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Original Article

The effect of nesting positions on pain, stress and comfort during heel lance in premature infants

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Abstract *Background:* Nesting positions are commonly used in procedural analgesic administration in premature neonates. The effectiveness of nesting positions is questioned. The aim of the this study was to assess the pain, stress, comfort and salivary cortisol and melatonin values in nesting positions during the heel lance procedure in premature infants at the NICU.
Methods: Experimental research; repeated measurement design. The sample comprised 33 premature neonates with gestational age of 31—35 weeks who had been hospitalized in the NICU. Nesting positions were given using linen or towels. The procedure of heel lance was recorded on camera. The camera recordings were evaluated according to the NIPS and the COMFORTneo scale. Saliva samples were obtained five minutes prior to and 30 min after the heel lance procedure. Salivary Cortisol and Melatonin were measured using the Salimetrics Cortisol Elisa Kit and the Salimetrics Melatonin Elisa Kit.
Results: The crying time, the mean NIPS score, the COMFORTneo score, the COMFORTneo NRS-pain scores and the COMFORTneo NRS-distress scores for premature neonates who were in the prone position during the procedure were significantly lower than the scores in the supine position ($p < 0.000$). Furthermore, the level of salivary cortisol five minutes prior to and 30 min after the heel lance procedure had significantly decreased in the prone position; however, there were insignificant differences in the mean levels of salivary melatonin between the positions.

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Conclusions: Nesting in the prone position has a pain reducing effect, enhancing comfort and reducing stress in premature infants.

1. Introduction

The survival rates of premature babies have increased with technological developments and the duration of stay at the Neonatal Intensive Care Unit (NICU) has increased.[1] During their stay at the NICU, premature infants are repeatedly exposed to painful procedures as a result of routine care.[2] In the long term, painful and stressful procedures may lead to physiological, psychological and behavioral sequelae in premature infants.[3] Within the scope of developmental supportive care attempts, some of the applications preformed during painful and stressful procedures include positioning and swaddling,[4–6] massage,[7] kangaroo care,[8,9] oral glucose and sucrose solutions,[10,11] non-nutritive sucking,[12,13] breastfeeding[10,14] and topical anesthetics.[15]

Positioning lies within the scope of developmental supportive care attempts, and it is one of the most important interventions used during painful and stressful procedures.[4,6] There are some studies on developmental supportive positions in preterm infants. These studies have emphasized that developmental supportive positions help avoid energy expenditure caused by unnecessary movements of the infant[16–19] and reduce unnecessary and excess sedation, and help wean from analgesia.[20]

Positions that are given to premature infants should be comfortable, safe, should enhance physiological stability, and help keep all extremities at the midline, while maintaining the flexion posture and support optimal neuromotor development.[3,20–23] To support infants keeping their hands together, to facilitate sleep and to support development of sensory systems, infants can be positioned by nesting.[16,20] Nesting positions maintain the flexion posture while maintaining intrauterine position and postures.[5,16,20] As a basic responsibility of the nurse and as one of the most important nursing strategies, positioning by nesting uses a non-pharmacological approach in order to reduce pain and stress and improve comfort. No study has examined the effect of the nesting positions on pain, stress and comfort, salivary cortisol and melatonin values. This study aims to examine the effects of nesting supine and prone positions on the infant's pain, stress and comfort during the heel lance procedure in premature infants hospitalized in the NICU.

2. Methods

This experimental study is a repeated measurement design research. The reason for carrying out the study in the same group is that pain is experimental (depending on experience) and every infant's response to pain is specific. The sample comprised 48 premature infants with gestational ages of 31–35 weeks who had been hospitalized at the NICU. The data for the research were collected between September 2013 and October 2014. All premature infants had been born through cesarean section and had oral feeding. Infants who had received sedatives, muscle relaxants, corticosteroids or analgesics, those who had major congenital malformations, those with apgar scores of lower than 6 at the first minute and lower than 8 at the fifth minute after birth, those with severe respiratory distress requiring mechanical ventilation or NCAP, and those with neurological, gastro-intestinal, cardiac and metabolic diseases were excluded from the study. The inclusion and the exclusion from participation in the study are presented in Fig. 1.

To determine the sample size, a pilot study with 10 premature infants was performed and the sample size was calculated using the power analysis by G*power. Considering a confidence level of 95% and a power of 80%, we estimated that 33 infants were required to determine the effect of positions on pain, stress and comfort response during the heel lance procedure in premature infants. We included 48 infants in the study, taking into account the loss of sample. Fifteen infants were excluded from the study (Fig. 1). Thirty-three premature infants submitted for heel lance were evaluated.

Supine and prone positions by nesting were given to the infants in the study. A nest maintains the preterm infant with limits (similar to that in the womb); thus the preterm infant has a surface to touch. Nesting maintains the flexion posture, while maintaining intrauterine position and postures. The nesting positions were given using sheets or towels on the infants. The study was applied to the heel lance procedure that is performed routinely in order to determine bilirubin and hematocrit levels. After having waited for 30 min for gastric emptying and stabilization following feeding, the supine positioning was applied on the 3rd postnatal day and prone positioning was applied on the 4th postnatal day. Having monitored the infant in the given position for 30 min, the heel lance procedure was performed. Salivary samples were obtained 5 min before and 30 min after the procedure. Four salivary samples were obtained from each infant. The heel lance procedure was recorded on camera. Camera recording was begun prior to the procedure and continued until the infant's crying was over. After the end of the data collection process, the recordings were evaluated by two scientists (observers), independent from each other; the observers were experienced in premature infants, newborn nursing and pain assessment. The observers assessed the pain, distress, and the comfort levels of the infants according to the Neonatal Infant Pain Scale (NIPS) and the COMFORTneo scale. To evaluate the inter-observer agreement, intra-

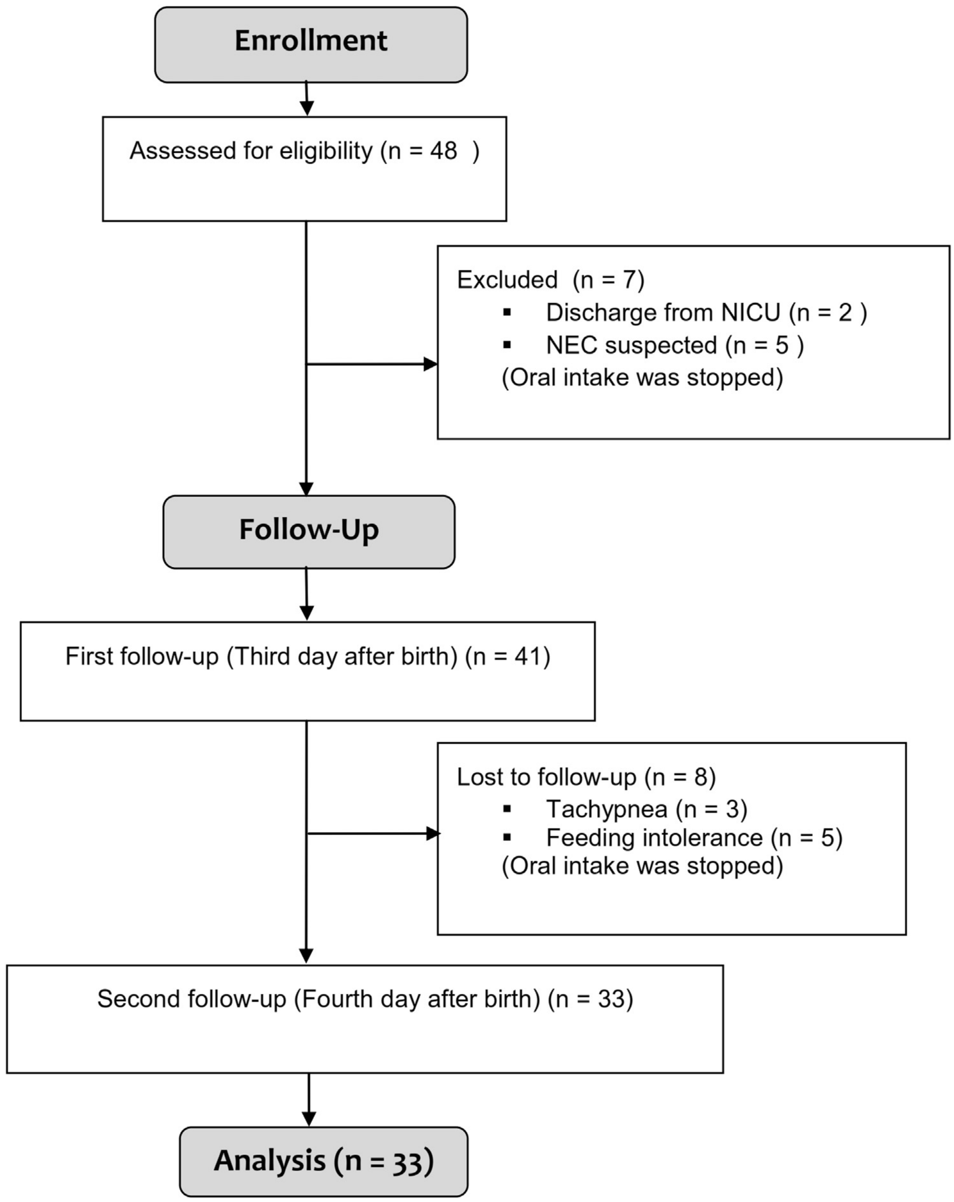

Figure 1 Selection of the study participants.

class correlation coefficients for the NIPS total score, the COMFORTneo scale total score, the COMFORTneo NRS-pain scores and the COMFORTneo NRS-distress scores were determined between the positions. Oxygen saturation and the heart rate were monitored using the Nellcor Pulse Oximetry device and recorded on the camera. Oxygen saturation, heart rate and crying time were determined from video recordings.

2.1. NIPS

The NIPS is composed of five behavioral and physiological parameters intended to assess infant pain. Behavioral parameters include facial expression, crying, arm movements, leg movements, state of arousal and physiological parameters including breathing patterns. Each behavior (except crying) has descriptors for the two possible scores of 0 and 1. Crying has three descriptors for a possible maximum score of 2. The total score is 0–7. A score of 0–2 means no pain/mild pain, a score of 3–4 means mild pain/moderate pain and scores of >4 indicate severe pain. The Cronbach's alpha coefficient was 0.95 before the procedure, 0.87 during the procedure and 0.88 after the procedure.[24]

2.2. COMFORTneo scale

The COMFORTneo scale consists of 7 items including alertness, calmness/agitation, respiratory response and crying, body movement, facial tension and muscle tone. The lowest score in the scale is 6 and the highest is 30. High scores indicate that the infant is uncomfortable. Furthermore, the COMFORTneo scale has a Numerical Rating Scale for pain and distress. NRS pain and NRS distress are scored after the COMFORTneo score by the caregiving nurse representing expert opinion. The rating is on a scale of 0–10, with 0 representing no pain or distress, and 10 representing the worst imaginable pain or distress. A score of 4–6 indicates moderate pain and distress, and a score of 7–10 indicates severe pain and distress.[25] The reliability and the validity of the Turkish version of the COMFORTneo scale were investigated. The Cronbach Alpha coefficient was determined as 0.85 for the primary observer and 0.82 for the assistant observer before care, and as 0.92 and 0.85, respectively, after care.[26]

2.3. Saliva sampling and analysis

Heel lancing was performed in the morning at the NICU where the study took place. The study data were gathered during the heel lancing in the morning, since it would not be ethical for the baby to take an extra heel lance procedure for the study. Furthermore, the transition from low light to bright light in the morning results in a increase in cortisol levels.[27,28] After a review of the literature, it was determined that the morning hours were preferred for intake of salivary cortisol sample.[18,29,30] For these reasons, the saliva sample collection was done between 9:00 and 11:00 in the morning. Saliva was obtained using Salimetrics Infant's Swab (SIS). The SIS was introduced under the tongue and moved around in the mouth and lip area. The sample collection time ranged from 60 to 90 s per sample. The SIS was then placed into an empty Salimetrics Swab Storage Tube. The saliva samples were centrifuged at 3000–3500 rpm for 15 min and stored at −80 °C for longer than 3 months. After the end of the data collection process, cortisol was measured using the Salimetrics Salivary Cortisol Elisa Kit (Kit numbers: 1–3002) and melatonin was measured using the Salimetrics Melatonin Elisa Kit (Kit numbers: 1–3402). Twenty-five μL and 100 μL of saliva, respectively, were required for the analyses of cortisol and melatonin. The salivas were collected from 33 infants. However, 32 (96.96%) samples were used for cortisol analysis. One sample was not used due to the inadequate amount of saliva. Ten (30.30%) samples were used for melatonin analysis. Twenty-three samples were not used due to inadequate amount of saliva.

2.4. Ethical approval

The study was approved by the Clinical Research Ethics Committee of Faculty of Medicine (No. 13–6.1/5), Scientific Ethics Committee of Faculty of Nursing (No. 2013/30), and informed consent was obtained from all parents prior to participation.

2.5. Data analysis

The Statistical Package for the Social Sciences (SPSS version 16.0) was used for the data analysis and a p value of <0.05 was considered statistically significant. The G*Power program was used for the Power analysis. The prevalence criteria (average, distribution of numbers and percentages, standard deviation, standard error) were used for evaluation of the descriptive data. The Shapiro–Wilk test was used in order to verify that the data were normally distributed, the Paired t test was used for parameters with normal distribution, and the Wilcoxon test was used for the parameters with abnormal distribution. For analysis of NIPS and COMFORTneo scale items according to positions, the McNemar Test was used for the scale items with two evaluation criteria, and the McNemar–Bowker Test was used for the scale items with more than two evaluation criteria. The Kruskal–Wallis Test was used to determine the correlation between gestational age and crying time. The Mann–Whitney U Test was used to determine the correlation between gender and crying time. The Intraclass Correlation Coefficient (ICC) was used to evaluate the consistency between the two observers.

3. Results

All 33 participants were premature infants, with an average gestational age of 33.03 ± 1.31 weeks (min: 31 weeks, max: 35 weeks). The mean birth weight of the infants was 1757 g ± 316 (min: 1230 g, max: 2450 g). 57.6% of the neonates were female. The mean fifth minute Apgar score was 8.96 ± 0.58 (min: 8, max: 10) (Table 1).

The infants' mean oxygen saturation ($SatO_2$) was 94.30 ± 5.63 in the prone position during the heel lance, and 86.63 ± 7.05 in the supine position during the heel lance. The mean $SatO_2$ significantly increased during prone positioning ($p = 0.000$) (Table 2). Furthermore, the mean $SatO_2$ significantly increased during the prone positioning five minutes prior to the heel lance, 30 min prior to the heel lance and 30 min after the heel lance ($p < 0.05$). The mean heart rate (HR) was 146.09 ± 15.82 during the prone positioning and 145.48 ± 11.69 during supine positioning, with

Table 1 Preterm infants characteristics and health status (n = 33).

Premature infant characteristics	
Sex (female/male; %)	57.6/42.4%
Gestational age (weeks), mean	33.03 ± 1.31 (min: 31.0, max: 35.0)
Birth weight (grams), mean	1757 g ± 316 g (min: 1230, max: 2450 g)
Birth length (cm), mean	42.5 cm ± 2.58 cm (min: 38, max: 47 cm)
One minute Apgar score, mean	7.69 ± 0.88 (min: 6, max: 9)
Five minutes Apgar score, mean	8.96 ± 0.58 (min: 8, max: 10)
Health status of premature infant (%)	
Prematurity only	27.3
PROM	21.2
Multiple pregnancy	21.2
TTN	9.1
IUGR	15.2
Hyperbilirubinemia	6.1

TTN: Transient tachypnea of the newborn.
IUGR: Intrauterine growth retardation.
PROM: Premature Rupture of Membranes.

Table 2 Behavioral and physiological responses of the premature infants during heel lance.

Premature infant responses (n = 33)	Supine	Prone	z Value/t Value	p Value
SpO_2 (mean)	86.63 ± 7.05	94.30 ± 5.63	−3.901[a]	0.000
Heart rate (mean)	145.48 ± 11.69	146.09 ± 15.82	1.417[b]	0.166
Crying time (sec)	49.66 ± 36.43 (Median: 55)	22.24 ± 25.52 (Median: 10)	−2.949[a]	0.003
NIPS (mean)				
First Observer	5.12 ± 2.50	2.63 ± 2.65	3.489[a]	0.000
Second Observer	4.69 ± 1.96	2.39 ± 2.35	4.013[a]	0.000
COMFORTneo score (mean)				
First Observer	22.42 ± 8.54	14.42 ± 7.38	3.509[a]	0.000
Second Observer	19.36 ± 5.69	13.30 ± 6.64	4.199[a]	0.000
COMFORTneo NRS-distress scores (mean)				
First Observer	5.42 ± 3.50	2.87 ± 3.03	3.496[a]	0.000
Second Observer	6.21 ± 1.88	3.78 ± 2.55	3.749[a]	0.000
COMFORTneo NRS-pain scores (mean)				
First Observer	6.09 ± 3.60	3.66 ± 3.41	2.974[a]	0.003
Second Observer	6.21 ± 1.96	3.72 ± 2.61	3.724[a]	0.000
*Salivary Cortisol Value (μg/dl)				
5 min prior to heel lance	1.19 ± 1.33	0.62 ± 0.72	−2.744[a]	0.006
30 min after heel lance	1.08 ± 1.19	0.49 ± 0.67	−3.459[a]	0.001
**Salivary Melatonin Value (pg/mL)				
5 min prior to heel lance	42.75 ± 41.02	46.13 ± 26.81	−0.459[a]	0.646
30 min after heel lance	44.29 ± 53.46	54.40 ± 32.79	−0.764[a]	0.445

* The saliva samples were adequate in 32 neonates for cortisol levels.
**The saliva samples were adequate in 10 neonates for melatonin levels.
[a] t Value (Paired Samples Test).
[b] z Value (Wilcoxon Test).

no significant difference between the positions ($p = 0.166$) (Table 2).

Premature infants in the supine position cried for a longer time during the heel lance than those in the prone position ($p = 0.003$). The crying time did not significantly differ in terms of weight, gender and gestational age ($p > 0.05$) (Table 2).

Comparison of the NIPS scores showed a significantly lower score in the prone position compared to the supine position ($p = 0.000$) (Table 2).

The COMFORTneo scores showed a significantly lower score in the prone position compared to supine position ($p = 0.000$). The COMFORTneo NRS-pain scores showed a significantly lower score in the prone position compared to the supine position ($p = 0.000$). The COMFORTneo NRS-distress scores showed a significantly lower score in the prone position compared to the supine position ($p = 0.000$) (Table 2).

The median salivary cortisol level during the prone positioning five minutes prior to the heel lance was significantly lower compared to the supine positioning ($p = 0.006$). Thirty minutes after the heel lance, the median salivary cortisol level during the prone positioning was significantly lower compared to the supine position ($p = 0.001$) (Table 2) (Fig. 2).

Five minutes prior to and 30 min after the heel lance, the median salivary melatonin level during the prone positioning showed no significant difference compared to the supine positioning ($p = 0.445$) (Table 2).

The inter-observer agreement regarding the NIPS total score, the COMFORTneo total score, the COMFORTneo NRS-pain scores and the COMFORTneo NRS-distress scores was found to be significant, and the agreement level was determined to be moderate and good ($p = 0.000$) (Table 3).

4. Discussion

The effects of painful stimuli on premature infants are known. We obtained results in this study to contribute to the literature examining the effects of nesting positions on premature infant's pain, comfort and stress. In the present study, 33 premature infants submitted for heel lance were evaluated. Heel lance was chosen as the painful stimulus for premature infants at the NICU. The nesting prone position increased the variation in partial oxygen saturation, but no significant difference in heart rate was observed. Other investigators who evaluated the physiological responses of premature infants to positions found that the prone position increased the oxygen saturation.[31–33] Similarly, Grunau et al. (2004) found no significant difference in the heart rate parameter between the prone and the supine positions.[4] Oxygen consumption increases and oxygen saturation decreases in stressful and painful procedures. Furthermore, an increase or decrease in heart rate may be expected. While prone position increased the respiratory function and oxygenation, it did not affect the heart rate in this study.

In this study, nesting prone position resulted in a better analgesic effect than nesting supine position, as assessed by Neonatal Infant Pain Scores. In contrast, in a study using FLACC, no significant difference was observed between the

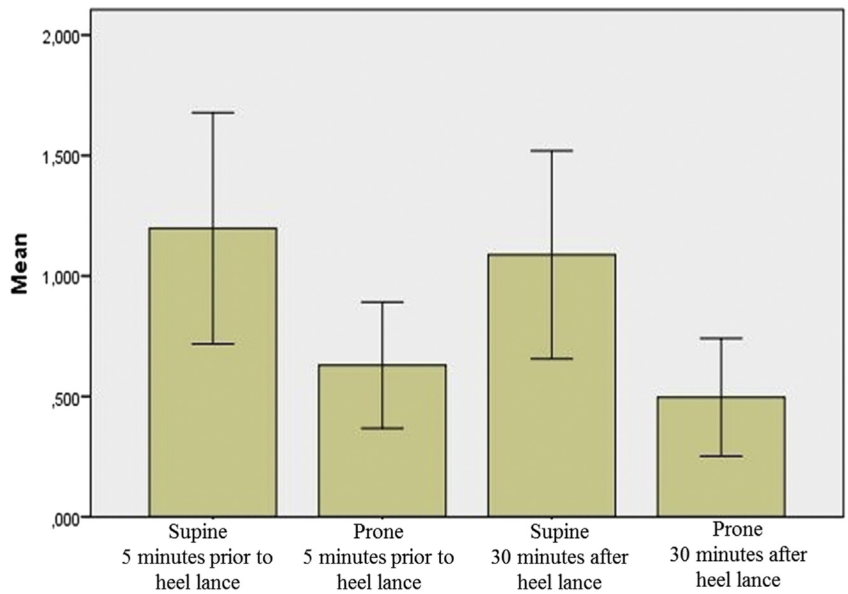

Figure 2 Salivary cortisol values (μg/dl) prior to and after the heel lance.

Table 3 Intraclass compliance (variability).

Positions	Variables	ICC	p
Supine	NIPS	0.734	0.000
	COMFORTneo score	0.566	0.000
	COMFORTneo NRS-pain scores	0.687	0.000
	COMFORTneo NRS-distress scores	0.602	0.000
Prone	NIPS	0.608	0.000
	COMFORTneo score	0.736	0.000
	COMFORTneo NRS-pain scores	0.656	0.000
	COMFORTneo NRS-distress scores	0.656	0.000

ICC: Intraclass Correlation Coefficient.

prone and supine positions in premature neonates submitted for heel lance.[4] In another study, the prone position was compared with kangaroo care and oral sucrose. Kangaroo care resulted in a better analgesic effect than the prone position and oral sucrose.[8] Morrow et al. (2010) determined that newborns, whom heel lance was performed while in the nurse's arms, had significantly lower NIPS scores compared to newborns in supine position.[6]

The COMFORTneo scores, the COMFORTneo NRS-pain scores and the COMFORTneo NRS-distress scores showed significantly lower scores in the prone position compared to the supine position by both observers during the procedure. The COMFORTneo scores determined scores of over 19, the COMFORTneo NRS-pain scores determined scores of over 6 and the COMFORTneo NRS-distress scores determined scores between 4 and 6, and these scores showed that premature infants were uncomfortable in the supine position. Few studies evaluated the comfort of premature infants in painful procedures. Van dijk et al. (2009) studied 286 premature neonates submitted for acute procedural pain. The Comfort-neo scores determined prior to the procedure showed a mean of 19.8 ± 3.8 and after the procedure, they showed a mean of 12.0 ± 3.4. The COMFORTneo NRS-pain scores and the COMFORTneo NRS-distress scores were determined to be over 4.[25] In contrast, another study determined a Comfort-neo score of between 9 and 14 in 86% of all painful procedures.[34] The results of this study demonstrated that the infants were comfortable in the majority of pain procedures, because several attempts were being implemented to reduce pain and improve comfort.

Neonatal pain and stress were conducted using saliva collection for measurement of the cortisol concentration.[18,30,35,36] Cortisol, which is one of the manifestations of physiological and hormonal changes caused by stress, is commonly measured in newborn infants, to demonstrate stress.[18,37] Saliva samples are suitable for assessment of salivary cortisol in the newborn and premature infants.[30,38] Salivary cortisol and melatonin could be used as a reliable, non-invasive, non-stress, pain-free alternative to serum cortisol.[18,30,39–41]

The level of salivary cortisol pre and post the heel lance significantly decreased in the prone position. Candia et al. (2014) determined the effect of positions on salivary cortisol values without any procedure. They explained that the level of salivary cortisol significantly decreased in the prone position compared to the lateral position and the supine position.[18] The results were similar to those our study.

The best indicator of circadian rhythm and activity of sleep in neonates is the level of melatonin. Obtaining saliva samples for measurement of melatonin value is non-stressful for premature neonates.[40] In this study, insignificant mean differences were observed in the level of salivary melatonin between positions. We think that the saliva samples were adequate in 10 neonates from whom saliva samples were obtained for melatonin levels. Therefore, there was no significance. We recommend obtaining more saliva for melatonin analysis.

In our study, prone positioning by nesting during the heel lance procedure in premature infants decreased the crying time significantly when compared to supine positioning. Studies related to developmental care applications during the heel lance procedure in premature infants have yielded similar results, which show that developmental care applications reduce the crying times of infants during invasive procedures. Bueno et al. (2012) compared the efficacy of expressed breast milk versus 25% glucose on pain responses during the heel lance. The result of the study demonstrated that a shorter duration of crying was observed in those with 25% glucose, in comparison to breast milk.[10] Kostandy et al. (2008) demonstrated decreases in the duration of crying in kangaroo care during the heel lance.[9] Another study found that the duration of the first cry was significantly shorter in the glucose group compared to the water group and the expressed breast milk group.[42] Prolonged crying increases the heart rate and oxygen consumption. For this reason, crying should be reduced by prone positions or other developmental supportive care attempts.

5. Limitations

Due to the time of use after opening the kit being short, saliva samples were frozen and analyzed at the end of the data collection process. Researchers could not be sure how much saliva was obtained during the study. One neonate's saliva samples were insufficient for salivary cortisol analysis. Twenty-three neonates' saliva samples were insufficient for salivary melatonin analysis. We recommend obtaining more saliva for measuring cortisol and melatonin levels in premature infants.

6. Conclusion

This study determined that nesting prone position reduced pain, stress, crying time and salivary cortisol level in premature infants at 31–35 weeks' gestation age. This study emphasized that the nesting prone position had pain-reducing, comforting and stress-relieving effects in premature infants at the NICU during heel lance procedures. It is recommended that studies be carried out in smaller premature infants using different invasive procedures in future studies.

Conflict of interest

Authors have reported no relevant financial and personal relationships with other individuals or organizations that could inappropriately affect their work.

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References

1. Altimier L, Phillips RM. The neonatal integrative developmental care model: seven neuroprotective core measures for family-centered developmental care. *Newborn Infant Nurs Rev* 2013;**13**:9–22.
2. Johnston CC, Fernandes AM, Campbell-Yeo M. Pain in neonates is different. *Pain* 2011;**152**:S65–73.
3. Coughlin M. *Age-appropriate care of the prematures and critically ill hospitalized infant: NANN guideline for practice*. Glenview, IL: National Association of Neonatal Nurses; 2011.
4. Grunau RE, Linhares M, Holsti L, Oberlander TF, Whitfield MF. Does prone or supine position influence pain responses in preterm infants at 32 weeks gestational age? *Clin J Pain* 2004;**20**:76–82.
5. Huang CM, Tung WS, Kuo LL, Ying-Ju C. Comparison of pain responses of premature infants to the heelstick between containment and swaddling. *J Nurs Res* 2004;**12**:31–40.
6. Morrow C, Hidinger A, Wilkinson-Faulk D. Reducing neonatal pain during routine heel lance procedures. *MCN Am J Matern Child Nurs* 2010;**35**:346–54.
7. Jain S, Kumar P, McMillan DD. Prior leg massage decreases pain responses to heel stick in premature babies. *J Paediatr Child Health* 2006;**42**:505–8.
8. Freire NB, Garcia JB, Lamy ZC. Evaluation of analgesic effect of skin-to-skin contact compared to oral glucose in preterm neonates. *Pain* 2008;**139**:28–33.
9. Kostandy RR, Ludington-Hoe SM, Cong X, Abouelfettoh A, Bronson C, Stankus A, et al. Kangaroo Care (skin contact) reduces crying response to pain in preterm neonates: pilot results. *Pain Manag Nurs* 2008;**9**:55–65.
10. Bueno M, Stevens B, de Camargo PP, Toma E, Krebs VL, Kimura AF. Breast milk and glucose for pain relief in preterm infants: a noninferiority randomized controlled trial. *Pediatrics* 2012;**129**:664–70.
11. Beken S, Hirfanoğlu IM, Gücüyener K, Ergenekon E, Turan O, Unal S, et al. Cerebral hemodynamic changes and pain perception during venipuncture: is glucose really effective? *J Child Neurol* 2014;**29**:617–22.
12. Liaw JJ, Yang L, Ti Y, Blackburn ST, Chang YC, Sun LW. Non-nutritive sucking relieves pain for preterm infants during heel stick procedures in Taiwan. *J Clin Nurs* 2010;**19**:2741–51.
13. Losacco V, Cuttini M, Greisen G, Haumont D, Pallás-Alonso CR, Pierrat V, et al. Heel blood sampling in European neonatal intensive care units: compliance with pain management guidelines. *Arch Dis Child Fetal Neonatal Ed* 2011;**96**:F65–8.
14. Marín Gabriel MÁ, del Rey Hurtado de Mendoza B, Jiménez Figueroa L, Medina V, Iglesias Fernández B, Vázquez Rodríguez M, et al. Analgesia with breastfeeding in addition to skin-to-skin contact during heel prick. *Arch Dis Child Fetal Neonatal Ed* 2013;**98**:F499–503.
15. Biran V, Gourrier E, Cimerman P, Walter-Nicolet E, Mitanchez D, Carbajal R. Analgesic effects of EMLA cream and oral sucrose during venipuncture in preterm infants. *Pediatrics* 2011;**128**:e63–70.
16. Vergara ER, Bigsby R. *Elements of neonatal positioning: developmental and therapeutic interventions in the NICU*. Baltimore: Brookes: Paul H. Publishing co; 2004. p. 177–203.
17. Chang YJ, Anderson GC, Dowling D, Lin CH. Decreased activity and oxygen desaturation in prone ventilated premature infants during the first postnatal week. *Heart Lung* 2002;**31**:34–42.
18. Cândia MF, Osaku EF, Leite MA, Toccolini B, Costa NL, Teixeira SN, et al. Influence of prone positioning on premature newborn infant stress assessed by means of salivary cortisol measurement: pilot study. *Rev Bras Ter Intensiva* 2014;**26**:169–75.
19. Carrier CT. Developmental support. In: Verklan MT, Walden M, editors. *Core curriculum for neonatal intensive care nursing*. 4th ed. Philadelphia: Saunders Elsevier; 2010. p. 213.
20. Hunter J. Therapeutic positioning: neuromotor, physiologic and sleep implications. In: Kenner C, McGrath JM, editors. *Developmental care of newborns and infants: a guide for health professionals*; 2010. p.285–312. Glenview: NANN.

21. Hunter J. Positioning. In: Kenner C, McGrath JM, editors. *Developmental care of the newborns and infants: a guide for health care professionals*. St. Louis, MO: Mosby; 2004.
22. Coughlin M, Lohman M, Gıbbıns S. Reliability and effectiveness of an infant positioning assessment tool to standardize developmentally supportive positioning practices in the neonatal ıntensive care unit. *Newborn Infant Nurs Rev* 2010;**10**:104—6.
23. Coughlin M. *Transformative nursing in the NICU: trauma-informed age appropriate care*. New York: Springer; 2014. p. 93—102.
24. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. *Neonatal Netw* 1993;**12**:59—66.
25. Van Dijk M, Roofthooft DW, Anand KJ, Guldemond F, de Graaf J, Simons S, et al. Taking up the challenge of measuring prolonged pain in (premature) neonates: the COMFORTneo scale seems promising. *Clin J Pain* 2009;**25**:607—16.
26. Kahraman A, Başbakkal Z, Yalaz M. Turkish validity and reliability of comfortneo scale. *Int Refereed J Nurs Res* 2014;**1**: 1—11.
27. Premkumar M, Sable T, Dhanwal D, Dewan R. Circadian levels of serum melatonin and cortisol in relation to changes in mood, sleep, and neurocognitive performance, spanning a year of residence in Antarctica. *Neurosci J* 2013;**2013**:254090.
28. Leproult R, Colecchia EF, L'Hermite-Balériaux M, Van Cauter E. Transition from dim to bright light in the morning induces an immediate elevation of cortisol levels. *J Clin Endocrinol Metab* 2001;**86**:151—7.
29. Neu M, Hazel NA, Robinson J, Schmiege SJ, Laudenslager M. Effect of holding on co-regulation in preterm infants: a randomized controlled trial. *Early Hum Dev* 2014;**90**:141—7.
30. Cabral DM, Antonini SR, Custódio RJ, Martinelli Jr CE, da Silva CA. Measurement of salivary cortisol as a marker of stress in newborns in a neonatal intensive care unit. *Horm Res Paediatr* 2013;**79**:373—8.
31. Goto K, Maeda T, Mirmiran M, Ariagno R. Effects of prone and supine position on sleep characteristics in preterm infants. *Psychiatry Clin Neurosci* 1999;**53**:315—7.
32. Heimann K, Vaessen P, Peschgens T, Stanzel S, Wenzl TG, Orlikowsky T. Impact of skin to skin care, prone and supine positioning on cardiorespiratory parameters and thermoregulation in premature infants. *Neonatology* 2010;**97**:311—7.
33. Gouna G, Rakza T, Kuissi E, Pennaforte T, Mur S, Storme L. Positioning effects on lung function and breathing pattern in premature newborns. *J Pediatr* 2013;**162**:1133—7.
34. Aukes DI, Roofthooft DWE, Simons SHP, Tibboe D, van Dijk M. Pain management in neonatal intensive care: evaluation of the compliance with guidelines. *Clin J Pain* 2015;**31**:830—5.
35. Antonini SR, Jorge SM, Moreira AC. The emergence of salivary cortisol circadian rhythm and its relationship to sleep activity in preterm infants. *Clin Endocrinol (Oxf)* 2000;**52**:423—6.
36. Takahashi Y, Tamakoshi K, Matsushima M, Kawabe T. Comparison of salivary cortisol, heart rate, and oxygen saturation between early skin-to-skin contact with different initiation and duration times in healthy, full-term infants. *Early Hum Dev* 2011;**87**:151—7.
37. Franck LS, Miaskowski C. Measurement of neonatal responses to painful stimuli: a research review. *J Pain Symptom Manage* 1997;**14**:343—78.
38. Herrington CJ, Olomu IN, Geller SM. Salivary cortisol as indicators of pain in preterm infants: a pilot study. *Clin Nurs Res* 2004;**13**:53—68.
39. Bagci S, Mueller A, Reinsberg J, Heep A, Bartmann P, Franz AR. Saliva as a valid alternative in monitoring melatonin concentrations in newborn infants. *Early Hum Dev* 2009;**85**: 595—8.
40. Bagci S, Mueller A, Reinsberg J, Heep A, Bartmann P, Franz AR. Utility of salivary melatonin measurements in the assessment of the pineal physiology in newborn infants. *Clin Biochem* 2010;**43**:868—72.
41. Maas C, Ringwald C, Weber K, Engel C, Poets CF, Binder G, et al. Relationship of salivary and plasma cortisol levels in preterm infants: results of a prospective observational study and systematic review of the literature. *Neonatology* 2014; **105**:312—8.
42. Ou-Yang MC, Chen IL, Chen CC, Chung MY, Chen FS, Huang HC. Expressed breast milk for procedural pain in preterm neonates: a randomized, double-blind, placebo-controlled trial. *Acta Paediatr* 2013;**102**:15—21.