

RESEARCH

Open Access



Effect of kangaroo mother care and white noise on physiological-stress parameters in heel lancing: randomized controlled study

Zila Özlem Kirbaş¹ , Elif Odabaşı Aktaş^{2*} , Bülent Bayraktar³ and Hava Özkan⁴

Abstract

Background Newborns are exposed to varying degrees of stressful interventions due to procedures such as heel lancing used in routine metabolic screenings. It is an examination of the effects of white noise and kangaroo care on some physiological parameters and stress markers (cortisol and glucose-regulated protein 78-GRP78) in heel lancing in newborns.

Methods Randomized controlled study was conducted at a gynecology service of a hospital between January and September 2023. 90 babies were divided into three groups: 30 babies in the Kangaroo Care Group (KCG), 30 babies in the White Music Group (WNG), and 30 babies in the Control Group (CG). All babies were randomly divided into groups. Stress parameters were measured by saliva collection method and physiological parameters by saturation device.

Results A statistically significant difference was determined between the total crying time, pulse and saturation values according to the groups ($p < 0.001$; $p = 0.001$). A statistically significant difference was determined between the mean values of cortisol and GRP78 measurements according to group and time interaction ($p < 0.001$). KCG was more effective in reducing total crying time and stabilizing pulse, saturation, salivary cortisol, GRP-78 values compared to WNG and CG.

Conclusion It was concluded that white noise and kangaroo care help reduce newborns' stress in the case of heel lancing.

Practical implications The practice of kangaroo care and the use of white noise methods may assist healthcare professionals as supportive methods in stress management during invasive procedures.

Trial Registration NCT06278441, registered on 19/02/2024.

Keywords Newborn, Glucose-regulated protein 78 (GRP78), Cortisol, Kangaroo care, White noise, Physiological parameters

*Correspondence:
Elif Odabaşı Aktaş
elifaktas@bayburt.edu.tr

¹Faculty of Health Sciences, Department of Nursing, Bayburt University, Bayburt, Türkiye

²Faculty of Health Sciences, Department of Midwifery Bayburt, Bayburt University, Bayburt, Türkiye

³Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Bayburt University, Bayburt, Türkiye

⁴Faculty of Health Sciences, Department of Midwifery, Ataturk University, Erzurum, Türkiye



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

Heel lancing from the newborn has an important role in the early diagnosis of diseases that can result in mental and developmental delay and even death within the scope of the newborn screening program. It is one of the basic preventive health services in nursing and midwifery practices carried out to save lives. However, newborns, who are often exposed to environmental stressors in the post-natal period, may experience pain and stress due to heel lancing, which is an invasive procedure [1]. For this reason, pharmacological and non-pharmacological methods reduce pain and stress in newborns during heel lancing. Non-pharmacological methods such as swaddling, massage, touching, white noise, and kangaroo care are a few of them. The calming effect of white noise applied to newborns in clinics and intensive care units has been supported by studies with its positive effects, such as feeding, starting breastfeeding, facilitating the transition to sleep, providing pain control, reducing blood pressure and stress, and reducing the length of hospital stay [2, 3]. Another non-pharmacological method used in interventions is kangaroo care. It has been observed that newborns who receive kangaroo care receive breast milk for longer, are less stressed, and are more comfortable. It has been proven that kangaroo care in newborns positively affects physiological and behavioral parameters during stressful invasive procedures and is effective on heart rate and crying duration [4].

In assessing the stress level in newborns, in addition to behavioral scales, it is possible to evaluate cortisol and glucose-regulated protein 78 (GRP78) levels in biological fluids such as blood and saliva. Cortisol is a hormone released from the adrenal cortex in response to stress. Taking samples to measure cortisol concentration in saliva is simple, non-invasive and stress-free [5]. Endoplasmic reticulum (ER) is an organelle that performs various functions such as lipid and protein synthesis, folding, and calcium homeostasis. Glucose-regulated protein 78 has a molecular weight of 78 kDa, known as HSP5, an endoplasmic reticulum stress marker [6–8]. Unfolded proteins, which tend to form protein clusters and accumulate hydrophobic amino acid residues within the protein, are associated with many diseases, especially cancer, diabetes, neurodegenerative and metabolic diseases. GRP78 plays a critical role in maintaining cell survival in stressful situations, such as decreased calcium in the ER and accumulation of non-glycosylated proteins [9, 10]. In the literature research, no studies examined the effects of white noise and kangaroo care on some stress markers (cortisol, GRP78) in newborns from whom heel lancing. Most research investigates the effect of kangaroo care and white noise music on relieving neonatal stress and pain [11–14]; however, very few studies compare the effectiveness of these methods and

their effect on the stress parameters cortisol. In fact, no study has been found evaluating the effectiveness of these methods regarding GRP78 [15–17]. We believe that with this study, healthcare professionals who are knowledgeable about the most effective methods for relieving stress during invasive procedures performed on newborns will provide better care. The current study is the first and serves as a reference. In this context, this study aimed to examine the effects of white noise and kangaroo care during heel lancing in newborns on some physiological parameters (pulse, saturation, crying duration) and stress markers (cortisol and GRP78).

Methods

Study design

This study, designed as a randomized controlled trial, was conducted in the gynecology department of a hospital in Türkiye between January and September 2023. In Türkiye, metabolic screenings for newborns are performed within the first 36–72 h after birth in hospitals, before discharge, and again on the 5th day at Family Health Centers [18]. We selected our sample group from the hospital setting to ensure accurate monitoring of physiological parameters and to reduce sample loss. Five neonatal nurses and three pediatricians staff the hospital's gynecology department. This trial is registered with ClinicalTrials.gov (NCT06278441, 19/02/2024).

Participants

The study population was selected from the obstetrics and gynecology service of the hospital, meeting specific inclusion criteria: gestational age of 37 weeks or more, birth weight over 2500 g, Apgar score of 8 or higher at both the 1st and 5th minutes, and no anatomical, physiological, or hearing-related congenital or acquired diseases. Infants on sedative medications and those with severe neonatal diseases were excluded. Based on reference study values, the minimum sample size required was calculated to be 57, with 95% confidence ($1-\alpha$), 95% test power ($1-\beta$), and an effect size of 57, $f=0.476$ [19]. To account for potential case losses, the biostatistician recommended increasing the sample size to 90 infants. These 90 newborns were then randomly assigned into three groups: White Noise Group (WNG), Kangaroo Care Group (KCG), and Control Group (CG), with 30 infants in each group. Randomization was conducted using a computer program (<http://www.randomizer.org>), and initial participants were assigned by a lottery method (WNG (1), KCG (2), CG (3)). Subsequent assignments were made based on the random numbers generated (Fig. 1: CONSORT Flow Diagram).

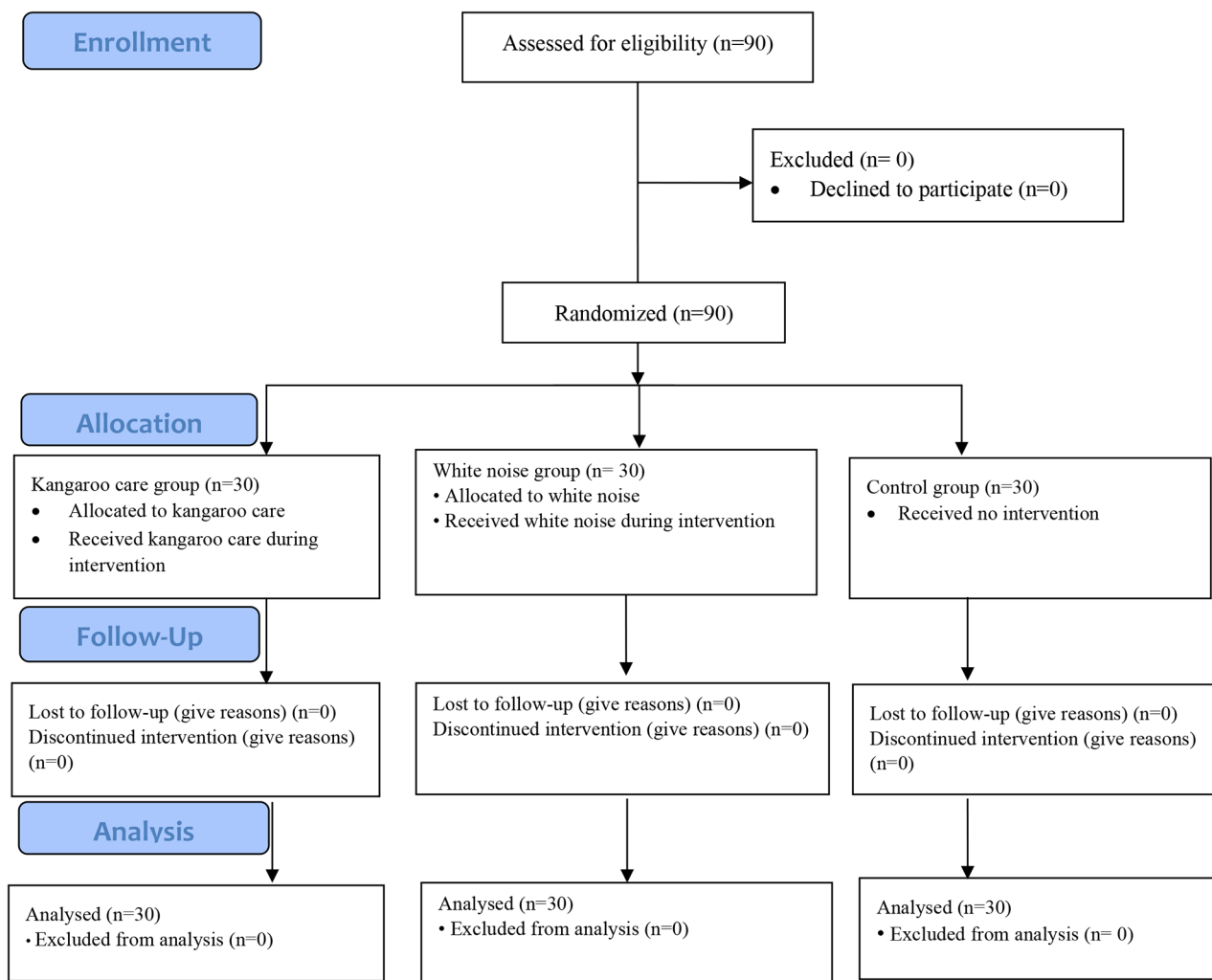


Fig. 1 Flow chart showing the recruitment of patients for the study

Data collection tools

Data were collected using a ‘Mother/Baby Information Form’, ‘Physiological and Stress Biomarkers Parameter Follow-up Form’, ‘Portable speaker’, ‘Decibel measurement device (Benetech GM1351/TURKIYE)’, ‘Orhan Osman’s Colic album - Don’t Let Your Baby Cry 2’.

‘Mother/Baby Information Form’ collected data on various demographics and health indicators. It included questions regarding the mother’s age, educational level, number of children, and mode of delivery. For the newborns, it gathered information on Apgar scores, gestational age, gender, birth weight, and length.

‘Physiological and Stress Biomarkers Parameter Follow-up Form’ was designed by the researchers to track physiological parameters (respiratory rate, oxygen saturation, and heart rate) and crying duration before, during, and after the procedure. Stress biomarkers (cortisol and GRP78) were measured using saliva samples collected before and after the procedure. Physiological parameters

were recorded one minute before the heel stick (when the infant was calm and stable), during the procedure, and one minute afterward. Oxygen saturation and heart rate were measured at the right wrist with a pulse oximeter, which is a commonly used non-invasive device in clinical settings (ST03694 Console Type Pulse Oximeter Device Nellcor Bedside). Crying duration during and after the procedure was timed with a stopwatch by the researcher. The stopwatch was started when the procedure began and stopped when the baby ceased crying. Saliva samples were collected using a Salimetrics SalivaBio Infant’s Swab (SIS), placed under the newborn’s tongue and around the mouth for 60–90 s, and then stored in a container. The form’s content was reviewed and refined based on feedback from three pediatric nursing experts.

‘Speaker’, “JBL” brand speaker was used for white noise for the newborns in the white noise group before, during and after the heel sting procedure.

'Sound Decibel Meter' was used to set the sound level on the white noise recorder below 50 dB [3, 20]. A BENE-TECH GM1351 model sound level meter was used to measure noise levels, which is a compact and accurate device suitable for this type of measurement. It is a Benetech brand, easy-to-use, sound level meter and decibel meter that can be attached to a tripod if desired via the screw hole on the bottom.

The song 'Don't Let Your Baby Cry 2' by Orhan Osman, featured in his album *Colic*, was utilized in this study. This track is included in previous literature and is part of the *Colic* album released by ON Music Productions. Orhan Osman created the album *Colic* by integrating the sounds of the womb from Dr. Harvey Karp's album *The Happiest Baby* with his own musical compositions. It should be added that this album falls under the category of white noise, which is known for its calming effects on infants [2, 21].

Interventions

Control Group (CG)

The heel lancing for the babies was conducted in a specially equipped intervention room. The room was well-lit, temperature-controlled, and equipped with all necessary medical supplies. A neonatal nurse was present to ensure the procedure was performed safely. The safety of the newborns was ensured throughout the procedure by continuously monitoring their vital signs and having a trained neonatal nurse present at all times. Heel lancing was performed by a single experienced nurse without support from other health professionals. The newborn was positioned comfortably on a padded stretcher before, during, and after the heel lancing. Heel blood sampling of newborns was done in the same intervention room to maintain a consistent and controlled environment. A saturation probe was attached to the right wrist of each baby to monitor its pulse and oxygen saturation levels before, during, and after the procedure. This group did not receive any intervention prior to the procedure. Saliva samples were collected immediately before the heel lance and 20 min afterward. To ensure reliability, the same nurse from the clinic performed all heel lance procedures. All interventions were carried out on the newborns between 7:00 and 10:00 in the morning to align with their natural circadian rhythms, which can influence stress and pain responses. Additionally, this time frame ensured the availability of necessary medical staff and minimized potential disturbances from routine hospital activities.

Kangaroo Care Group (KCG)

In the Kangaroo Care Group (KCG), each participant received kangaroo care during the blood collection procedure. The baby was dressed only in underwear and

a diaper. Following the guidelines of the World Health Organization (WHO) for kangaroo care [22], the mother unbuttoned her shirt and placed the baby on her bare chest. Throughout the blood collection, the baby was allowed to move their arms and legs freely. The mother supported the baby with one hand on their back and the other on their bottom. The ambient room temperature was carefully maintained between 24 and 26 degrees Celsius for comfort. The mother reclined in a semi-fowler position with her back propped by a pillow, ensuring the baby faced her to facilitate bonding. The baby remained wrapped in a blanket. Kangaroo care was started 5 min before the heel prick and continued throughout the procedure, ending 30 min after the procedure was completed. During this time, a saturation probe was affixed to the baby's right wrist to monitor pulse and oxygen saturation levels before, during, and after the blood collection. Crying durations were meticulously recorded using a stopwatch throughout the entire procedure. For all groups, the stopwatch was started at the moment the heel lancing began and stopped when the newborn stopped crying. Saliva samples were collected immediately before the heel lancing and again 20 min afterward for subsequent analysis.

White Noise Group (WNG)

After the newborns in this group were brought to the intervention room, white noise at 50 dB was played through a speaker positioned 1 m away. The sound level was calibrated to 50 dB using a decibel meter. Heel lancing was performed once the newborns had been exposed to the white noise and had calmed down. Physiological parameters of the newborns were monitored and recorded before, during, and after the heel lancing procedure. Crying times were tracked with a stopwatch and recorded on a follow-up form by the researchers. The stopwatch was started at the moment the heel prick began and stopped when the newborn stopped crying, not when the procedure was completed. Saliva samples were collected just before and 20 min after the procedure to measure the peak levels of stress biomarkers, as previous research indicates that these levels typically peak around 20 min post-stressor [23].

Saliva collection and measurement

To collect saliva samples, the research team used Salimetrics® SalivaBio Infant Swabs (Carlsbad, CA). They held one end of the swab and placed the other end against the newborn's buccal mucosa or sublingually to absorb saliva. Immediately after collection, the swabs were placed into labeled storage tubes [24]. The collected saliva samples were then transferred to Eppendorf tubes and centrifuged at 1500 g for 15 min in a refrigerated centrifuge (NF 1200R, NUVE, Ankara, TURKIYE) in the laboratory.

The samples were subsequently stored at -30°C until the analyses for cortisol hormone and GRP78 were performed. For the measurements, human-specific commercial kits were used: cortisol levels were measured using BT LAB kits (Cat.NoE1003Hu, CHINA), and GRP78 levels were measured using ELISA kits from SinoGeneClon (Product code: SG-20252, CHINA).

Ethical considerations

Bayburt University Ethics Committee approved the study (Decision no: (2022/334) and it is registered into ClinicalTrials.com (NCT06278441). In addition, administrative approval was obtained from the Provincial Health Directorate of the place where the study was conducted. An informed consent form was signed after being informed about the purpose and confidentiality of the data obtained. Because neonates were minor human participants, informed consent was obtained from a parent and/or legal guardian in our study. Neonates of parents and/or legal guardians who did not give written consent were not included in the study. The study protocol adheres to the ethical guidelines outlined in the Declaration of Helsinki.

Data analysis

Data were analyzed in IBM SPSS V23. Compliance with normal distribution was examined with skewness and kurtosis coefficients (± 2) [25]. Group and time used generalized Linear Models to compare data complying with normal distribution, and multiple comparisons were analyzed with the Tukey test. Group and time used a Robust ANOVA test to compare data that did not comply with normal distribution, and multiple comparisons were analyzed with Bonferroni Correction. Analysis results were presented as frequency (percentage) for categorical variables, mean $\pm$ standard deviation, and median (minimum–maximum) for quantitative variables. The significance value was taken as $p < 0.05$.

Results

A comparison of parameters by groups is given in detail in Table 1. A statistically significant difference exists between the median values of total crying time according to groups ($p < 0.001$). CG's total crying time median was 2.53, KCG 0.49, and WNG 1.17. No statistically significant difference exists between the distributions of other parameters according to groups ($p > 0.05$, Table 1).

Mean pulse rate was 131 before the procedure, 156 during the procedure, 132 after the procedure; mean saturation was 96 before the procedure, 90 during the procedure, 93 after the procedure. It is given in detail in Tables 2 and 3. A statistically significant difference was obtained between the median values of pulse measurements according to groups ($p < 0.001$). The median CG

pulse measurement was 138, KCG 132, and WNG 143. KCG differed from other groups. A statistically significant difference was obtained between the median values of pulse measurements according to time ($p < 0.001$). The median heart rate measurement before the procedure was 131; during the procedure, it was 156; and after the procedure, it was 132. The procedure orders differed from other times (Table 2).

A statistically significant difference was obtained between the median values of pulse measurements according to the group and time interaction ($p < 0.001$). While the highest median value of 175 was obtained during the CG procedure, the lowest median value of 126.5 was obtained after the CG procedure (Table 2).

A statistically significant difference was obtained between the median values of saturation measurements according to groups ($p = 0.001$). The median saturation measurement of KG was 93, KCG was 94, and WNG was 91. All groups differed from each other. A statistically significant difference was obtained between the median values of saturation measurements according to time ($p < 0.001$). The median saturation measurement before the procedure was 96; during the procedure, it was 90; and after the procedure, it was 93. All times it differed from each other (Table 3).

A statistically significant difference was obtained between the median values of saturation measurements according to the group and time interaction ($p < 0.001$). While the highest median value of 96 was obtained for both KCG and WNG before the procedure, the lowest median value of 90 was obtained for both CG and WNG during the procedure (Table 3).

There is a statistically significant difference between the mean values of cortisol measurement according to the groups. The difference was obtained ($p < 0.001$). The mean of CG cortisol measurement was 2.88, 2.38 in the KCG group, and 2.56 in the WNG. CG differed from other groups. A statistically significant difference was obtained between the average values of cortisol measurements according to time ($p = 0.049$). While the average cortisol measurement before the procedure was 2.68, it was accepted as 2.53 after the procedure (Table 4).

A statistically significant difference was obtained between the mean values of cortisol measurements according to the interaction of group and time ($p < 0.001$). The highest mean value of 3.09 was obtained after the CG procedure. In contrast, the lowest mean value of 2.08 was obtained after the KCG procedure (Table 4).

A statistically significant difference was obtained between the mean values of GRP78 measurement according to the groups ($p < 0.001$). The mean of CG GRP78 measurement was 0.50, 0.42 in the KCG group, and 0.44 in the WNG group. All groups differed from each other. A statistically significant difference was obtained between

Table 1 Comparison of parameters by groups

	Control Group	Kangaroo Care Group	White Noise Music Group	Total	Test Statistics	p
MOTHER						
Educational Status						
Literate	1 (3,3)	2 (6,7)	0 (0)	3 (3,3)	4.659	0.832*
Primary School	3 (10)	2 (6,7)	2 (6,7)	7 (7,8)		
Middle School	11 (36,7)	6 (20)	9 (30)	26 (28,9)		
High School	7 (23,3)	10 (33,3)	10 (33,3)	27 (30)		
University and above	8 (26,7)	10 (33,3)	9 (30)	27 (30)		
Number of children						
1	10 (33,3)	10 (33,3)	9 (30)	29 (32,2)	5.603	0.729*
2	9 (30)	14 (46,7)	12 (40)	35 (38,9)		
3	6 (20)	3 (10)	6 (20)	15 (16,7)		
4	5 (16,7)	2 (6,7)	3 (10)	10 (11,1)		
5	0 (0)	1 (3,3)	0 (0)	1 (1,1)		
Delivery Type						
Vaginal birth	19 (63,3)	14 (46,7)	17 (56,7)	50 (55,6)	1.710	0.425**
Cesarean birth	11 (36,7)	16 (53,3)	13 (43,3)	40 (44,4)		
Mother's Age	30,83 ± 5,82	30,33 ± 6,19	29,8 ± 6,21	30,32 ± 6,02	0.217	0.805 [‡]
	29,5 (21–42)	29,5 (21–42)	28 (21–42)	29 (21–42)		
BABY						
Baby gender						
Girl	21 (70)	15 (50)	17 (56,7)	53 (58,9)	2.570	0.277**
Boy	9 (30)	15 (50)	13 (43,3)	37 (41,1)		
Birth Weight (kg)	3324,67 ± 418,91	3333,5 ± 388,08	3227,33 ± 249,58	3295,17 ± 359,01	0.931	0.628 [‡]
	3305 (2495–4130)	3240 (2760–4050)	3245 (2890–4050)	3250 (2495–4130)		
Birth Height (cm)	50,13 ± 1,74	49,7 ± 1,18	50,2 ± 1,45	50,01 ± 1,47	1.02	0.365 [‡]
	50 (48–54)	50 (48–52)	50 (48–53)	50 (48–54)		
Total Crying Time	2,53 ± 0,96	0,59 ± 0,3	1,21 ± 0,37	1,44 ± 1,02	64.546	< 0.001
	2,53 (1,03–4,13) ^a	0,49 (0,28–1,3) ^b	1,17 (0,48–2,21) ^c	1,16 (0,28–4,13)		

*Fisher-Freeman-Halton test; **Pearson ki kare test [‡] One-Way Variance Analysis; [‡]Kruskal Wallis H test; ^{a–c}: There is no difference between groups with the same letter; Mean ± Standard Deviation; Median (Minimum - Maximum)

the average values of GRP78 measurements according to time ($p < 0.001$). While the mean GRP78 measurement before the procedure was 0.47, it was obtained as 0.44 after the procedure (Table 5).

A statistically significant difference was obtained between the mean values of GRP78 measurements according to the interaction of group and time ($p < 0.001$). The lowest average value of 0.37 was obtained after the KCG procedure, while the highest average value of 0.52 was obtained after the CG procedure (Table 5).

Discussion

In the study where we examined the effects of white noise and kangaroo care during heel lancing on some physiological parameters and stress markers of newborns, there was a significant difference in crying times between KCG WNG and CG. Crying time was highest in CG and lowest in KCG. Considering these results, kangaroo care and white noise calm the newborn and reduce crying times during heel lancing. In the study of Karakoç and Türker [11], it was reported that white noise reduced crying

duration and pain. In another study, KCG was found to have less crying duration than CG [12]. Crying is the most obvious reaction of newborns during painful and stressful procedures [11]. Studies conducted with healthy newborns found that those who received kangaroo care cried less than those who did not [26, 27]. In another study conducted with newborns, less crying time was found in heel lancing in the mother's lap compared to control and swaddling positions [28]. Together with our study, all these results were obtained from non-pharmacological methods. Studies show that white noise and kangaroo care reduce newborns' pain, stress, and crying times.

In the study, we observed that all groups showed an increase in pulse values during the procedure, with the highest increase noted in the CG, followed by WNG and KCG, respectively. However, KCG exhibited less decrease in saturation during the procedure, with rapid increases observed after the procedure compared to CG and WNG. Our findings indicate that KCG potentially enhances cardiovascular and respiratory stability in infants, which

Table 2 Descriptive statistics and multiple comparison results of heart rate measurements by group and time

	Time			Total
	Before procedure	During procedure	After procedure	
Mean±SD	133.41±11,98	157.43±16,52	134.19±12,89	
Median (Min-Max)	131 (112–170)	156 (126–194)	132 (112–171)	
Group				
Control Group	133.5 (120–169) ^{AB}	175 (138–194) ^C	126.5 (112–161) ^A	138 (112–194) ^a
Kangaroo Care Group	128.5 (112–170) ^A	141 (126–172) ^{BD}	128 (112–171) ^A	132 (112–172) ^b
White Noise Music Group	130.5 (116–145) ^A	162 (142–170) ^E	142 (128–154) ^D	143 (116–170) ^a
Total	131 (112–170) ^a	156 (126–194) ^b	132 (112–171) ^a	138 (112–194)
	Test statistics*		p	
Group	21.4		<0.001	
Time	102.3		<0.001	
Group*Time	59		<0.001	

^{a–b}: There is no difference between groups with the same letter; ^{A–E}: There is no difference between interactions with the same letter; Median (Minimum - Maximum); *Robust ANOVA; the median method was used as the comparison method

Table 3 Descriptive statistics and multiple comparison results of saturation measurements by group and time

	Time			Total
	Before procedure	During procedure	After procedure	
Mean±SD	95.37±2,42	89.98±2,4	93.28±2,38	
Median (Min-Max)	96 (90–99)	90 (80–95)	93 (89–98)	
Group				
Control Group	95 (91–99) ^{AB}	90 (80–95) ^{CD}	94 (91–97) ^A	93 (80–99) ^a
Kangaroo Care Group	96 (92–99) ^B	91,5 (87–95) ^E	95 (93–98) ^{AB}	94 (87–99) ^b
White Noise Music Group	96 (90–99) ^{AB}	90 (87–93) ^C	91 (89–94) ^{DE}	91 (87–99) ^c
Total	96 (90–99) ^a	90 (80–95) ^b	93 (89–98) ^c	93 (80–99)
	Test statistics*		p	
Group	6.92		0.001	
Time	55.16		<0.001	
Group*Time	58.63		<0.001	

^{a–c}: There is no difference between groups with the same letter; ^{A–E}: There is no difference between interactions with the same letter; Mean±Standard Deviation; Median (Minimum - Maximum); *Robust ANOVA; the median method was used as the comparison method

Table 4 Comparison of cortisol measurements by group and time

	Time		Total
	Before procedure	After procedure	
Group			
Control Group	2,66±0,52 ^B	3,09±0,63 ^A	2,88±0,61 ^a
Kangaroo Care Group	2,68±0,49 ^B	2,08±0,42 ^C	2,38±0,54 ^b
White Noise Music Group	2,71±0,54 ^{AB}	2,40±0,51 ^{BC}	2,56±0,54 ^b
Total	2,68±0,51	2,53±0,67	2,6±0,6
	F	p	PES
Group	13,84	<0,001	0,137
Time	3,93	0,049	0,022
Group*Time	15,54	<0,001	0,152

^{a–b}: There is no difference between groups with the same letter; ^{A–C}: There is no difference between interactions with the same letter; Mean±Standard Deviation; F: Variance Analysis test statistic; $R^2=26,49$; Adjusted $R^2=24,38$; PES: Partial Eta Squared

Table 5 Comparison of GRP78 measurements by group and time

	Time		Total
	Before procedure	After procedure	
Group			
Control Group	0,47±0,07 ^B	0,52±0,07 ^A	0,50±0,08 ^a
Kangaroo Care Group	0,47±0,05 ^B	0,37±0,05 ^D	0,42±0,07 ^b
White Noise Music Group	0,47±0,05 ^B	0,42±0,05 ^C	0,44±0,06 ^c
Total	0,47±0,06	0,44±0,09	0,45±0,08
	F	p	PES
Group	28,08	<0,001	0,244
Time	15,58	<0,001	0,082
Group*Time	23,06	<0,001	0,210

^{a–c}: There is no difference between groups with the same letter; ^{A–D}: There is no difference between interactions with the same letter; Mean±Standard Deviation; F: Variance Analysis test statistic; $R^2=40,38$; Adjusted $R^2=38,67$; PES: Partial Eta Squared

aligns with existing literature suggesting that kangaroo care reduces adverse stress effects [13, 26]. This may be attributed to mechanisms such as increased maternal-infant bonding, regulation of autonomic nervous system activity, and improved thermal regulation, all contributing to physiological stability [29].

In the study of Çaksak [21], when white noise, fetal position, and both applications were evaluated together during the procedure, it was found that using both white noise and fetal position together had the most significant calming effect on newborns. Karakoç and Türker [11] found that white noise significantly reduced heart rate and stabilized oxygen saturation levels in infants and white noise exposure during painful procedures like heel lancing resulted in lower heart rate variability and quicker recovery to baseline levels. These findings suggest that white noise can effectively mitigate stress responses in newborns by promoting autonomic stability and enhancing overall physiological resilience. Gokulu et

al. [14] found a statistically significant difference in pulse and saturation rates in healthy newborns before and after heel lancing. Kostandy and Ludington-Hoe [27] reported that kangaroo care caused a decrease in pulse and saturation rates during heel lancing in two-day-old healthy newborns. As a result, while our study differs from other studies in terms of physiological parameters, it is parallel to some. Considering the differences in working environments, newborns appear to have different physiological responses to pain and stress during the procedure.

In our study, a significant difference was obtained in salivary cortisol measurements according to the time interaction of the groups. After the procedure, the highest cortisol values were detected in CG, while the lowest value was seen in KCG. A study evaluating salivary cortisol concentrations for procedural pain monitoring in newborns found that salivary cortisol levels increased after blood collection [30]. Stoye et al. [15] reported in their study that cortisol levels in preterm newborns before vaccination were higher than morning cortisol levels and that this would cause a source of stress in the preterm newborn. In the context of our study, this finding suggests that newborns may experience heightened stress responses prior to medical procedures, such as heel lancing. Elevated cortisol levels indicate that the infants were already in a state of heightened arousal and stress before the procedure, which could potentially amplify their physiological responses during the heel lancing. This highlights the importance of interventions like kangaroo care and white noise in mitigating stress and stabilizing physiological parameters. By providing a calming environment and reducing stress, these interventions may help to lower cortisol levels and improve overall outcomes for newborns undergoing painful procedures. Gao et al. [16] examined the effectiveness and safety of combining sucrose with massage, music, non-nutritive sucking, and gentle human touch in the treatment of preterm infants with recurrent procedural pain. The study concluded that combined interventions remained effective and safe in reducing recurrent procedural pain and reduced cortisol levels at discharge. It also showed that it supports early neurobehavioral development in preterm infants. Although our study parallels the studies in the literature, we can say that KC, one of the interventions, is more effective in reducing stress than the other intervention. The 'developmental origins of health and disease' hypothesis proposes that environmental exposures early in life, during critical periods for development, can influence health throughout life [31]. However, a single study has never assessed HPA axis activity in newborns due to the high individual variability of salivary cortisol concentrations. We suggest that it should be based not on value but on repeated measurements. Our study showed that painful procedures performed on newborns increase the

stress level. It was observed that this increase was mainly in CG, not KCG or WNG, after the procedure.

GRP78 is an endoplasmic reticulum stress marker, an essential endoplasmic reticulum chaperone, and a vital regulator of the unfolded protein response. ER homeostasis is critical in maintaining cellular functions such as protein synthesis, folding, and secretion. Disruption of ER homeostasis causes ER stress and triggers the unfolded protein response, which can lead to apoptosis in cells under constant stress, especially cancer, diabetes, and many metabolic and neurodegenerative diseases. In addition to playing an essential role in early embryonic development, GRP78 plays a role in age-related diseases [6, 7]. In our current study, the average saliva GRP78 level in newborn groups was the lowest in the Kangaroo Care group and the highest in the control group. No studies on GRP78 as a stress marker in newborns have been found in the literature. Our current study is the first and serves as a reference in this sense. There are several different studies in the literature evaluating GRP78 [32, 33].

Strengths and limitations

The first limitation of the study is that the study was conducted in a single health center, and the results obtained cannot be generalized to this sample group. Another limitation is that no intervention was applied to the CG to evaluate the effectiveness of the study. Finally, one of our limitations is that not all researchers could be blinded due to the nature of the study. Specifically, the researchers who administered the interventions (kangaroo care and white noise) could not be blinded because they were directly involved in providing the interventions. However, the researchers who recorded the physiological parameters and analyzed the data were blinded to the group assignments to minimize bias. This limitation means that there may be some degree of observer bias in the administration of the interventions, although efforts were made to ensure objective data collection and analysis.

Implications for practice

Kangaroo care and white noise music techniques are effective, simple and safe methods to reduce stress during the heel lancing procedure of newborns. These interventions provide a good way for healthcare professionals to use during the heel lancing procedure. Among these interventions, kangaroo care application has a more positive effect on newborn physiological parameters, cortisol, and GRP78 levels compared to white noise. In our study, we found that white noise also had positive effects on crying time, pulse and saturation levels, and cortisol and GRP78 levels of newborns throughout heel lancing. However, kangaroo care was more effective in stabilizing these parameters and reducing stress in newborns. This

conclusion aligns with our findings that both interventions are beneficial, but kangaroo care provides greater overall physiological and biochemical stability.

Conclusion

As a result, white noise and kangaroo care during heel lancing had significant effects on various physiological parameters in newborns. Both interventions decreased crying duration and pulse levels while helping to maintain higher saturation levels. Specifically, kangaroo care was more effective in reducing cortisol and GRP78 levels compared to white noise. These findings suggest that while both interventions are beneficial in mitigating stress and stabilizing physiological parameters, kangaroo care provides a greater overall reduction in stress indicators and improved physiological stability in newborns. In examining the stress response in newborns, cortisol and GRP78 levels can be assessed, given their potential use as stress biomarkers and their importance for a better understanding of neonatal stress physiology. Additionally, due to the scarcity of available information and the fact that this study is the first study conducted in newborns, further experimental and prospective studies are needed to determine the clinical significance of the findings.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12887-024-05033-1>.

Supplementary Material 1

Supplementary Material 2

Author contributions

ZOK Conceptualization; Investigation; Writing - original draft; Funding acquisition; Methodology; Validation; Writing - review & editing; Visualization; Software; Formal analysis; Project administration. EOA Supervision; Resources; Writing - review & editing; Writing - original draft; Data curation. BB Software; Formal analysis; Project administration; Data curation; Supervision; Resources. HO Writing - original draft; Writing - review & editing.

Funding

This research received no external funding.

Data availability

The data sets used for this study are available from the corresponding author on reasonable request.

Declarations

Ethical considerations

Bayburt University Ethics Committee approved the study (Decision no: (2022/334) and it is registered into ClinicalTrials.com (NCT06278441). In addition, administrative approval was obtained from the Provincial Health Directorate of the place where the study was conducted. An informed consent form was signed after being informed about the purpose and confidentiality of the data obtained. Because neonates were minor human participants, informed consent was obtained from a parent and/or legal guardian in our study. Neonates of parents and/or legal guardians who did

not give written consent were not included in the study. The study protocol adheres to the ethical guidelines outlined in the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Conflict of interest

No conflict of interest is declared by the authors.

Received: 16 May 2024 / Accepted: 26 August 2024

Published online: 06 September 2024

References

1. Courtois E, Droutman S, Magny JF, et al. Epidemiology and neonatal pain management of heelsticks in intensive care units: Epipain 2, a prospective observational study. *Int J Nurs Stud*. 2016;59:79–88.
2. Akcan E. The Effect of Amniotic Fluid, Breast Milk and Lavender Smell on Pain During Heel Blood Collection in Newborns (thesis). Kayseri: Erciyes University. 2014.
3. İmseytoğlu D, Yıldız S. Music therapy in neonatal intensive care units. *Flornce Nightingale Nurs Magazine*. 2012;20(2):160–5.
4. Johnston C, Campbell-Yeo M, Disher T, et al. Skin-to-skin care for procedural pain in neonates. *Cochrane Database Syst Rev*. 2017;16:2.
5. Blair C, Berry DJ, Investigators FLP. Moderate within-person variability in cortisol is related to executive function in early childhood. *Psychoneuroendocrinology*. 2017;81:88–95.
6. Little E, Ramakrishnan M, Roy B, Gazit G, Lee AS. The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. *Crit Rev Eukaryot Gene Expr*. 1994;4(1):1–18.
7. Ma Y, Hendershot LM. Herp is dually regulated by both the endoplasmic reticulum stress-specific branch of the unfolded protein response and a branch that is shared with other cellular stress pathways. *J Biol Chem*. 2014;279(14):13792–9.
8. Wang M, Wey S, Zhang Y, Ye R, Lee AS. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. *Antioxid Redox Signal*. 2009;11(9):2307–16.
9. Misra UK, Deedwania R, Pizzo SV. Activation and cross-talk between akt, NF- κ B, and unfolded protein response signaling in 1-LN prostate cancer cells consequent to ligation of cell surface-associated GRP78. *J Biol Chem*. 2006;281(19):13694–707.
10. Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. *Cancer Res*. 2007;67(8):3496–9.
11. Karakoç A, Türker F. Effects of white noise and holding on pain perception in newborns. *Pain Manage Nurs*. 2014;15(4):864–70.
12. Karapınar Y. Comparison of the Effects of Skin-to-Skin Contact, Breastfeeding, and Sucrose-Soaked Pacifiers on Pain during Heel Blood Collection in Newborns. Gaziosmanpaşa University Institute of Health Sciences, Master's Thesis. 2019.
13. Özdel D, Sarı HY. Effects of the prone position and kangaroo care on gastric residual volume, vital signs and comfort in preterm infants. *Japan J Nurs Sci*. 2020;17(1):12287.
14. Gokulu G, Bilgen H, Özdemir H, et al. Comparative heel stick study showed that newborn infants who had undergone repeated painful procedures showed increased short-term pain responses. *Acta Paediatr*. 2016;105(11):520–5.
15. Stoye DQ, Boardman JP, Osmond C, et al. Saliva cortisol diurnal variation and stress responses in term and preterm infants. *Archives Disease Childhood-Fetal Neonatal Ed*. 2022;107(5):558–64.
16. Gao H, Xu G, Li F, et al. Effect of combined pharmacological, behavioral, and physical interventions for procedural pain on salivary cortisol and neurobehavioral development in preterm infants: a randomized controlled trial. *Pain*. 2021;162(1):253–62.
17. Howard C, Powell AS, Pavlidis E, et al. No effect of a musical intervention on stress response to venepuncture in a neonatal population. *Acta Paediatr*. 2020;109(3):511–7.

18. Kirbaş ZÖ, Odabaşı Aktaş E. Metabolic screenings in newborn and the importance of informing mothers. 3. Int Istanbul Curr Sci Res Congress Febr 8–9, 2023. Istanbul-TÜRKİYE.
19. Ünal T. The Effect of Mother's Voice and Father's Voice Played to Newborns During the Heel Blood Collection Procedure on Pain Level and Physiological Parameters. (Master's thesis) Düzce University. 2020.
20. World Health Organization (WHO). (1999). Guidelines for Community Noise. Noise sources and their measurement. Accessed Jan 17, 2024. <http://www.who.int/docstore/peh/noise/guidelines2.html>
21. Çakşak A. The effect of white noise, manual fetal positioning, and the combination of both applications on pain in term babies during heel blood collection. Eskişehir: Eskişehir Osmangazi University Health Sciences Institute, Department of Nursing; 2017.
22. World Health Organisation (WHO). Kangaroo mother care: A practical guide. Geneva: Department of Reproductive Health and Research. 2003. Accessed Jan 17, 2024. <https://www.who.int/publications/i/item/9241590351>
23. Kahraman A, Başbakkal Z. Yenidoğan Bebeklerin Stresini Değerlendirmede Bir Yöntem; Tükürük Kortizol Düzeyi. Balıkesir Sağlık Bilimleri Dergisi. 2017;6(3):136–41.
24. Salimetrics LLC. Collection Methods: SalivaBio Infant's Swab (SIS). 2018.
25. George D, Mallery M. SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update (10a ed.) Boston: Pearson. 2010.
26. Avcin E, Kucukoglu S. The effect of breastfeeding, kangaroo care, and facilitated tucking positioning in reducing the pain during heel stick in neonates. *J Pediatr Nurs*. 2021;61:410–6.
27. Kostandy RR, Ludington-Hoe SM. Kangaroo care (skin-to-skin) for clustered pain procedures: Case study. *World J Neurosci*. 2016;6(1):43.
28. Inal S, Aydın Yılmaz D, Erdim L. The effectiveness of swaddling and maternal holding applied during heel blood collection on pain level of healthy term newborns; randomized controlled trial. *Early Child Dev Care*. 2022;192(13):2066–77.
29. Sehgal A, Nitzan I, Jayawickreme N, Menahem S. Impact of skin-to-skin parent-infant care on preterm circulatory physiology. *J Pediatr*. 2020;222:91.7.e2.
30. Olszewska M, Pointinger-Tomasik S, Kwinta P. Assessment of salivary cortisol concentrations for procedural pain monitoring in newborns. *J Perinat Med*. 2023;51(4):564–72.
31. Lacagnina S. The developmental origins of health and disease (DOHaD). *Am J Lifestyle Med*. 2020;14(1):47–50.
32. Cirrik S, Esnafoglu E, Adiguzel O. Evaluation of plasma GRP78 levels in patients with autism spectrum disorder. *Dusunen Adam*. 2021;34(1):83–8.
33. Esnafoglu E, Cirrik S. Evaluation of plasma GRP78 levels as an indicator of endoplasmic reticulum stress in the patients with autism spectrum disorder. *Psychiatry Clin Psychopharmacol*. 2018;28:19–20.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.