

The effect of bubble nasal continuous positive airway pressure application on saliva cortisol levels in full-term neonates

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Abstract

Background Neonates with respiratory distress are commonly treated with bubble nasal continuous positive airway pressure (nCPAP) and undergo many procedures that cause stress-induced pain. Salivary cortisol is a biomarker of alteration in the hypothalamic-pituitary-adrenal axis caused by repeated and long-term exposure to stress.

Objective To analyze the effect of bubble nCPAP use on salivary cortisol levels in full-term infants.

Methods This study used a one-group pre-test/post-test design to compare salivary cortisol levels before and 30 minutes after bubble nCPAP application. Salivary cortisol was measured using an ELISA method. Pain was also assessed at the same time points using the *Neonatal Infant Pain Scale* (NIPS) score. We included infants of 37 0/7 to 42 6/7 weeks' gestational age with respiratory distress necessitating bubble nCPAP application. Infants with birth weight of <2,500 grams, major congenital anomalies, an APGAR score of <5 at 5 minutes, shock, and infants who had undergone surgery were excluded.

Results A total of 38 subjects participated in this study. Prior to bubble nCPAP application, median cortisol levels were significantly lower [1.65 (range 0.1 to 66.5) ng/mL] than after bubble nCPAP application [6.8 (range 0.4 to 92) ng/mL] ($P < 0.05$). There were no significant differences in cortisol increase based on gender, type of birth, or salivary cortisol sampling time. There were significant differences in pain level after the 30 minute nCPAP application.

Conclusion An increase in cortisol levels and pain scale scores during administration of bubble nCPAP indicates a pain response in full-term neonates; therefore, this procedure should be accompanied by pain or stress management. [*Paediatr Indones.* 2024;64:22-7; DOI: 10.14238/pi64.1.2024.22-7].

Keywords: salivary cortisol; nCPAP; term neonates

Respiratory distress at birth is a common complication in neonates, whether full-term, preterm, or post-term. It accounts for 25% of NICU deaths.^{1,2} In Indonesia, respiratory disorders are the main cause of mortality in infants admitted to the NICU with an incidence of 36.5%, followed by prematurity (32.4%), sepsis (12%), hypothermia (6.3%), blood disorders or jaundice (5.6%), post-maturity (2.8%), and congenital abnormalities (1.4%).³ Neonates with respiratory disorders are generally admitted to the neonatal intensive care unit (NICU) and undergo many procedures that cause stress and pain.⁴ Nasal continuous positive airway pressure (nCPAP) is the standard of care for resuscitation and continued support of neonates of all gestational ages who breathe spontaneously, but have respiratory problems.⁵

Nasal CPAP potentially causes physiological stress in neonates, primarily due to the use of nCPAP prongs.⁶ Untreated pain during this critical period of

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brain development can have immediate and long-term consequences on the baby.⁷ Therefore, neonates using nCPAP should undergo pain evaluation. Assessment of pain in neonates can be done using several scoring systems. However, these type of assessments are subjective.⁸

Salivary cortisol is a biomarker of changes in the hypothalamic-pituitary-adrenal axis caused by repeated and long-term exposure to pain.⁹ Salivary cortisol is considered to be more objective for assessing pain in neonates compared to commonly used scoring systems such as NIPS.⁸ In addition, salivary cortisol measurements are easy to perform, painless, and non-invasive. Salivary cortisol is also reflective of free cortisol compared to total cortisol, which can be influenced by plasma-binding protein concentrations.¹⁰ Moreover, neonates are considered to have no adrenal rhythm until 2-3 months after birth, which is related to the immaturity of the adrenal cortex and central nervous system.¹¹

A study showed an increase in skin cortisol levels in premature infants under severe and mild stress stimulation on the third day and the highest difference on the seventh day.¹² The other study reported by Rebelato et al, salivary cortisol levels of infants using nCPAP were lower compared to infants treated with invasive ventilators.¹³ Limited studies have reported the effect of bubble nasal cpap on cortisol levels. Therefore, the aim of this study was to assess the effect of nasal bubble CPAP on cortisol levels as a hormonal stress response in full-term infants with respiratory distress.

Methods

This study employed a one-group pre-test/post-test design to evaluate salivary cortisol levels of full-term neonates before and 30 minutes after application of bubble nCPAP. From May 2021 to January 2022, this study was conducted in NICU patients at Dr. Wahidin Sudirohusodo Hospital, Hasanuddin University Hospital, Tadjuddin Chalid Hospital, and Kartini Mother and Child Hospital, Makassar. The study was approved by the Ethics Committee for Biomedical Research in Humans, Faculty of Medicine, Universitas Hasanuddin. All parents or guardians of participants provided written informed consent to participate in

the study.

Inclusion criteria were infants of 37 0/7 - 42 6/7 weeks' gestational age with respiratory distress necessitating bubble nCPAP application (bilateral prong interface, *Fisher & Paykel*). Exclusion criteria were birth weight of <2,500 grams and newborns with major congenital abnormalities, an APGAR score of <5 at 5 minutes, shock, or infants who had undergone surgery.

Before installing bubble nCPAP and other invasive devices, saliva specimens were obtained from subjects without stimulating salivary secretion using a sterile cotton bud placed under the bottom end of the tongue for approximately 30 seconds. Between 10 and 50 μL of saliva was required. Cortisol levels in saliva are constant at room temperature for three weeks and do not require additional treatment. Specimens were maintained at a temperature of -10 to -80°C in the *Hasanuddin University Medical Research Center (HUMRC)* laboratory until analysis, with a maximum storage period of 6 months. The saliva specimen should not be contaminated with blood or milk; therefore, the participant fasted 2 hours to avoid milk contamination in the saliva, and the retrieval of the saliva was done gently. The second retrieval of salivary cortisol was after 30 minutes of the bubble nCPAP application. Cortisol concentrations (ng/mL) were measured in the saliva specimens using an enzyme-linked immunosorbent assay (ELISA) technique (*DBC Saliva Cortisol Kit*; DBC-Diagnostics Biochem Canada, Ontario, Canada) at the HUMRC laboratory.

Neonatal Infant Pain Scale (NIPS) scores were also calculated before and after 30 minutes of bubble nCPAP application. NIPS is a multidimensional scale used to assess the behavioral pain response in both full-term and preterm infants. NIPS evaluates six variables to assess procedural pain: five behavioral indicators (facial expression, crying, state of arousal, arm and leg positions) and one physiological indicator (breathing pattern). Each indicator was assigned a value of 0 or 1, with the exception of crying, which was assigned a value between 0 and 2, resulting in a total score between 0 and 7. The pain scale was divided into three categories: no pain (NIPS score 0-2,) mild pain (NIPS score 3-4), and severe pain (NIPS score >4).¹⁴ Other characteristics, including gender, birth weight, and vital signs, were recorded.

Statistical analyses were completed by using

SPSS version 26 (IBM, Armonk, New York). Data were grouped and analyzed by univariate and bivariate analyses. A P value of <0.05 were considered to be statistically significant.

Results

Thirty-eight subjects were eligible for the study. The characteristics of subjects are shown in **Table 1**.

Table 2 shows that there were no significant differences in salivary cortisol levels by gender, mode of delivery, and specimen collection time, both before and after bubble nCPAP application. When compared to before bubble nCPAP application, cortisol levels after application were increased across all characteristics groups.

Figure 1 demonstrates the difference in cortisol levels before and after 30-minute bubble nCPAP application. Median saliva cortisol levels was 1.65

(range 0.1 to 66.5) ng/mL prior to bubble nCPAP application and was 6.8 (range 0.4 to 92) ng/mL 30 minutes after application (P<0.05).

NIPS scoring revealed that almost all subjects were pain-free prior to nCPAP application; however, following application, the number of subjects with mild to moderate pain increased. Six subjects had severe pain scores (**Table 3**). There were significant differences in pain level using the Neonatal Infant Pain Scale (NIPS) score after 30 minutes of nCPAP application.

Discussion

Cortisol in saliva is a biomarker of alterations in the hypothalamic-pituitary-adrenal (HPA) axis induced by chronic and recurrent pain exposure.⁹ Salivary cortisol is regarded as more objective than the commonly used scoring system, NIPS, for assessing pain in neonates.⁸

Table 1. Characteristics of subjects

Characteristics	(N=38)
Gender, n	
Male	20
Female	18
Mode of delivery, n	
Vaginal	9
Cesarean section	29
Specimen collection time, n	
Morning	5
Afternoon	17
Night	16
Median birth weight (range), gram	2,750 (2,500-3,000)
Median gestational age (range), weeks	38 (37-40)

Table 2 . Analysis of characteristics and salivary cortisol levels before and after bubble nCPAP application

Variables	Median cortisol level (range), ng/mL			
	Before bubble nCPAP application	P value	After bubble nCPAP application	P value
Gender				
Male	1.55 (0.10-12.00)	0.409*	6.8 (0.40-46.00)	0.740*
Female	1.65 (0.30-66.50)		6.5 (0.80-92.00)	
Mode of birth				
Vaginal	0.80 (0.20-6.90)	0.543*	12.40 (0.80-46.00)	0.787*
Cesarean section	2.10 (0.10-66.50)		3.90 (0.40-92.00)	
Specimen collection time				
Morning	1.55 (0.40-2.80)	0.673**	16.00 (2.10-46.00)	0.721**
Afternoon	0.80 (0.10-47.00)		6.50 (0.40-51.00)	
Night	2.10 (0.20-66.50)		8.15 (1.00-92.00)	

*Mann-Whitney test; **Kruskal Wallis test

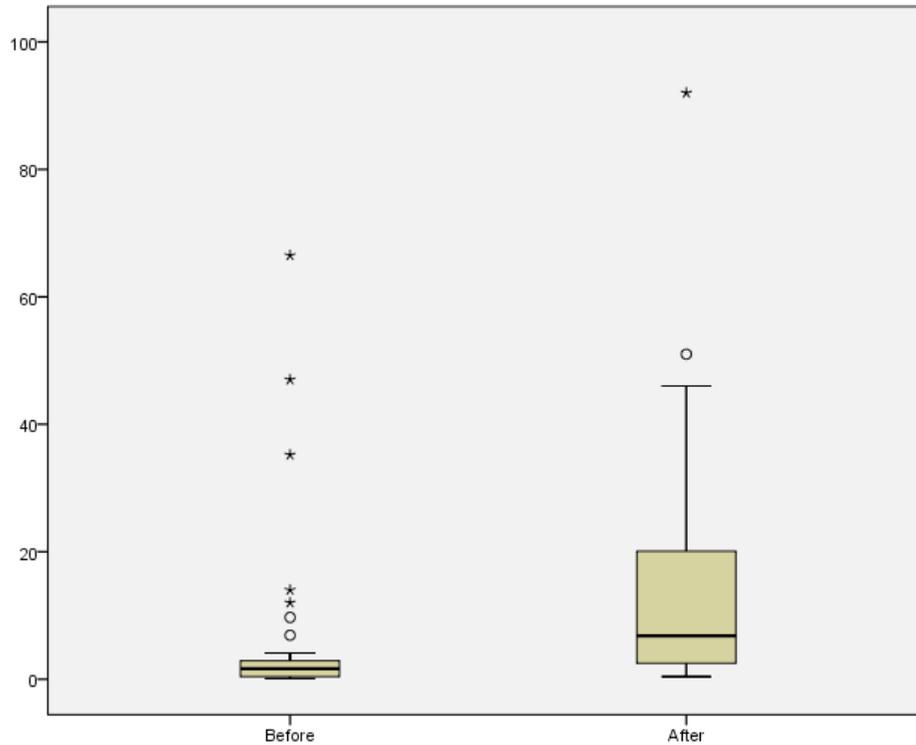


Figure 1. Cortisol levels prior to and after 30 minute bubble nCPAP application

Table 3. NIPS before and after the bubble nCPAP application

Pain scale	Bubble nCPAP application		P value
	Before	After	
No pain, n (%)	37 (97.4)	2 (5.3)	<0.001*
Mild to moderate pain, n (%)	1 (2.6)	30 (78.9)	
Severe pain, n (%)	0 (0)	6 (15.8)	

*Wilcoxon test

Nasal CPAP is one of the most common respiratory support treatments applied to neonates during NICU stays. According to several studies, placement of the nCPAP nasal prong causes moderate to severe pain.¹⁵ Salivary cortisol levels significantly increased after bubble nCPAP application, and this increase was not associated with gender, mode of delivery, birth weight, or specimen collection time. These changes in cortisol levels indicate that the procedure was painful, therefore, it may be beneficial to soothe infants during this procedure.

Sarapuk *et al.*¹⁶ studied 60 premature infants and found a relationship between the installation of a breathing apparatus, one of which was CPAP, and increased median salivary cortisol levels when compared to infants who did not use an assistive

breathing device [1.00 (range 0.38-2.44) g/dL] *vs.* [0.335 (range 0.156-1.236) g/dL; P=0.022]. In a previous study, preterm newborns treated in the NICU had various stress factors, one of which was the installation of nCPAP compared to controls. Namely, mean cortisol levels in 43 infants treated in the NICU [4.3151 (SD 2.6492) mg/dL] were higher than controls [1.0166 (SD 0.8300) ng/mL].¹⁰ Another study divided 90 premature infants with respiratory distress into 3 groups, those who used ventilators, nCPAP, and head boxes. The mean cortisol levels were 39.22 ng/mL for mechanical ventilation, 28.96 ng/mL for nCPAP, and 25.88 ng/mL for head box.¹⁷ In addition, Grunau *et al.* found a relationship between pain and cortisol levels in infants (P<0.05) in 76 full-term and premature infants.¹⁸ Our findings were

in agreement with other studies; the use of nCPAP in infants can cause stress and pain, as reflected by an increase salivary cortisol levels. Cortisol is by far the most frequently used biomarker for acute pain. It is generally considered a good biomarker because the concentration in saliva is not affected by salivary flow rate, and is resistant to degradation by enzymes and clotting.¹⁹ This study showed a significant increase in the number of subjects with mild to moderate pain levels as well as severe pain after the procedure, based on the NIPS scale. This finding was in agreement with a study which assessed infants with a pain scale and found that the level of moderate pain was highest after nCPAP installation.¹⁵ Similarly, the installation of nCPAP contributed to the highest percentage of moderate pain.²⁰

Oftentimes, severely ill infants survive and flourish in a highly stressful environment. In addition to painful stimuli, many painless stimuli, such as commotion and illumination intensity, can induce stress in hospitalized neonates. About 5 minutes after exposure to a stressor such as pain, the hypothalamus secretes corticotropin-releasing hormone, which stimulates the pituitary gland to release adrenocorticotrophic hormone. Adrenocorticotrophic hormone stimulates the cortex of the adrenal glands to secrete glucocorticoids, one of which is cortisol, a stress indicator hormone. Multiple studies have demonstrated that variations in cortisol excretion may result from painful or non-painful stress stimuli. Cortisol secretion is elevated as a result of psychological and physical stress responses to stimuli such as medical interventions, systemic diseases, hypoglycemia, and frigid exposure. Therefore, cortisol is considered to be a biological indicator of stress.^{10,20-22}

Infants experience variable degrees of discomfort or pain from mild to severe procedures in the NICU. Neonatal pain can induce stress. It is known that recurrent exposure to pain during the neonatal period has both short- and long-term consequences, including alterations in pain sensitivity and reactivity, as well as in the stress stimulation system in general. Stress caused by the NICU environment can increase plasma cortisol concentrations. Cortisol is a glucocorticoid that exerts physiological effects on carbohydrate, protein, and fatty acid metabolism and plays a crucial role in stress-related physiological responses. The evaluation of salivary cortisol concentrations is a

suitable method for assessing neonatal stress.^{13,23}

Our study had limitations, including the presence of other factors that may have affected infant salivary cortisol levels, such as sepsis, other invasive procedures, and noise, which can increase infant salivary cortisol levels. These factors were not accounted for in our design or analysis.

In conclusion, there is an increase in cortisol levels as a hormonal biomarker of stress or pain and pain scale score during administration of bubble nCPAP, indicating that this procedure causes pain in full-term neonates. Therefore, pain or stress management should accompany this procedure.

Conflict of interest

None declared.

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