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RESEARCH

Effect of mechanical ventilation modes on salivary cortisol levels in preterm infants

Mekanik ventilasyon modlarının prematüre bebeklerin tükürük kortizol düzeylerine etkisi

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Abstract

Purpose: The aim of this study was to examine the relationship between mechanical ventilation modes and stress levels by measuring salivary cortisol levels in preterm infants.

Materials and Methods: The study group comprised 65 preterm infants on respiratory support and 43 control preterm infants. The Neonatal Infant Pain Scale (NIPS) was used to evaluate pain. To determine the stress level, salivary cortisol levels were studied in saliva samples taken in the morning and evening on the 4th postnatal day.

Results: In the study group, morning and evening cortisol levels of the infants were measured 8.33±5.7 ng/ml and 8.05±5.6 ng/ml, respectively. In the control group, morning and evening cortisol values of the infants were measured 1.50±0.7 ng/ml and 1.48±0.7 ng/ml, respectively. The morning and evening salivary cortisol levels of the infants in the study group were significantly higher than those of the infants in the control group. In the invasive mechanical ventilation group infants, morning and evening cortisol levels of the infants were measured 12.46±5,3 ng/ml and 12.0±5,2 ng/ml, respectively. In the non-invasive mechanical ventilation group, morning and evening cortisol values were measured 4.57±2.7 ng/ml and 4.41±2.7 ng/ml, respectively. Both morning and evening salivary cortisol levels of infants on invasive mechanical ventilation were higher than those of infants on non-invasive mechanical ventilation.

Conclusion: Increased salivary cortisol levels in invasive modes indicate that intubation causes pain and stress in newborns.

Keywords:. Neonatal, salivary cortisol, invasive, non-invasive mechanical ventilation

Öz

Amaç: Bu çalışmada prematüre bebeklerin tükürük kortizol düzeyini ölçerek mekanik ventilasyon modları ile stres düzeyleri arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Çalışma grubunu solunum desteği alan 65 prematüre bebek, kontrol grubunu ise 43 prematüre bebek oluşturdu. Ağrının değerlendirilmesinde Yenidoğan Bebek Ağrı Ölçeği (NIPS) kullanıldı. Stres düzeyini belirlemek amacıyla doğum sonrası 4. gün sabah ve akşam alınan tükürük örneklerinde tükürük kortizol düzeylerine bakıldı.

Bulgular: Çalışma grubundaki bebeklerin sabah kortizol değerleri 8,33±5,7 ng/ml, akşam kortizol değerleri 8,05±5,6 ng/ml, kontrol grubundaki bebeklerin sabah kortizol değerleri 1,50±0,7 ng/ml, akşam kortizol değerleri 1,48±0,7 ng/ml ölçüldü. Çalışma grubundaki bebeklerin sabah ve akşam tükürük kortizol düzeyleri kontrol grubundaki bebeklere göre anlamlı düzeyde yüksek bulundu. Çalışma grubundaki bebeklerden invaziv mekanik ventilasyon uygulanan bebeklerin sabah kortizol değerleri 12,46±5,3 ng/ml, akşam kortizol değerleri 12,0±5,2 ng/ml, non-invaziv mekanik ventilasyon uygulanan bebeklerin sabah kortizol değerleri 4,57±2,7 ng/ml, akşam kortizol değerleri 4,41±2,7 ng/ml ölçüldü. İnvaziv mekanik ventilasyon uygulanan bebeklerin hem sabah hem de akşam tükürük kortizol düzeyleri, non-invaziv mekanik ventilasyon uygulanan bebeklerin kortizol düzeylerinden yüksekti.

Sonuç: İnvaziv yöntemlerde artan tükürük kortizol düzeyleri, entübasyonun yenidoğanlarda ağrı ve strese neden olduğunu göstermektedir.

Anahtar kelimeler: Yenidoğan, tükürük kortizol, invaziv, non-invaziv mekanik ventilasyon

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INTRODUCTION

As the respiratory system of premature infants is insufficient, respiratory muscles are weak, and the development of alveoli and alveolar capillaries is limited[1,2]. They generally need respiratory support with invasive modes such as Pressure Support (PS) mode and Synchronised Intermittent Positive Pressure Ventilation (SIPPV) mode or nasal Synchronised Intermittent Mandatory Ventilation (SIMV) and nasal Continous-Positive Pressure Ventilation (nCPAP) mode.

Stress is a critical condition that can lead to negative consequences in the neurological development of infants. Stress is defined as a factor that can develop secondary to emotional and physical causes. The neonatal intensive care environment and invasive, non-invasive interventions applied to the baby are strong sources of stress and trauma. In particular, preterm infants are vulnerable to the impact of early-life pain/stress[3]. It is challenging to evaluate pain and stress in a newborn baby. The main problem in the evaluation and grading of pain in newborns is that they do not have a verbal expression for pain[4]. Because newborns cannot describe pain and stress, many scales have been published. The most widely used of these are the "Neonatal Infant Pain Scale (NIPS)" and the CRIES scale[5-7].

Although pain varies depending on various factors, it should be evaluated regularly to determine the presence of pain and the effectiveness of the treatment applied. In particular, pain during mechanical ventilation makes it difficult to adapt to ventilation. Pain management in premature infants on mechanical ventilation may positively affect the clinical course of newborns by protecting their energy and reducing their oxygen needs[8]. The physiological consequences of stress are regulated by the central nervous system with stimulation of the hypothalamic pituitary axis (HPA) system. In response to stress, there is an increase in the secretion of glucocorticoids, especially the hormone cortisol[9]. Moreover, neonatal pain/stress contributes to altered HPA function up to school age in children born very preterm in the long term [10].

Increases in heart rate, respiratory rate, and blood pressure, deterioration in blood gasses and changes in skin color and pupil size in premature infants with pain are the most common physiological changes[6]. The stress level of newborn infants can be evaluated using behavioral scales, but biochemical measurements can also be used[11]. Cortisol level measurements in premature infants are widely used to detect stress levels. This is a reliable method for evaluating pain and stress in preterm infants[11-14]. One of the biochemical tests used is the measurement of salivary cortisol levels. The test is non-invasive, easily accessible, and inexpensive[11]. Saliva cortisol reflects the plasma free cortisol level and is independent of the amount of saliva[12].

In this study, we evaluated the relationship between mechanical ventilation modes and prolonged pain and stress in newborns by measuring the salivary cortisol levels of premature infants on invasive or non-invasive mechanical ventilation modes. We hypothesized that the salivary cortisol levels of premature infants on invasive mechanical ventilation have higher salivary cortisol levels than those of premature infants on non-invasive mechanical ventilation modes.

MATERIALS AND METHODS

Sample

The study was designed as a prospective and cross-sectional study. This study was conducted between January 2022 and May 2022 at Çukurova University, Faculty of Medicine, Neonatal Intensive Care Units. The study group consisted of 65 premature infants who received respiratory support, and the control group consisted of 43 premature infants who were on room air. Babies born at 34 weeks of gestation and below were included in the study. Newborns with major congenital malformation, congenital infection, congenital heart disease, surgical problems, newborns who underwent a painful procedure (aspiration, vein/heel blood collection, catheterization, intubation, etc.) 1 h before sampling, and newborns treated with hydrocortisone or dexamethasone were excluded from the study.

Ethics approval was obtained from the Çukurova University Faculty of Medicine, Non-Invasive Ethics Committee (Decision No: 2020/101), and written parental consent was obtained. The Declaration of Helsinki, which regulates human biomedical research, was complied with throughout the study.

Procedure

The infants' date of birth, gender, gestational age, mode of delivery, birth weight, antenatal steroid

therapy, 1st and 5th minute Apgar score, development of RDS and whether surfactant is applied, need for ventilator therapy, invasive and non-invasive mechanical ventilation modes, presence of sepsis, and caffeine treatment were recorded. Immediately before sampling, NIPS score, pulse, body temperature, respiratory rate, oxygen saturation, systolic– diastolic blood pressure, and NIPS score were recorded[15,16].

Because cortisol levels in newborn infants reach their lowest levels when they are 3-5 days old, saliva samples were taken from all infants on the 4th postnatal day[17]. There are studies in the literature that the test should be performed twice a day because morning cortisol levels are higher and the test is easily applicable[18]. Therefore, saliva samples were collected from all infants in the morning and evening. The infants were lying in the supine position in an incubator in a dim light and quiet environment. Saliva samples were collected without the use of any salivation stimulating agents. The infants appeared not to be stressed by this procedure. To prevent bias throughout the study, saliva samples were taken by the same health personnel between 07.00-09.00 in the morning and 22.00-23.00 in the evening. Saliva samples were collected with sterile disposable 3 mL volume Pasteur pipettes (LP Italiana, Via Carlo Reale, PA 20157 Milano). Samples were then transferred to Salimetrics brand saliva storage tubes (Salimetrics, LLC USA). The samples were frozen at -20^{0}C and stored in an upright position at -80^{0}C until laboratory analysis.

Laboratory analysis

Saliva cortisol levels were measured in the laboratory of Çukurova University Faculty of Medicine, Department of Biochemistry. The salivary cortisol level was measured using the enzyme immunoassay method. DiaMetra (Diametra S.r.l, Via Pozzuolo 14, Spello Italy) brand salivary cortisol ELISA kit was used. "DiaMetra Cortisol Saliva Elisa kit" is a competitive immunoenzymatic colorimetric method. The samples were first melted at 4°C and centrifuged at 3000 rpm for 15 min in a refrigerated centrifuge, then kept at -20°C for 1 h, melted 4°C, and centrifuged again at 3000 rpm for 15 min. The supernatant portions of the centrifuged samples were used to obtain cortisol from saliva. These samples were divided into two tubes, and extreme values were measured again. With the "DiaMetra Cortisol Saliva Elisa kit", calibrators at varying concentrations were used to calculate the cortisol concentrations in the sample content, and salivary cortisol values were calculated as "ng/mL" via the "4 parameter logistic standard curve".

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 25.0 package program was used for statistical analysis of the data. Categorical measurements are summarized as numbers and percentages, and continuous measurements are presented as mean and standard deviation (median and minimum-maximum where appropriate). The Shapiro– Wilk test was used to determine whether the parameters in the study showed normal distribution. Chi-square and Fisher exact tests were used to compare categorical expressions. The independent Student's t-test was used for normally distributed parameters, and the Mann –Whitney U test was used for non-normally distributed parameters. Spearman's rho correlation test was used to determine the relationship between continuous measurements. The statistical significance level was set at 0.05 in all tests. In the power analysis, Type 1 error = 0.05 when power = 80% effect size d = 0.5, the minimum number of samples reached was found to be 102.

RESULTS

There were 32 girls and 33 boys in the Study group and 23 girls and 20 boys in the Control group. Demographic data for the infants are given in Table 1. No statistically significant difference was found between the study and control groups regarding infants' gender, mode of delivery, gestational weeks, and birth weight. Sixteen (37.2%) of the infants in the control group and 19 (29.2%) infants in the study group received antenatal steroid treatment (p=0.386). The 1st and 5th minute Apgar scores of the infants in the Study group were significantly lower than the Apgar scores of the Control group (p<0.001; p<0.001, respectively) (Table 1).

The mean NIPS Score, peak heart rate, and respiratory rate of the infants in the Study group were significantly higher than those in the Control group (p<0.001 for all, respectively). The mean O_2 saturation and systolic blood pressure values of the infants in the study group were found to be significantly lower than those of the infants in the control group (p=0.003; p<0.001, respectively) (Table 2).

Table 1. Demographic findings of the infants

Parameter	Study Group (n=65)	Control Group (n=43)	p
Gender Girl (%)	32 (49.2)	23 (53.5)	0.665[a]
Boy (%)	33 (50.8)	20 (46.5)	
Mode of Delivery-C-Section (%)	49 (75.4)	36 (83.7)	0.300[a]
Antenatal steroids (%)	19 (29.2)	16 (37.2)	0.386[a]
Gestational age (weeks)*	31.6±1.6 (29-34)	32.2±1.2 (30-34)	0.105[b]
Birth weight (g)*	1939±543 (900-3000)	1985±285 (1400-2600)	0.616[c]
Apgar 1st min*	6.8±0.8 (5-8)	7.5±0.6 (6-9)	<0.001[b]
Apgar 5th min*	7.9±0.7 (7-9)	8.9±0.7 (8-10)	<0.001[b]

a: chi-square test, b: Mann –Whitney U test, c: Independent Student's t-test, * Values are given mean±SD, Min– Max in parentheses.

Table 2. Comparison of infants' vital signs and NIPS scores between groups

Parameter	Study Group (n=65)	Control Group (n=43)	p
	Mean±SD (Min-Max)	Mean±SD (Min-Max)	
NIPS Scores	0.51±0.7 (0-3)	0.12±0.3 (0-1)	<0.001[b]
Heart rate (/min)	137.1±9.4 (108-160)	131.1±6.1 (118-141)	<0.001[c]
O_2 Sat (%)	98.8±1.3 (95-100)	99.5±0.7 (98-100)	0.003[b]
Systolic blood pressure (mmHg)	69.2±9.5 (48-97)	74.9±6.2 (60-86)	<0.001[b]
Diastolic blood pressure (mmHg)	42.9±7.8 (26-60)	42.9±3.1 (38-50)	0.677[b]
Respiratory rate (/min)	57.3±4.9 (45-70)	51.5±5.4 (41-61)	<0.001[c]
Body temperature (°C)	36.6±0.2 (36.1-37.2)	36.7±0.2 (36.1-37.2)	0.266[b]

b: Mann –Whitney U test, c: Independent student t-test

RDS was in 17 (26.2%) patients in the Study group, whereas no patient had RDS in the Control group (p<0.001). Clinical sepsis was detected in 19 (29.2%) infants in the Study group and in 6 (13.9%) infants in the Control group. Although the incidence of sepsis in the infants in the Study group was observed to be higher than that in the Control group, the difference was not statistically significant (p=0.065). Antibiotic, surfactant, and caffeine treatments were higher in the infants in the Study group than in the Control group (p<0.001; p=0.003; p=0.004, respectively).

In the study group, 31 of the infants (47.7%) were on invasive mechanical ventilation and intubated, whereas 34 of the infants (52.3%) were on non-invasive modes. For infants with invasive mechanical ventilation, 2 (6.5%) infants were treated with HFOV, 17 (54.8%) infants with PSV, and 12 (38.7%) infants with SIPPV. Among non-invasive mechanical ventilation infants, 9 (26.5%) were treated with

nCPAP, 3 (8.8%) with nSIPPV, 22 (64.7%) with nSIMV. The mean salivary cortisol values in the morning and evening were found to be significantly higher in the infants in the Study group than in the infants in the Control group (p<0.001; p<0.001, respectively) (Table 3) (Figure 1).

Infants in the invasive mechanical ventilation group had significantly higher morning and evening salivary cortisol levels than those in the non-invasive mechanical ventilation group (p<0.001; p<0.001, respectively). In addition, the morning salivary cortisol levels of the infants in the invasive and non-invasive groups were significantly higher than the evening salivary cortisol levels (p=0.029, p=0.011, respectively) (Table 3). No significant difference was found between morning and evening salivary cortisol values in infants who underwent PSV and SIPPV modes on invasive mechanical ventilation (p=0.402; p=0.391, respectively). No statistically significant difference was observed in the changes in salivary cortisol values in the morning and evening in infants who underwent PSV and SIPPV mechanical ventilation (p=0.170; p=0.182, respectively) (Table 3).

Infants in the nSIMV group with non-invasive mechanical ventilation had higher morning and evening salivary cortisol levels compared with infants in the non-invasive nCPAP mode (p=0.031; p=0.038, respectively). In the non-invasive mechanical ventilation group, the morning salivary cortisol levels of the infants in the nSIMV group were higher than the evening salivary cortisol levels (p=0.017). No significant difference was found between the morning and evening salivary cortisol levels of infants in the nCPAP group (p=0.575) (Table 3).

Table 3. Differences in salivary cortisol levels (ng/mL) in infants in morning and evening

	Morning	**Evening**	**p+**
	Mean±SD (Min-Max)	Mean±SD (Min-Max)	
Study Group (n: 65)	8.33±5.7 (1.53-25.0)	8.05±5.6 (1.43-25.2)	0.003
Control Group (n: 43)	1.50±0.7 (0.17-3.56)	1.48±0.7 (0.2-3.7)	0.341
p++	<0.001**	<0.001**	
Study Group (n:65)			
Invasive MV (n: 31)	12.46±5.3 (6.28-25.02)	12.0±5.2 (5.88-25.21)	0.029
Non-invasive MV (n: 34)	4.57±2.7 (1.53-10.75)	4.41±2.7 (1.43-10.65)	0.011
p**	<0.001**	<0.001**	
Δ salivary cortisol	-0.42±1.0 -	0.15±0.34	0.073
Invasive MV Group (n: 31)			
PSV (n: 17)	12.9±5.2 (6.81-25.02)	12.6±4.9 (6.97-25.21)	0.170
SIPPV (n: 12)	11.3±5.6 (6.28-24.31)	10.9±5.7 (5.88-23.91)	0.182
p**	0.402	0.391	
Non-invasive MV Group (n: 34)			
nCPAP (n: 9)	2.73±1.1 (1.53-4.80)	2.66±1.3 (1.43-5.03)	0.575
nSIMV (n: 22)	4.67±2.4 (1.85-9.91)	4.47±2.3 (1.88-10.16)	0.017
p**	0.031*	0.038*	

MV: Mechanical ventilation, +: Paired sample t-test, ++: Mann –Whitney U test, * p<0.05, ** p<0.001

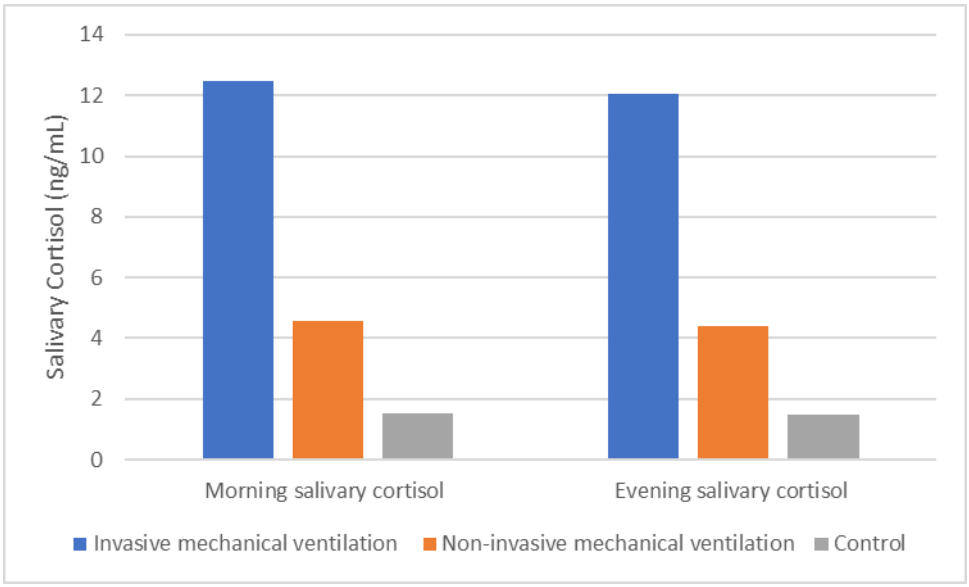

Figure 1. Difference in salivary cortisol levels between the groups in the morning and evening (ng/mL)

A moderate positive correlation was found between infants' NIPS scores and morning (r=0.555) and evening (r=0.552) cortisol values (p<0.001s; p<0.001, respectively). Although the level of salivary cortisol change (Δ cortisol) in the morning and evening was higher in the invasive mechanical ventilation group than in the non-invasive mechanical ventilation group, the difference was not statistically significant (p=0.073) (Table 3).

DISCUSSION

Neonatal intensive care units are stressful for infants because of exposure to noise, light, invasive and non-invasive interventions, and separation from the mother. Various scales have been defined to measure the level of stress and pain[19]. In addition, some biochemical analysis can be performed for this purpose. Because it is a non-invasive and painless procedure, it has been recommended to measure salivary cortisol levels more recently[12]. The primary aim of our study was to compare the stress levels of preterm infants who underwent mechanical ventilation by analyzing salivary cortisol levels and to evaluate the significance of these parameters by examining their correlation with pain scales.

In this study, we have shown that salivary cortisol levels were higher in the ventilated group than in the control group. In addition, intubated patients had higher levels compared with noninvasively ventilated ones.

Antenatal steroid treatment may affect postnatal saliva levels. In this study, antenatal steroid treatment was administered to 19 (29.2%) mothers in the study group, whereas 16 (37.2%) mothers in the control group were treated. There was no difference in the rates of antenatal steroid administration between the groups.

Cortisol levels in saliva are critical indicators of plasma free cortisol. Salivary cortisol levels are not affected by salivary secretion rate. Saliva collection is an easy and non-invasive method. Baid et al.[20] in their study, they stated that although there are many hormones in saliva, only salivary cortisol levels can be measured significantly. Calixto et al.[15]stated that there is a significant correlation between salivary cortisol and blood cortisol levels; therefore, salivary cortisol measurement can be used for various conditions and diseases.

Invasive mechanical ventilation was applied to 31 (47.7%) infants in the study group, and non-invasive mechanical ventilation was applied to 34 (52.3%) of them. Because mechanical ventilation is also a source of stress and pain in newborns, pain management should be appropriately managed. It causes acute pain in preterm infants due to procedures such as oral,

intranasal, and endotracheal aspiration, insertion of nasal cannulas, and postural drainage[21]. Cone et al.[22] conducted a study to evaluate the pain and stress caused by endotracheal aspiration methods in 10 infants receiving conventional mechanical ventilation. In this study, the stress and pain created by aspiration methods were evaluated using pain scales similar to those used in our study. They observed that these methods did not significantly affect salivary cortisol levels.

In our study, we determined that infants who underwent mechanical ventilation had higher morning and evening salivary cortisol levels than infants in the control group. These results indicate a significant response to pain and stress in infants. In addition, we observed that morning cortisol levels were significantly higher than evening cortisol levels in both groups. This finding is an expected result because cortisol secretion is physiologically higher in the morning and decreases toward the evening hours.

In most studies in the literature, it was stated that appropriate analgesia should be provided to newborns receiving invasive mechanical ventilation[23-26]. Analgesia and sedation suitable for infants are administered to our intensive care unit when needed. The infants included in the study were not receiving any sedative or analgesic therapy when the samples were collected.

Studies comparing invasive and non-invasive mechanical ventilation modes in terms of salivary cortisol levels are scarce in the literature. Therefore, more extensive and comprehensive studies are required to elucidate this issue. In Morelius et al.[27] on salivary cortisol levels in preterm and term infants after changing diapers, salivary cortisol levels were significantly higher in preterm infants. In our study, salivary cortisol levels were quite high in all groups. However, because there were no term newborns in our study, no comparison of preterm and term infants could be made.

The primary aim of our study was to examine the relationship between ventilation modes and salivary cortisol levels. Synchronized modes are preferred in infants because they facilitate the adaptation of the newborn to mechanical ventilation. In the analysis, we studied invasive and non-invasive mechanical ventilation modes and observed that there was no significant difference between salivary cortisol levels in different modes in patients who underwent invasive mechanical ventilation. In the non-invasive MV group, the cortisol level in the group followed up with the nCPAP mode was significantly lower than that in the patients followed up with the nSIMV mode. To the best of our knowledge, there is no study on this subject in the literature. This difference may be because nCPAP is an application made with continuous support rather than PIP application. Additionally, the general condition of the patients followed up with the nCPAP mode is better than that of the patients followed up with the nSIMV mode, which may account for this difference. In this study, we used NIPS to assess pain. In our study, it was observed that the mean NIPS Score, heart rate, and respiratory rate of the infants in the invasive MV group were higher than those in the non-invasive MV group. We detected a positive correlation between NIPS scores and morning and evening salivary cortisol levels. Therefore, both the NIPS scores and salivary cortisol levels are significant tests in the assessment and rating of pain.

Our study has some limitations. First, our study was conducted in a single center with a relatively small sample size. Subgroup analysis of the HFOV mode, which was applied in very few cases to the ventilation modes, could not be performed in this study. There were cases in our study group who were followed up on mechanical ventilation for many reasons, such as RDS, pneumonia, or sepsis, which may be attributed to stress and pain. Analysis with a homogenous group could be more meaningful.

In conclusion, pain and stress in newborns may cause many pathologies in the short and long term. Behavioral scales and biochemical analyses are needed to evaluate stress and pain. The selection of the appropriate mechanical ventilation mode on a case-by-case basis is important in terms of stress and pain management. The results showed that non-invasive mechanical ventilation modes should be preferred. All efforts should be made to terminate invasive mechanical ventilation as soon as possible. Pain in newborns is an issue that should not be ignored. Physicians and nurses have significant duties in pharmacological and non-pharmacological management.

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REFERENCES

1. Kelly MM. The basics of prematurity. J Pediatr Health Care. 2006;20:238-44.
2. Warren JB, Anderson JM. Newborn respiratory disorders. Pediatr Rev. 2010;31:487.
3. Zhao T, Griffith T, Zhang Y, Li H, Hussain N, Lester B et al. Early-life factors associated with neurobehavioral outcomes in preterm infants during NICU hospitalization. Pediatr Res. 2022;92:1695-1704.
4. Slater R, Cantarella A, Franck L, Meek J, Fitzgerald M. How well do clinical pain assessment tools reflect pain in infants? PLoS Med. 2008;5:e129.
5. Collados-Gómez L, Camacho-Vicente V, González-Villalba M, Sanz-Prades G, Bellón-Vaquerizo B. Neonatal nurses' perceptions of pain management. Enferm Intensiva. 2018;29:41-7.
6. Maxwell LG, Malavolta CP, Fraga MV. Assessment of pain in the neonate. Clin Perinatol. 2013;40:457-69.
7. McPherson C, Miller SP, El-Dib M, Massaro AN, Inder TE. The influence of pain, agitation, and their management on the immature brain. Pediatr Res. 2020;88:168-75.
8. Asadi-Noghabi F, Tavassoli-Farahi M, Yousefi H, Sadeghi T. Neonate pain management: what do nurses really know? Glob J Health Sci. 2014;6:284.
9. Sheng JA, Bales NJ, Myers SA, Bautista AI, Roueinfar M, Hale TM et al. The hypothalamic-pituitary-adrenal axis: Development, programming actions of hormones, and maternal-fetal interactions, Front Behav Neurosci. 2021;14:601939.
10. Brummelte S, Chau CM, Cepeda IL, Degenhardt A, Weinberg J, Synnes AR, et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. Psychoneuroendocrinology. 2015;51:51-63.
11. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34:163-71.
12. 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.
13. 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;26:69-75.
14. Gunes T, Koklu E, Ozturk MA, Koklu S, Cetin N. Evaluation of serum cortisol levels in a relatively large and mature group of ventilated and nonventilated preterm infants with respiratory distress syndrome. Am J Perinatol. 2006;23:335-9.
15. Calixto C, Martinez FE, Jorge SM, Moreira AC, Martinelli CE. Correlation between plasma and salivary cortisol levels in preterm infants. J Pediatr. 2002;140:116-8.
16. 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.
17. Rokicki W, Forest MG, Loras B, Bonnet H, Bertrand J. Free cortisol of human plasma in the first three months of life. Biol Neonate. 1990;57:21-9.
18. Hudson-Barr D, Capper-Michel B, Lambert S, Palermo TM, Morbeto K, Lombardo S. Validation of the pain assessment in neonates (PAIN) scale with the neonatal infant pain scale (NIPS). Neonatal Netw. 2002;21:15-21.
19. Cabral DM, Antonini SRR, Custódio RJ, Martinelli CE, Da Silva CAB. Measurement of salivary cortisol as a marker of stress in newborns in a neonatal intensive care unit. Horm Res Paediatr. 2013;79:373-8.
20. Baid SK, Sinaii N, Wade M, Rubino D, Nieman LK. Radioimmunoassay and tandem mass spectrometry measurement of bedtime salivary cortisol levels: a comparison of assays to establish hypercortisolism. J Clin Endocrinol Metab. 2007;92:3102-7.
21. Boyle EM, McIntosh N. Sedation and Analgesia. In Manual of Neonatal Respiratory Care (Ed S Donn): 473-84. New York, Springer, 2012.
22. Cone S, Pickler RH, Grap MJ, McGrath J, Wiley PM. Endotracheal suctioning in preterm infants using four-handed versus routine care. J Obstet Gynecol Neonatal Nurs. 2013;42:92-104.
23. Pillai Riddell RR, Stevens BJ, McKeever P, Gibbins S, Asztalos L, Katz J, Ahola S, et al. Chronic pain in hospitalized infants: health professionals' perspectives. J Pain. 2009;10:1217-25.
24. Simons SH, van Dijk M, Anand KS, Roofthooft D, van Lingen RA, Tibboel D. Do we still hurt newborn infants?: A prospective study of procedural pain and analgesia in neonates. Arch Pediatr Adolesc Med. 2003;157:1058-64.
25. Açıkgöz A, Yıldız S. Effects of open and closed suctioning systems on pain in newborns treated with mechanical ventilation. Pain Manag Nurs. 2015;16:653-63.
26. Carbajal R, Eriksson M, Courtois E, Boyle E, Avila-Alvarez A, Andersen RD, et al. Sedation and analgesia practices in neonatal intensive care units (EUROPAIN): results from a prospective cohort study. Lancet Respir Med. 2015;3:796-812.
27. Mörelius E, Hellström-Westas L, Carlén C, Norman E, Nelson N. Is a nappy change stressful to neonates? Early Hum Dev. 2006;82:669-76.