Original Research Article

Association of Maternal Hair Cortisol Level with Neonatal Facial Pain Expression Based on Gender of Newborns

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ABSTRACT

Introduction: Mother's mental and physical conditions during pregnancy affect the development of fetus. This study aimed to investigate the role of neonates' gender in pain responses and its association with maternal stress during pregnancy and programming. **Materials and Methods:** This study was conducted on 105 neonates (53 females and 52 males) born in Gorgan, Iran. The pain stimulus was intramuscular injection of vitamin K, a routine procedure for infants at birth. The first painful injection was performed for all children under the same conditions. The neonatal infant pain scale was used to assess pain intensity in newborns. Maternal stress during pregnancy was evaluated by measuring hair cortisol levels. Statistical analysis was done using Kolmogorov-Smirnov, independent t-test and Pearson correlation tests. **Results:** Mean pain intensity score before, during and after injection was not significantly different between male and female newborns. Mean pain score during injection was significantly higher in male neonates whose mothers had high cortisol levels compared to male neonates whose mothers had normal cortisol levels (P-value = 0.01). **Conclusions:** These data indicate gender indifferences in response to pain at birth and imply the differential effect of maternal hair cortisol levels on pain score of male neonates.

KEYWORDS: Hydrocortisone, Infant, Newborn, Pain, Pregnancy

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INTRODUCTION

Mother's mental and physical conditions during pregnancy affect the development of fetus [1]. This effect may go on to later developmental stages after birth and even after puberty [2-4]. Exposure of fetus to abnormal conditions at a certain time of intrauterine development induces long-term effects on physiological characteristics after birth, a process known as programming [5]. Consequences of programming are associated with amount of confounding factor, duration of exposure and the stage of fetal development [6]. Since certain diseases including cardiovascular disease, diabetes, addiction, stress response and pain tolerance may be influenced by programming, recent studies have focused on the role of programming in the pathogenesis of such diseases [7-10]. Maternal stress during pregnancy has been reported to affect the development of the hypothalamic-pituitary-adrenal axis in the fetus and some related behaviors such as pain response [11]. To study the role of gender in pain response, elimination of indirect factors including some learned behavior and socio-cultural issues by measuring the pain reaction in neonates could be beneficial. However, the reports of pain response in male and female neonates have been contradictory. In premature neonates, there was no correlation between gender and response to pain [12, 13], while female hospitalized term neonates treated with antibiotics, showed a stronger response to pain compared to the male counterparts [14]. Comorbidities, pharmaceutical and diagnostic interventions, immaturity, and the scale used for evaluation of pain response might influence neonate's response to pain [15].

Maternal stress during pregnancy is another important confounding factor that has

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received less attention in studies. Given the crucial and documented role of stress during pregnancy on the development of fetal hypothalamic-pituitary-adrenal axis and the role of this axis on the physiological response to stimuli such as pain, this study aimed to evaluate the difference in male and female neonates' response to pain considering the long-term psycho-neuro-hormonal state of the mothers.

MATERIALS AND METHODS

After receiving approval from the Research Ethics Committee (code: 356191122764) of the Golestan University of Medical Sciences, 105 neonates (53 females and 52 males) born in the Sayad Shirazi Teaching Hospital in Gorgan (North of Iran) were included in this study. The study was conducted between August 2015 and March 2016. Written informed consent was obtained from mothers prior to participation in the study. The study was done on mothers with gestational age of 28 to 41 weeks and 6 hours age, and healthy, awake and quiet newborns with no history of injection with an Apgar score of above 7 at one and five minutes before first breastfeeding. Exclusion criteria were as follows: drug use during pregnancy or birth, cesarean section, birth defects, respiratory or cardiovascular problems, neural defects, Apgar score of less than 7 at one and five minutes after birth, resuscitation at birth, maternal mental disorder and a history of divorce.

The pain stimulus was intramuscular injection of vitamin K, a routine procedure necessary for all infants at birth. All study subjects were assessed in the care room after completion of primary measurements. The first painful injection was performed for all children under same conditions. All intramuscular injections were performed by trained personnel and in the same area (vastus lateralis). An insulin syringes with needle 26G was used for injection of 10 mg of vitamin K. All newborns had diapers and genders of the subjects remained unknown during the recording and evaluation of responses to pain. A single-blind experiment was conducted to analyze the behavior of the infants, during which the observer was unaware of the infant's gender. For each subject, only one injection was made and all injections were performed in the morning between 8 and 11 a.m. The pain response was video-recorded a minute before the injection when the newborn was at rest and continued until two minutes after the injection. Videos were captured by Canon PowerShot SX710 HS camera.

The neonatal infant pain scale (NIPS) was used to assess the intensity of pain. The scale includes facial changes (calm 0, facial expressions 1), cry (no cry 0, whimper 1, vigorous cry 2), movements of the feet (still 0, movement 1), respiration (relaxed 0, change in breathing 1), and state of arousal (sleep or calm 0, restlessness paddle 1). According to this scale, a minimum score of zero means no pain and maximum score of 7 means severe pain [16].

Maternal hair cortisol levels were measured as described below.

First, hair washing and steroid extraction procedures were performed according to a method described by Davenport et al. [17]. In brief, each hair segment was placed into a 15 ml Falcon tube, then 2.5 ml isopropanol were added, and the tube was gently mixed on an overhead rotator for 3 min. After decanting, the wash cycle was repeated two more times. The hair samples were allowed to dry for at least 12 h. The hair segments were powdered using a Retsch ball mill (5 min at 30 Hz). Next, 50 mg of the powered hair was carefully weighed out and transferred into a 2 ml cryo vial (Eppendorf, Germany). After adding 1.5 ml of pure methanol, the vial was rotated slowly for 24 h for steroid extraction. After centrifuging the samples at 10, 000 rpm for 2 min, 1 ml of the clear supernatant was transferred into a new 2 ml cryo vial. Alcohol was evaporated at 60 ^{o}C under constant stream of nitrogen for almost 20 min until the samples were completely dried. Finally, 0.4 ml of phosphate buffer was added and the tube was vortexed for 15 s. We used 20 µl from the content of each vial for cortisol measurement using an EIA kit, designed for

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quantification of salivary cortisol. The normal range of maternal hair cortisol level was defined as 0.1 to 0.7 μg/dl.

All data were analyzed using SPSS (version 21). Descriptive statistics were expressed as mean ± standard deviation (SD). Normal distribution of data was assessed by Kolmogorov-Smirnov test. Independent t-test was used to compare differences between means from two groups. Relationship between cortisol level of mothers and pain score of newborns (after - before injection scores) was investigated using the Pearson's correlation test. P-values less than 0.05 were considered as statistically significant.

RESULTS

There was no significant difference in the birth weight, Apgar score at first and fifth minute, mother's age, father's age, gestational age and maternal weight of male and female neonates. In addition, no significant difference was observed between mean pain scores before, during and after the injection. The mean cortisol level of mothers with female neonates was significantly higher than that of mothers with male neonates (Table 1).

Table 1. Demographic characteristics of the study subjects

	Female	Male	P-value
Birth weight (gr)	3174.64 ± 338.3	3152.73 ± 358.66	0.74
Apgar score at first minute	8.02 ± 0.41	8 ± 0.34	0.8
Apgar score at fifth minute	9.06 ± 0.3	9.06 ± 0.24	0.98
Age of mother (year)	25.47 ± 5.18	25.4 ± 6.09	0.95
Age of father (year)	29.22 ± 6.64	29.59 ± 6.63	0.78
Gestational age (week)	39.13 ± 1.07	38.59 ± 3.4	0.28
Weight of mother (kg)	72.89 ± 16.41	70.75 ± 12.35	0.45
Pain before injection	0.48 ± 0.12	0.68 ± 0.15	0.29
Pain during injection	4.56 ± 1.36	4.08 ± 1.5	0.11
Pain after injection	0.83 ± 0.15	0.94 ± 0.18	0.66
Hair cortisol level (μg/dl)	**1.14 ± 0.12**	**0.79 ± 0.08**	0.02

Mean pain score during injection was significantly higher in male neonates whose mothers had high cortisol levels compared to male neonates whose mothers had normal cortisol levels (P-value = 0.01). However, no significant relationship was found between cortisol levels and pre- and post-injection pain scores of both male and female newborns (Table 2).

Table 2. Mean ± SD of pain scores based on maternal hair cortisol levels in male and female newborns

	Hair cortisol level	Pain score before injection	Pain score during injection	Pain score after injection
Female	Normal	0.6±0.3	4.8±0.32	0.6±0.04
	High	0.4±0.11	4.6±0.25	0.9±0.17
	P-value	0.49	0.67	0.17
Male	Normal	1±0.29	3.52±0.37	1±0.34
	High	0.7±0.19	4.66±0.28	0.95±0.25
	P-value	0.14	0.01	0.92

A positive correlation was found between maternal cortisol levels and pain score of male newborns during the injection. However, this correlation was not observed

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in female newborns. Moreover, the pre-injection and post-injection pain scores had no significant relationship with maternal cortisol levels. In both genders, maternal cortisol levels had no significant correlation with pain scores before and after the injection (Table 3).

Table 3. Correlation between maternal cortisol levels and pain scores in male and female newborns

	Hair cortisol level / Pain	Before injection	During injection	After injection
Female	r	-0.14	-0.3	0.14
	P-value	0.35	0.85	0.38
Male	r	-0.22	0.39	-0.08
	P-value	0.17	0.01	0.61

DISCUSSION

In this study, we compared the response to pain in healthy male and female term infants by considering the psycho-neuro-hormonal status of their mothers during pregnancy, and found no statistically significant difference. Study of Gaynsbvrg et al. reported a significant difference between pain response of healthy term female and male newborns based on the Neonatal Facial Coding System (NFCS), but found no difference based on the NIPS [18], which is consistent with our findings. In this study, pain response of male infants was significantly different based on their maternal cortisol levels. Measurement of serum, saliva and hair cortisol level has been widely accepted as indicators of psycho-neuro-hormonal status. Since cortisol circadian rhythm limits the application of single measurement from serum or saliva, hair cortisol measurement has been proven to be more practical [19-22]. The average cortisol level of mothers with female neonates was significantly higher than that of mothers with male neonates. DiPietro et al. assessed salivary cortisol level of 120 pregnant women by consecutive weekly measurements, and reported that salivary cortisol level of mothers with a male fetus was higher in early weeks of pregnancy, while after week 30, salivary cortisol level was higher in mothers with female fetus [23]. Gender of fetus affect the placental glucocorticoid receptor isoforms and seems to be involved in prematurity [24, 25]. Our data showed no significant difference between the pain score of male and female newborns. However, when considering the mothers' cortisol levels, it was observed that the pain score was higher in male newborns whose mothers had high hair cortisol levels. This finding is consistent with study of Bolten in 2013, on the effects of prenatal cortisol level in the prenatal programming of emotion regulation [26]. In 2012, Lee reported that late gestational maternal serum cortisol is inversely associated with fetal brain growth [27]. In study of Duma, the number of glucocorticoid sensitive genes was higher in men, indicating that the response to anti-inflammatory action of glucocorticoid in men could be stronger than in women [29]. However, a study suggested that unlike female placenta, male placenta is more resistant to exogenous glucocorticoid [25]. Various aspects of pain including stimulus threshold, avoidance reactions, facial expressions and recovery time should be considered for interpretation of results, even in cases without learned-pain reactions including neonates. The interaction of the hypothalamic-pituitary-adrenal axis of the fetus and mother, the different function of male and female placenta in glucocorticoid metabolism, the type and severity of prenatal stress and the impact of each of these factors on intrauterine development and programming augment the pain complexity. Therefore, generalization of the results is limited.

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CONCLUSION

These data indicate gender indifferences in response to pain at birth and imply the differential effect of maternal hair cortisol levels on pain score of male neonates.

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