present findings is difficult to explain. Two possible underlying mechanisms were suggested. One is the direct effects of longer second-stage labor and some complications related to more bleeding at the third-stage on the fetus, and the other is the effect that is mediated by the mother experiencing these complications. The intrauterine stressful environment may affect in the secretion of fetal cortisol as the infant attempts to cope with a stressful situation. Furthermore, mothers who experience both physical and emotional stress expend a lot of energy during labor, and their hypothalamic–pituitary–adrenal (HPA) axis is then activated in response to the stressful situation [2, 11, 20]. Maternal stress subsequently stimulates the fetal adrenal glands to secrete cortisol, and the infant's HPA axis is also activated. In contrast, adequate stress is necessary for maintaining maternal/fetal/newborn well-being and establishing infant's breathing. Breathing immediately after birth is initiated by mechanical, chemical, thermal, and sensory factors that stimulate the respiratory center in the medulla of the brain and trigger respirations [1, 21]. The secreted fetal cortisol is an important factor to accelerate absorption of the lung fluid during breathing. Taken together, experiencing spontaneous labor, the normal process of labor and or passing though the birth canal may induce adequate stress for both the mother and fetus.

The present authors acknowledge some limitations. First, this study was not an experimental one and included only healthy full-tem infants. Although the present study demonstrated the positive associations of the length of second-stage labor and bleeding immediately after birth, with birth stress measured by salivary cortisol levels in healthy full-term infants, the present results should not be interpreted as advocating shortening the duration of second-stage of labor by using practices, such as directed pushing, such as the Valsalva method, episiotomy, or vacuum assistance. There is no consensus regarding the assessment of the infant's condition based on salivary cortisol levels immediately after birth. The present study could not propose a cutoff value of salivary cortisol level for predicting too much stress in newborn infants because the study subjects were only healthy full-term infants. However, measuring salivary cortisol levels is a test that may be evaluated less invasively and directly evaluate the degree of infant's stress immediately after birth. Therefore, further studies, including infants delivered in different situations, such as mode of delivery, gestational age, and some complications of pregnancy, are needed. In addition, the association between salivary cortisol level and infant's prognosis should be examined. Second, other factors may be associated with increased salivary cortisol levels because the coefficient of determination R^2 was 0.339 in this multiple regression model, and the model only explained 30% with the entered relevant factors. Thus, the data should be interpreted under some cautions.

Conclusion

The present study indicated that infant salivary cortisol levels immediately after birth were associated with the length of second-stage labor and bleeding during third-stage labor even in healthy full-term infants. This finding also provides support for the use of salivary cortisol levels in evaluating infant stress.

Acknowledgments

The authors are most thankful to all of the infants and parents who participated in this study. They also appreciate the cooperation of M. Yamada, M.D., S. Michigami, M.D., H. Kondo, M.D., T. Ishimaru, M.D., and the staff nurses for generously collecting data for our study.

The authors also wish to acknowledge the support provided by grants from Meijiyasuda Mental Health Foundation and Yamajifumiko Nursing Research Fund, and JSPS KAKENHI (Grant Number JP 15K20750). The study sponsors did not have any involvement.

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