

Assessment and Correction of Stress in Preterm Infants and Their Mothers

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What is already known on this topic?

- Premature birth is accompanied by the stress in infants due to their immaturity and presence of constant stressors of the neonatal intensive care unit (NICU).
- Parents of preterm infants experience stress in the NICU.

What this study adds on this topic?

- Kangaroo mother care with skin-to-skin contact reduces the stress and normalizes salivary cortisol levels in both the infant in the NICU and the mother.

ABSTRACT

Objective: To investigate how skin-to-skin contact influences the stress levels in preterm infants in the neonatal intensive care unit and their mothers.

Materials and Methods: The study included 60 preterm infants with gestational age less than 32 weeks, who were treated in the neonatal intensive care unit, and their mothers. The overall design was a baseline-response design. Saliva was collected before (baseline) and after skin-to-skin contact to measure free cortisol by enzyme immunoassay method.

Results: Infant baseline salivary cortisol level was 0.587 [0.239; 1.714] $\mu\text{g/dL}$. It was significantly higher in neonates who had invasive ventilation (1.00 [0.38; 2.44]) $\mu\text{g/dL}$ compared to non-ventilated infants (0.335 [0.156; 1.236]) $\mu\text{g/dL}$, $P = .022$ and was positively correlated with the duration of ventilation ($r = 0.70$; $P = .0000$). Maternal baseline salivary cortisol level was 0.212 [0.123; 0.378] $\mu\text{g/dL}$. There was significant decreasing of salivary cortisol after Kangaroo mother care with skin-to-skin contact in infants (0.162 [0.111; 0.231]) $\mu\text{g/dL}$, $P < .001$ and mothers (0.096 [0.077; 0.156]) $\mu\text{g/dL}$, $P < .001$.

Conclusion: Preterm infants in the neonatal intensive care unit and their mothers experience stress which can be confirmed by the increased baseline cortisol levels. Kangaroo mother care with skin-to-skin contact reduces the stress and normalizes salivary cortisol levels in both the infant in the neonatal intensive care unit and the mother.

Keywords: Maternal stress, preterm infants, salivary cortisol, stress

INTRODUCTION

Premature birth is accompanied by stress in infants due to their immaturity and presence of constant stressors, which they are exposed to in the neonatal intensive care unit (NICU) from the first days of life.^{1,2} Preterm neonates not only suffer from the diseases that often accompany premature birth but are also exposed to many painful and invasive treatment procedures and daily care manipulations together with the numerous environmental stimuli.^{1,3,4} Also, premature infants in the NICU are usually separated from the mother, and this is the most important stressor for a child.¹ It has been proven that this severe stress stops when the baby returns to the mother.⁵

When a child is born, stress helps him/her to adapt to extrauterine life, especially, it stabilizes the functioning of the respiratory and cardiovascular systems and induces lung maturation.⁶ However, excessive stress experienced by an immature infant when the brain is particularly sensitive to environmental influences has both early and long-term consequences for the child's development.

Parents of preterm infants in the NICU also experience considerable stress due to anxiety, fear, powerlessness, and parental role alterations.⁵ Mother-infant separation, that is,

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breaking their natural relationship that occurs in utero and increases after childbirth, is the strongest stressor for mothers. An important link between maternal psychological status and infant development has been proven,^{7,8} and this emphasizes the important role of the mother in the child's development and the need to support a "mother-infant" relationship.

Considering the plenty of factors that accompany premature birth and its long-term side effects, it is necessary to implement the stress-reducing methods in routine care. Kangaroo mother care with skin-to-skin contact (SSC) recommended for premature neonates, among many other benefits, can also minimize the negative effects of stress and mother-infant separation in the NICU.^{3,9}

The objective of the study is to investigate how skin-to-skin contact influences the stress levels in preterm infants in the NICU and their mothers.

MATERIALS AND METHODS

Participants, Ethics and Methods

The study included 60 preterm infants with gestational age (GA) less than 32 weeks. Infants were recruited from the NICU, which is level III NICU. Prematurity (GA $\leq$ 32 weeks) was the criteria for inclusion in this study. Exclusion criteria were the following: chromosomal disorders, congenital malformations, and absence of parents' consent.

The overall design was a baseline-response design. The baseline is defined as the state before the intervention (SSC) is introduced. The response refers to the stress response in relation to the intervention (SSC) introduced. All individuals act as their controls between the baseline level and the study intervention results. All infants included in the study had an SSC with their mother under the nurses' supervision. Naked neonates were positioned prone on the mother's bare chest, dressed only with diapers. Mothers were provided with comfortable chairs and advised to wear front open gowns.

Sample Collection and Cortisol Assay

Saliva was collected before (baseline) and after SSC. Saliva specimens were collected from 1:00 PM to 3:00 PM due to the diurnal rhythm of maternal cortisol. Salivary samples were taken on the fourth to tenth day of the infant's life. Saliva samples were collected without the usage of any salivation stimulating agents. Infant saliva samples were collected using cotton sponges; after that, it was extracted from the sponges by centrifugation (2 minutes at 2000× g). Mother's samples were collected with Salivette (Sarstedt, Rommelsdorf, Germany). After extraction, samples were frozen and stored at -20 °C. Enzyme Immunoassay for the quantitative determination of free cortisol in human saliva was used to analyze cortisol concentrations in the samples (IBL International GmbH, Hamburg, Germany)

Human Research Statement

Ethics approval was obtained from appropriate I.Horbachevsky Ternopil National Medical University ethics committee, and research was conducted in accordance with the World Medical Association's Helsinki Declaration. The informed consent was obtained from all the participants who took part in the study.

Statistical Analysis

All computations were performed using StatSoft STATISTICA Version 13 (Tulsa, Okla, USA). Quantitative data are presented as the median and interquartile range (IQR; 25th–75th percentiles). For qualitative parameters, absolute and relative frequencies are presented. The Wilcoxon matched pairs test (for 2 dependent groups) and Mann–Whitney *U* test (for 2 independent groups) were used to compare numerical data. Significance was assumed at $P < .05$. Correlations were analyzed using Spearman's rank correlation coefficient. The required sample size was calculated using G*Power Software sample size calculator.

RESULTS

A total of 60 infants with a GA of 31.0 [29.0; 32.0] weeks were enrolled in the study. There were 30 (50%) boys and 30 (50%) girls. Table 1 summarizes the patients involved in the study group.

Preterm Infant's and Maternal Stress Levels in NICU

Infant baseline salivary cortisol level was 0.587 [0.239; 1.714] μ g/dL. It was significantly higher in neonates who underwent invasive ventilation (1.00 [0.38; 2.44]) μ g/dL compared to non-ventilated infants (0.335 [0.156; 1.236]) μ g/dL, $P = .022$ and was positively correlated with the duration of invasive ventilation (Spearman $r = 0.70$; $P = .0000$) (Figure 1).

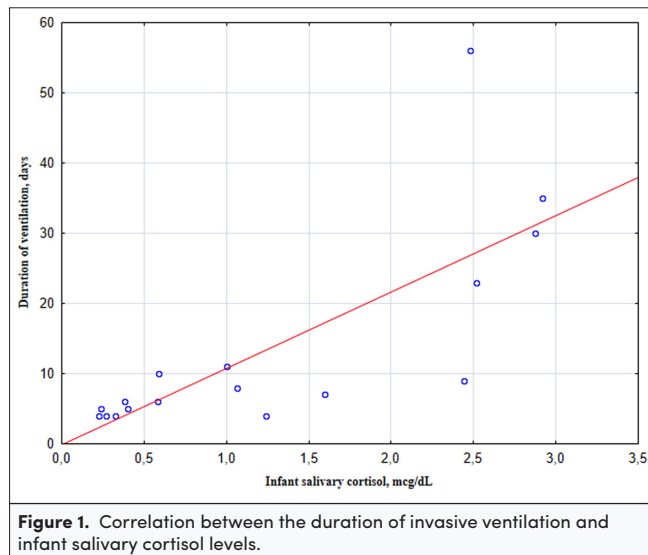
No significant correlations were found between the infant baseline cortisol level and GA ($P = .366$), birth weight ($P = .912$), and chronological age ($P = .957$). There was no difference in the baseline salivary cortisol depending on the gender ($P = .98$), mode of delivery ($P = .96$), need for primary resuscitation ($P = .118$), incidence of respiratory distress syndrome ($P = .626$), and early-onset sepsis ($P = .278$).

Maternal baseline salivary cortisol level was 0.212 [0.123; 0.378] μ g/dL. There was no difference in maternal baseline cortisol

Table 1. Summary of the Patients Involved in the Study

	Study Group, n = 60
Gestational age, weeks (Me [Lq; Uq])	31.0 [29.0; 32.0]
Birth weight, grams (Me [Lq; Uq])	1475.0 [1225.0; 1775.0]
Percentile of BW for GA (Me [Lq; Uq])	53.5 [31.5; 70.0]
SGA (n (%))	5 (8.1%)
Gravida	
1 (n (%))	24 (40.0)
≥ 2 (n (%))	36 (60.0)
Parity	
1 (n (%))	29 (48.3)
≥ 2 (n (%))	31 (51.7)
Delivery mode	
Vaginal delivery (n (%))	18 (30.0)
C-section (n (%))	42 (70.0)
Primary resuscitation (n (%))	35 (58.3)
RDS (n (%))	48 (80.0)
Early-onset sepsis (n (%))	20 (33.3)
Invasive ventilation (n (%))	17 (28.3)
CPAP (n (%))	24 (40.0)
Nasal cannulas (n (%))	18 (30.0)

GA, gestational age; SGA, small for gestational age; RDS, respiratory distress syndrome; CPAP, continuous positive airway pressure; BW, birth weight.



level depending on the infant gender ($P = .99$), GA ($P = .361$), the number of fetuses ($P = .204$), and infant need of ventilation ($P = .114$).

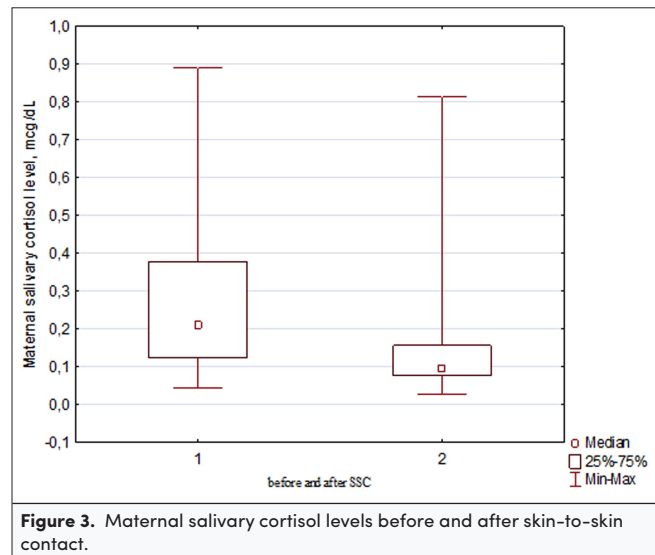
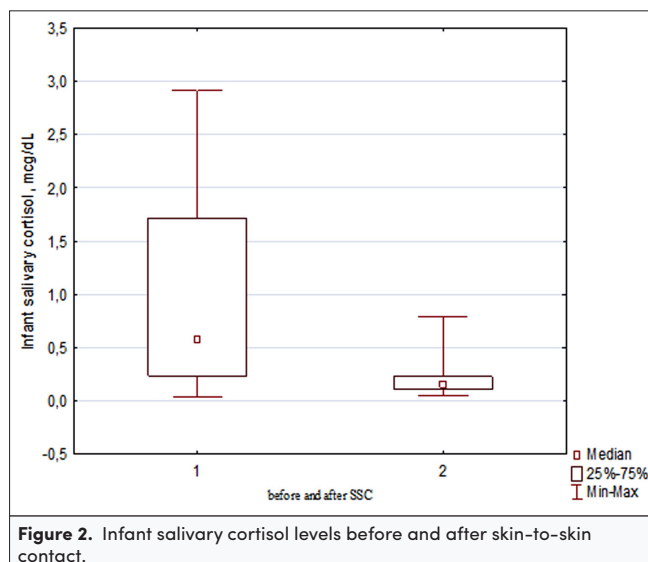
Kangaroo Mother Care with Skin-to-Skin Contact Impact on the Preterm Infants' and Their Mothers' Stress

There was significant decreasing of salivary cortisol after Kangaroo mother care with SSC, both in infants (0.162 [0.111; 0.231]) $\mu\text{g/dL}$, $P < .001$ and their mothers (0.096 [0.077; 0.156]) $\mu\text{g/dL}$, $P < .001$ (Figures 2 and 3). Only 5 (8.3%) infants and 6 (10.0%) mothers had increased salivary cortisol after SSC ($P = .144$ in infants and $P = .005$ in mothers).

We found no significant correlations between maternal and infant cortisol levels before ($r = +0.13$; $P = .470$) and after SSC ($r = +0.31$; $P = .147$).

DISCUSSION

Our study found that preterm infants in the NICU and their mothers experience stress, which is confirmed with the increased



baseline cortisol levels, both in children and mothers. Very pre-term neonates undergo repeated painful treatment and caregiving procedures during the period of rapid brain development and stress system programming.¹ This is a period when their sensory systems are immature, and thus the imbalance of excitatory versus inhibitory processes can lead to increased nociceptive signaling in the central nervous system.¹⁰ The immature central nervous system of preterm infants is particularly vulnerable to intensive external stimuli, and in combination with this, mother's separation and pleasant stimuli absence (mother's touch, voice, and odor) have been associated with altered brain microstructure and hormonal status disbalance.¹⁰ A lot of researches showed stress related to procedural neonatal pain during heel lance, eye-screening examination, physical examination, and caregiving interventions that was associated with the increased levels of cortisol.^{2,5,11} Our study was not associated with some exact procedural pain, as the sample collection was performed against the background of all routine procedures.

The invasive ventilation period can be described as a very stressful and uncomfortable period,¹² and the less mature the infant, the longer ventilation he/she needs.¹³ There is no data on how painful and stressful invasive ventilation is itself, but it is obvious that it is accompanied by plenty of potential painful interventions like intubation and sometimes reintubation, often endotracheal suctioning, skin lesions due to the adhesive materials changes. Routine use of sedatives seems insufficient to prevent high stress scores.¹² Also, various modes of ventilation can potentially increase the level of stress. In particular, infants additionally suffer from the need to "fight the ventilator" at mandatory asynchronous modes.¹⁴ A reduction in stress hormone concentration, namely adrenaline, was also seen over a short time period in preterm infants with GA less than 32 weeks when mode of ventilation was changed from conventional mandatory ventilation to patient-triggered ventilation.¹⁵ With the wide introduction of high frequency ventilation in the neonatal practice, there were reports of increased stress manifestation, namely discomfort, agitation, restlessness, increased pain scores, and decreased sleep time appeared in literature.¹² However, there are no data on laboratory determination of hormonal status in children with high frequency and conventional ventilation.

Stress and pain experienced in the neonatal period have been suggested to influence the hypothalamic-pituitary-adrenal (HPA) axis in the later period.¹⁶ Thus, chronic activation of the HPA axis in early life is considered to result in long-term programming of the HPA axis to function in a dysregulated manner.³ Higher baseline salivary cortisol that positively correlated to the amount of experienced pain has been reported in preterm infants at 8 months corrected age as compared with the full-term healthy neonates.¹⁷ Thus, the higher salivary cortisol level, found in the present study, could be an early sign of a disturbance in the HPA system related to stress in preterm infants in the NICU. Also, stress in preterm newborns has been associated with long-term consequences such as disorders in cognitive, motor, and behavioral development, anxiety, depression, autism spectrum disorder, high blood pressure, and metabolic syndrome.^{18,19} Therefore, preterm infants who had long-term mechanical ventilation require special support in stress mitigation and follow-up monitoring to prevent early and long-term effects of prolonged stress hormones elevations.

The current study showed that mothers of preterm infants in the NICU also experienced high levels of stress. These findings coincide with other researches^{8,18} that pointed that the mother-infant separation and inability to perform maternal role were the greatest stress factors for mothers.⁸ Most of these studies examined the behavioral manifestations of maternal stress using questionnaires, and there are only a few data on hormonal status evaluation in mothers after preterm birth and admission of their infants to NICU.^{5,18}

Stress in the NICU leads to the search for evidence-based interventions that would effectively reduce stress and its negative consequences for infants and their mothers. Kangaroo mother care with SSC is one of the developmental care approaches that has been used to mitigate the physiological and behavioral stress and treat the negative consequences of maternal separations from infants.²⁰ Our study provides valuable insights about laboratory confirmation of positive SSC effect on infant and maternal hormonal stress. Cortisol level decreasing in infants indicates that SSC has buffering effects on stress reactivity. Constantly receiving the warmth from the mother's skin and familiar olfactory stimuli, having autonomic stability and calm sleep, feeling comfort and safety on mother's chest, and hearing her heartbeat together with the incredible power of maternal love and tender embrace may facilitate the maturation of the HPA axis and decrease infant stress reactivity during SSC.^{18,21} Our results are consistent with the theory that maternal contact and gentle touch have an impact on the infant's brain system that manages stress.²¹

The stress regulatory effect of SSC was investigated by other authors. Thus, Castral et al²² investigated the salivary cortisol level in infants before and after heel prick that was performed at SSC, and they found no increase in the cortisol level.

Similar to research in premature infants, maternal salivary cortisol levels after SSC significantly reduced when compared with baseline levels in our study that coincides with the results of other authors.^{18,23} Decreased feelings of stress and anxiety state in mothers of preterm infants during and after SSC were also

reported in the researches that studied behavioral patterns of maternal stress. Mothers often say that the inability to hold and hug the child causes the most stress. Therefore, SSC, which allows the mother to hold the baby on her chest and feel his/her every movement and heartbeat will undoubtedly bring the mother not only pleasure but also will help to mitigate the negative effects of parental role alteration and enhance the feelings of the maternal parental role.²⁴ Also, it has been proven that the feeling of comfort and stress reduction by mothers is continued even after SSC that confirms a prolonged effect of SSC on maternal stress.²⁴ Therefore, it is also necessary to study the duration of the beneficial effect of SSC on maternal hormonal status.

Some infants (8.3%) and mothers (10.0%) in our study responded to SSC with the increase of cortisol levels. We have found some researches that pointed to either increased or decreased cortisol levels during the SSC—a decrease was revealed in 45.7% and an increase in 54.3% of newborns.²⁵ We have much fewer “nonresponders” to stress buffer effects of SSC, which requires further study of these features. Therefore, it is valuable to identify the unique needs of each baby. According to the developmental care principles, it is important to provide the infant with opportunities for rest and recovery and to individualize the technique of this manipulation in keeping with the capabilities and needs of each child. Some babies may be ready for SSC soon after birth and others may need to have several days or even weeks in an incubator or cot because they are unwell and unstable.²⁶ Each infant's status and need should be assessed before SSC begins to ensure that the procedure is beneficial to each baby. It is also necessary to prepare the mother for this intervention and to make sure that she feels confident enough to hold her infant quickly after birth or maybe the mother will need time to learn her infant and become more comfortable with the environment.²⁶

Environmental factors, such as sharing the time and environment, are considered to increase the correlation between maternal and child cortisol levels.⁵ We found no significant correlations between maternal and infant cortisol levels before and after SSC. The lack of correlation may be explained by the fact that mothers were not constantly present with infants, did not stay overnight at the NICU, and were not involved in all caregiving procedures around the clock.^{5,27} The objective support to these data was provided by Morelius et al⁵ who compared the influence of family-centered care, that is, when parents were constantly present with infants in relation to the standard care by measuring the salivary cortisol before and after nappy change as a stressful intervention. No significant differences regarding cortisol reactivity were found; however, there was a correlation between the basal and reactive cortisol levels in infants and mothers in the family-centered care group compared to the standard care group.⁵ It is considered that the human touch and constant closeness to the parent day and night facilitate the preterm infant's biological brain development and the HPA axis maturation.²⁷ These findings indicate that parents must stay close to their infants to be able to cherish and support the baby and thus, achieve the mother-infant synchronization and stress reduction.

CONCLUSION

Preterm infants in the NICU and their mothers experience stress which is confirmed by the elevated baseline cortisol levels. Baseline cortisol level is significantly higher in neonates who had invasive ventilation and is positively correlated with the duration of ventilation.

Kangaroo mother care with SSC reduces the stress and normalizes salivary cortisol levels in both the infant in the NICU and the mother. Only a small number of infants and mothers responded to SSC with increased cortisol levels, indicating the need to individualize the technique of this intervention according to the capabilities and needs of each child and the readiness of each mother.

Thus, Kangaroo mother care with SSC should be routinely used in preterm infants care.

Ethics Committee Approval: This study was approved by Ethics committee of I. Horbachevsky Ternopil National Medical University, (Approval No: 49).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – I.S., H.P.; Design – I.S., H.P.; Supervision – H.P.; Resources – I.S., H.P.; Materials – I.S., H.P.; Data Collection and/or Processing – I.S., H.P.; Analysis and/or Interpretation – I.S., H.P.; Literature Search – I.S., H.P.; Writing Manuscript – I.S., H.P.; Critical Review – I.S., H.P.

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

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