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The effect of multi-sensory stimuli used during vaccination on pain and physiological parameters in infants: A randomized controlled trial

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ABSTRACT

Purpose: The study examine the effect of Multi-Sensory Stimulus Method (MSSM) during vaccination in infants on pain and physiological parameters.
Methods: The study was a randomized controlled trial between March and December 2023. A total of 96 infants aged 2–6 months were included, divided into 3 intervention and 1 control group. The MSSM was applied to the first intervention group by the nurse, and to the second intervention group by the mother. Third group infants were only breastfed without MSSM, while the control group (CG) received standard care. Data were collected via forms, pain scale, and physiological parameters. Pain, peak heart rate (HRP), and oxygen saturation (SpO_2) were evaluated at 5 points: 5 min before, 2 min before, immediately before and after vaccination, 2 min after, and 5 min after the vaccination. Process was video-recorded. Data analyzed with descriptive stats, ANOVA, Chi-Square, Bonferroni tests ($p < 0.05$).
Results: No significant differences among groups in terms of descriptive characteristics ($p > 0.05$). Significant differences found in total FLACC scores and SpO_2 values measured throughout the procedure, while no statistically significant difference was observed in HRP values ($p < 0.05$). MSSM by mother/nurse had lower FLACC scores than breastfeeding and control groups. Crying duration in the CG was longer than MSSM groups was applied by the mother or nurse.
Conclusion: MSSM applied by mothers and nurses during vaccination reduced pain, helped maintain SpO_2, and no effect on HRP.
Implications to practice: MSSM supports atraumatic and family-centered care practices, its use is recommended during painful procedures.
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Introduction

Vaccination programs are essential due to their ability to reduce the mortality and morbidity rates of preventable diseases (Zhou et al., 2024). According to the CDC's Work in Global Immunization 2024 vaccination report, it is estimated that approximately 4 million deaths are prevented each year worldwide through childhood immunization, and more than 50 million deaths could be prevented between 2021 and 2030 (Fast Facts on Global Immunization | Global Immunization | CDC, 2024). However, injections administered as part of routine immunization schedules are also a common cause of pain (Ouach et al., 2019). The fear of pain that develops during childhood may lead to vaccine avoidance behavior later in life (McLenon & Rogers, 2019). Considering the short- and long-term negative effects of vaccine-related pain, various non-pharmacological interventions have been studied to reduce pain during injections. These methods include oral sucrose administration (Gad et al., 2019), the use of Buzzy devices (Redfern et al., 2018), non-nutritive sucking, breastfeeding, and 50 % dextrose solution (Nieto García et al., 2019). These interventions have the potential to reduce not only pain but also procedural stress, fear of injections, and vaccine avoidance behaviors (Komaroff & Forest, 2020). However, most existing studies involve interventions performed solely by healthcare

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personnel, without maternal participation, and typically apply only a single technique (Nieto García et al., 2019; Redfern et al., 2018).

One of the non-pharmacological pain management techniques that has proven effective in reducing pain is the MSSM, also referred to as "multisensory stimulation" or "sensorial saturation" (Locatelli & Bellieni, 2018). Multi-sensory stimulation (MSS) involves the simultaneous use of several stimuli such as massage, sound, smell, and eye contact to activate a larger area of the brain, saturate sensory channels, and thus reduce the perception of painful stimuli (Zeraati et al., 2016). This approach is most effectively implemented by combining taste (oral administration of sweet solutions such as sucrose or glucose), speech (drawing the infant's attention through words), and touch (providing gentle massage) (Locatelli & Bellieni, 2018). The method is easy to learn and can be effectively implemented by the mother, pediatric provider, or nurse (Bellieni et al., 2007).

A review of the literature reveals that MSSM has been reported as an effective, practical, and cost-efficient approach in various contexts such as painful examinations in preterm newborns (Anand et al., 2011), heel-prick procedures (Bellieni et al., 2001), venipuncture in infants (Fitri et al., 2020), and reducing stress during bathing in preterm infants (Soltani et al., 2022; Anand et al., 2011; Behnam Vashani et al., 2015; Bellieni et al., 2001; Fitri et al., 2020; Soltani et al., 2022). However, studies investigating the effect of maternal involvement and the use of MSS in pain management during vaccination procedures remain limited (Karimi et al., 2022; Lan et al., 2021; Shayani & Marães, 2023; Wu et al., 2022). Moreover, no study in the literature has examined the use of breast milk as the sweet component in MSS or compared maternal versus healthcare personnel administration.

This study aimed to investigate the effect of MSSM to infants during vaccination on pain and physiological parameters.

Material and methods

Study design

This study was conducted as a randomized controlled experimental trial to investigate the effect of MSS on pain and physiological parameters in infants during vaccination.

Hypothesis

Infants receiving MSS during vaccination will have lower levels of pain, HRP, and crying duration, and higher SpO_2 compared to those in the CG and Breastfeeding Group (BG). Furthermore, in the group where the MSS is administered by the mother, the FLACC pain score will be lower, SpO_2 levels higher, HRP values lower, and crying duration shorter compared to the group where the nurse administers the MSS.

Participants

Based on a similar study in the literature, a minimum sample size of 80 was calculated with an effect size of d = 0.71, 99 % power, and a 1 % significance level (Sayed et al., 2018). To prevent potential loss of participants, a 20 % increase was targeted, and 24 infants were included in each group, totaling 96 infants. Each infant was included in the study only once, during a single vaccination session at either 2, 4, or 6 months of age (Figs 1).

Inclusion criteria

Infants without any diagnosed health issues (Infants without health issues were defined as those without chronic or congenital conditions, neurological, cardiac, respiratory, or acute illnesses; transient conditions such as resolved jaundice or neonatal hypoglycemia were not considered exclusion criteria), born between 38 and 42 weeks of gestation, included in the Ministry of Health's routine vaccination schedule, breastfed by their mothers, and scheduled for the combination vaccine (DTaP-IPV-Hib).

Exclusion criteria

Infants who had received analgesics, muscle relaxants, or sedatives within the past 4 h, and those who could not complete the procedure due to protocol deviations (e.g., interruption of the intervention due to excessive restlessness).

Randomization

Randomization was performed using block randomization according to vaccination ages at 2, 4, and 6 months (separate blocks for each age group; www.random.org was used).

Data collection tools

For data collection, the Infant and Mother Identification Information Form and the Multiple Sensory Stimulus Application Method form were used. To collect pain-related data, the Infant Follow-up Form, FLACC Pain Scale, a camera, and a tripod were utilized. For the measurement of physiological parameters, a pulse oximeter, weighing scale, length measuring tape, and head circumference measuring tape were used. Crying duration was assessed from video recordings using a stopwatch and recorded in seconds. To accurately assess infants' pain levels and crying durations before, during, and after vaccination, and to evaluate inter-rater reliability, recordings were made using an "Apple" brand smartphone (Apple iPhone 13256GB).

Infant and mother identification information form

This form, prepared in accordanc e with the literature (Bekar & Efe, 2022; Bellieni, 2012; Kassab et al., 2020), consisted of 15 multiple-choice and open-ended questions including information such as the infant's age, body weight, height, head circumference, and gender.

Infant follow-up form

This form was developed by reviewing the relevant literature (Bekar & Efe, 2022; Bellieni, 2012; Kassab et al., 2020) and included sections for recording the infant's SpO_2 level, heart rate (HR), total FLACC pain scale score, and duration of crying.

FLACC pain scale

The FLACC Pain Scale was developed by Voepel-Lewis et al. (2010). This unidimensional scale evaluates five behavioral indicators (facial expression, leg movement, activity level, crying, and consolability). The total score ranges from 0 to 10, with higher scores indicating higher levels of pain (Voepel-Lewis et al., 2010). In the study conducted by Şenaylı et al., pain in children aged 1 month to 9 years was assessed, and the Turkish version of the scale was deemed appropriate for use. The Cronbach's alpha reliability coefficient of the scale ranges between 0.83 and 0.86 (Şenaylı et al., 2006).

Multiple sensory stimulus application method

It has been observed in the literature that there is no standardized duration or combination for the application of MSSM (Karimi et al., 2022; Lan et al., 2021; Zeraati et al., 2016). In this study, the MSSM applied to the infants consisted of four sensory stimuli, as follows:

- **1. Breastfeeding (Tactile Sense):** Initiated by the mother 20 min before the vaccination and ended just before the injection.

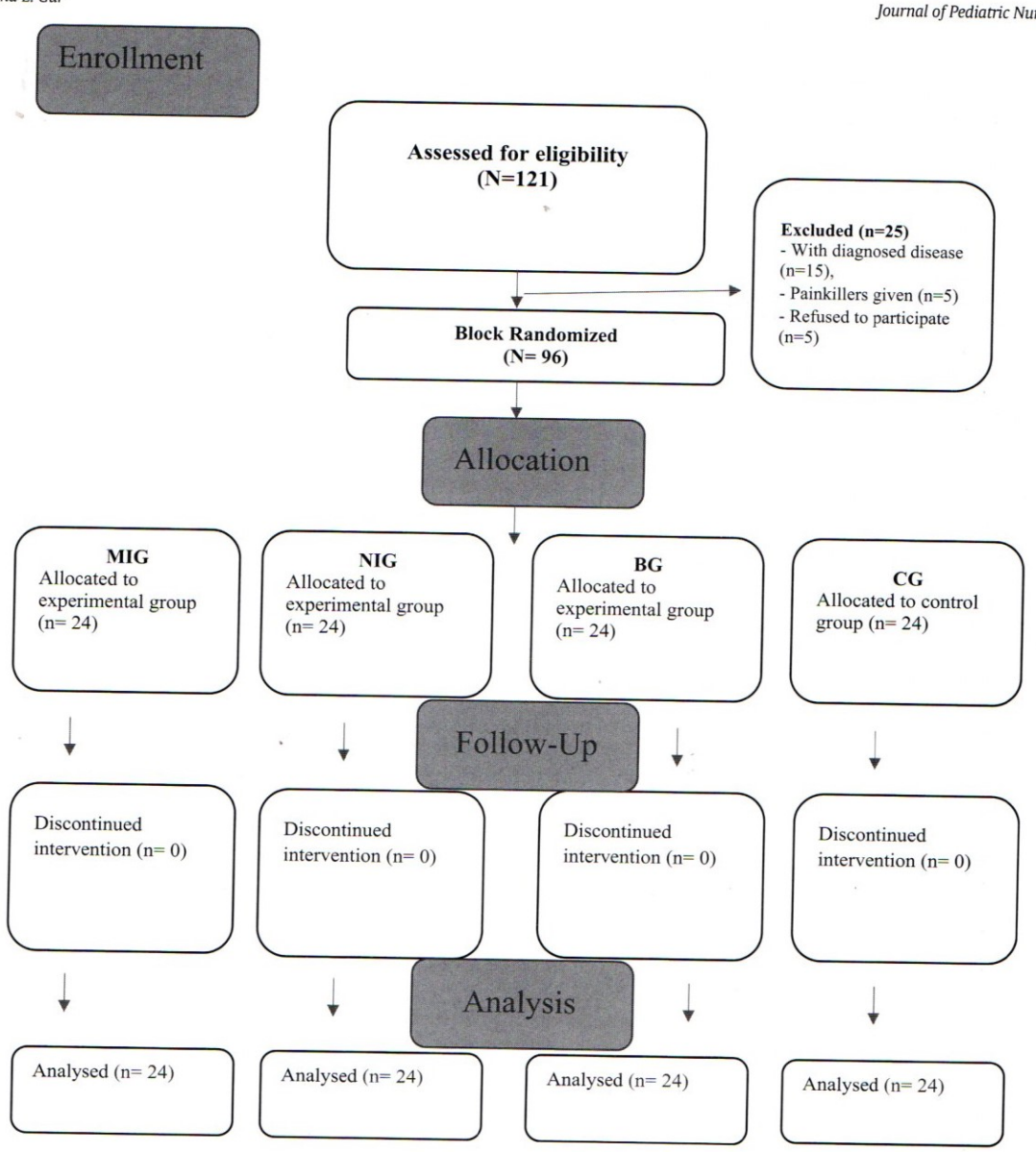


Fig. 1. CONSORT Diagram (Grant et al., 2018).

- **2. Touch – Gentle Massage (Tactile Sense):** Started 5 min before the injection and continued until 5 min after. The practitioner supported the infant's back and buttocks while gently caressing the face.
- **3. Talking – Sound (Auditory Sense):** Implemented through soft-toned speech, lullabies, or songs addressed to the infant (Bahman Bijari et al., 2012; Fitri et al., 2020; Im & Kim, 2009). Auditory stimulus was provided by mothers through singing lullabies or speaking softly to their infants. No specific song standard was applied. The sound level was kept within the natural range of parent–infant communication under routine clinical conditions, and no decibel measurements were taken with a device.
- **4. Eye Contact (Visual Sense):** Continuous eye contact was maintained between the mother and the infant throughout the procedure.

The three stimuli other than breastfeeding were administered for a total of 10 min. The entire application lasted 25 min, and the application form was provided to the mothers in advance for instructional purposes (Fig 2).

Application protocol

Preparation before application. Parents were asked to complete the "Infant and Mother Identification Information Form". The infants' body weight, height, and head circumference were measured and recorded. The mothers assigned to perform the MSSM were instructed on the method verbally and practically, asked to repeat the procedure, and corrections were made if necessary. Once the mother confirmed she was ready, a pulse oximeter was placed on the infant's right wrist, and video recording was initiated.

During implementation. Intervention Groups:

- MIG (Mother Intervention Group): MSSM components were applied by the mother. Guidance was provided when necessary.
- NIG (Nurse Intervention Group): Only breastfeeding was performed by the mother; the other three stimuli were applied by the nurse.
- BG: Infants were breastfed in their mother's arms for 20 min. No MSSM was applied.

Stimulus	Start-End Time	Duration (min)	Description
1. Breastfeeding (Tactile)	20 minutes before vaccination – just before vaccination	Pre-vaccination duration	The infant is calmed during breastfeeding.
2. Touch – Gentle Massage	5 minutes before vaccination – 5 minutes after vaccination	10 minutes	The infant's back and buttocks are supported, and the face is gently caressed.
3. Talking – Sound			The caregiver speaks to the infant in a soft tone and sings lullabies or songs to soothe them.
4. Eye Contact			Eye contact is established with the infant within the field of vision.
			After vaccination, eye contact, gentle touch, and verbal interaction are continued.

Fig. 2. Multisensory stimulus application process.

- CG: Infants were soothed in their mother's arms before vaccination. Minimal touching was allowed if necessary.

In all groups, SpO_2 and HRP values were measured 5 min, 2 min, and immediately before vaccination.

After the application. SpO_2 and HRP values were also recorded at the 2nd and 5th minutes post-vaccination. FLACC Pain Scale scoring was evaluated by two independent observers based on video recordings at 5 min, 2 min, and immediately before, as well as immediately after, 2 min after, and 5 min after vaccination. The duration of crying was measured using a stopwatch from the video recordings. Ethically, mothers in the BG and CG groups were given the MSSM form to apply during future vaccinations.

Procedure

All procedures, except anthropometric measurements, were conducted in the vaccination room. The interventions were carried out by the nurse researcher who was familiar with the method and an author of the study. One of the observers evaluating the FLACC scale had academic experience, while the other was an experienced field nurse. In infants receiving multiple vaccines, the procedure was implemented during the administration of the DTPa-IPV-Hib vaccine; the other vaccines were administered after the procedure was completed. Infants were placed on a stretcher immediately before the injection, and all vaccines were administered to the right vastus lateralis muscle by the same nurse.

Data analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (number, percentage, mean, median, standard deviation) were used in the analysis. The Shapiro-Wilk test was employed to assess the normality of the data; parametric tests were used for normally distributed data. The Chi-Square test was used for comparisons of categorical variables between groups, and One-Way ANOVA was used for numerical variables. Two-Way Repeated Measures ANOVA was employed to evaluate within-group and between-group changes over time. If a significant difference was found in more than two dependent measurements, Bonferroni-adjusted multiple comparison tests were performed to identify the time interval of the difference. A significance level of $p < 0.05$ was accepted for all analyses.

Ethical and legal aspects of the study

For this study, ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee (Date: 15.03.2023, Decision No: 2023/14), and institutional permission was granted by the hospital where data collection took place (Date: 14.04.2023, Reference No: E-19274766-199-657,073). Prior to data collection, verbal information about the study was provided to the parents of all infants who participated in the research, and written consent was obtained using the Informed Voluntary Consent Form. Permission to use the FLACC pain scale was obtained via email from the original developer. The study was registered on ClinicalTrials.gov with the number NCT06291519.

Results

Comparison of descriptional characteristics

According to the results in Table 1, no significant difference was found between the groups in terms of the descriptive characteristics of the infants and parents ($p > 0.05$). The groups were found to be similar in terms of the infants' gestational age, gender, mode of delivery, body weight, height, head circumference, as well as the parents' age, number of children, family type, educational status, income level, and the infant's primary caregiver.

Assessment of the distribution and comparison of pain levels, evaluation of inter-observer agreement

According to Table 2 and Fig. 3, the total FLACC Pain Scale scores measured during the procedure showed a statistically significant difference between the groups ($p < 0.05$). The CG group's FLACC scores at the 2nd minute before the procedure, just before the procedure, and at the 2nd and 5th minutes after the procedure were higher than those of the MIG group. The CG group also had higher FLACC scores at all time points compared to the NIG group. The BG group's FLACC score at the 2nd minute after the procedure was higher than that of the NIG group.

Within-group comparisons

In the MIG, NIG, and BG groups, the FLACC scores measured during the procedure showed significant changes over time ($p < 0.05$): In all three groups, the FLACC score measured immediately after the procedure was significantly higher than the scores at all other time points. Although the FLACC score at the 2nd minute after the procedure remained elevated, it was lower than the score measured immediately after the procedure.

According to the data presented in Table 3, there was a statistically significant, positive, and strong correlation between the two observers' ratings at all time points ($p < 0.05$). The agreement was evaluated using the Intraclass Correlation Coefficient (ICC), and the inter-observer reliability was found to be high (e.g., ICC = 0.95, $p < 0.001$).

Comparison and distribution of findings related to physiological parameters

SpO_2 values (between-group comparison)

According to Table 4 and Fig. 4, the SpO_2 values measured during the procedure showed significant differences between the groups

Table 1
Distribution and Comparison of Infants' and Parents' Descriptive Characteristics ($n = 96$).

Variables	MIG	NIG	BG	CG	Test Value/*p*
	n %	n %	n %	n %	
Baby Gestational Age					
38	16 66.7	16 66.7	15 62.5	19 79.2	4.429**/*0.942*
39	2 8.3	4 16.7	3 12.5	2 8.3	
40	5 20.8	4 16.7	5 20.8	3 12.5	
41	1 4.2	0 0.0	1 4.2	0 0.0	
Age (Month)					
2	8 33.3	8 33.3	8 33.3	8 33.3	0.000**/*1.000*
4	8 33.3	8 33.3	8 33.3	8 33.3	
6	8 33.3	8 33.3	8 33.3	8 33.3	
Gender					
Girl	15 62.5	13 54.2	13 54.2	10 41.7	2.133**/*0.601*
Boy	9 37.5	11 45.8	11 45.8	14 58.3	
Mode of Birth					
Caesarean s.	21 87.5	19 79.2	21 87.5	21 87.5	1.023**/*0.884*
Normal	3 12.5	5 20.8	3 12.5	3 12.5	
Number of Child					
One	11 45.8	17 70.8	7 29.2	9 37.5	
Two	7 29.2	5 20.8	10 41.7	7 29.2	11.164**/*0.210*
Three	5 20.8	2 8.3	5 20.8	7 29.2	
Four	1 4.2	0 0.0	2 8.3	1 4.2	
Mother Education					
Primary	7 29.2	3 12.5	8 33.3	8 33.3	
High School	10 41.7	8 33.3	7 29.2	8 33.3	5.343**/*0.498*
University	7 29.2	13 54.2	9 37.5	8 33.3	
Father Education					
Primary	8 33.3	3 12.5	6 25.0	7 29.2	
High School	8 33.3	5 20.8	6 25.0	6 25.0	5.881**/*0.449*
University	8 33.3	16 66.7	12 50.0	11 45.8	
Income Status					
Income<Expense	1 4.2	1 4.2	3 12.5	0 0.0	
Income = Expense	22 91.7	21 87.5	19 79.2	20 83.3	5.236**/*0.510*
Income>Expense	1 4.2	2 8.3	2 8.3	4 16.7	

Variables	MIG	NIG	BG	CG	Test Value *p* Bonferroni[1]
	$\bar{x}\pm$ SS	$\bar{x}\pm$ SS	$\bar{x}\pm$ SS	$\bar{x}\pm$ SS	
Baby's Weight (gr)	6523.75 ± 1294.49	6135.42 ± 1122.44	6205.83 ± 1449.18	6285.42 ± 1117.82	0.436*** *0.728*
Baby's Height (cm)	61.71 ± 4.91	59.04 ± 3.78	59.62 ± 4.45	60.75 ± 4.68	1.686*** *0.176*
Head Circumference (cm)	40.92 ± 2.02	40.00 ± 1.84	40.17 ± 1.90	40.42 ± 2.12	0.982*** *0.405*
Mother Age	29.96 ± 5.57	30.25 ± 5.28	31.96 ± 4.92	32.12 ± 6.06	1.018*** *0.388*
Father Age	33.29 ± 5.36	33.42 ± 7.33	34.58 ± 7.62	37.58 ± 8.44	1.806*** *0.152*
How long the baby breastfed before vaccination (min)	0.00 ± 0.00	5.00 ± 0.00	0.00 ± 0.00	87.92 ± 34.64	**149.25***** **0.000*** **(4 > 1, 2, 3)**

*p < 0.05, **Chi-square analysis or Fisher's Exact test, *One-way analysis of variance (ANOVA).
Bonferroni[1]: (1 = MIG; 2 = NIG; 3 = BG; 4 = CG).

($p < 0.05$). In detail: The NIG group had significantly higher SpO_2 values than the CG group at the 2nd minute before the procedure and just before the procedure. The MIG group had higher SpO_2 values than the CG group immediately after the procedure and at the 2nd minute after the procedure. Both the MIG and NIG groups had significantly higher SpO_2 values than the CG group at the 5th minute after the procedure.

Looking at within-group comparisons; In the MIG group, the SpO_2 value immediately after the procedure was significantly lower than at all other time points ($p < 0.05$). In the NIG group, the SpO_2 values at the 2nd minute before and immediately after the procedure were lower than the other measurements ($p < 0.05$). In the BG group, the lowest SpO_2 value was recorded immediately after the procedure, and the 2nd minute after the procedure was also lower than some other time points ($p < 0.05$). In the CG group, the SpO_2 values before the procedure were lower, the lowest point was immediately after the procedure, and the subsequent measurements were significantly higher ($p < 0.05$).

Heart Rate Peak (HRP) values (between-group comparison)

According to Table 5, there was no statistically significant difference between the groups in terms of the HRP values measured during the procedure ($p > 0.05$). This finding indicates that Hypothesis 3 (difference in HRP values between groups) was not supported.

When the within-group changes in HRP values during the procedure were analyzed, statistically significant differences were found in all groups over time ($p < 0.05$). In the MIG group, the HRP value measured immediately after the procedure was significantly higher than the value at the 5th minute before the procedure. In the NIG group, the HRP values measured immediately after the procedure and at the 2nd minute after the procedure were higher than all pre-procedure measurements.

Table 2
Distribution and Comparison of FLACC Pain Scale Total Score Mean Values ($n = 96$).

Time of Measurement	Groups				Test Value p	Bonferroni[2]
	MIG	NIG	BG	CG		
	$\bar{x}\pm$ SS *Med*	$\bar{x}\pm$ SS *Med*	$\bar{x}\pm$ SS *Med*	$\bar{x}\pm$ SS *Med*		
Bp 5. min	0.08 ± 0.28	0.00 ± 0.00	0.17 ± 0.38	0.29 ± 0.46	3.376**	**4 > 2**
	0.00	*0.00*	*0.00*	*0.00*	***0.022****	
Bp 2. min	0.04 ± 0.20	0.00±0.00	0.17 ± 0.38	0.29 ± 0.46	4.144**	**4 > 1, 2**
	0.00	*0.00*	*0.00*	*0.00*	***0.008****	
Ibp	0.04 ± 0.20	0.00 ± 0.00	0.17 ± 0.38	0.38 ± 0.49	6.314**	**4 > 1, 2**
	0.00	*0.00*	*0.00*	*0.00*	***0.001****	
Iap	6.04 ± 1.55	6.17 ± 1.40	6.63 ± 1.35	6.96 ± 1.04	2.364**	
	5.50	*6.50*	*7.00*	*7.00*	*0.076*	
Ap 2. min	1.25 ± 1.03	1.13 ± 0.99	2.08 ± 1.06	2.79 ± 1.35	11.664**	**4 > 1,2**
	1.00	*1.00*	*2.00*	*3.00*	***0.000****	**3 > 2,**
Ap 5. min	0.42 ± 0.65	0.38 ± 0.58	0.58 ± 0.78	1.04 ± 0.81	4.448**	**4 > 1, 2**
	0.00	*0.00*	*0.00*	*1.00*	***0.006****	
Test Value	2727.199***	320.610***	358.591***	367.263***		
p	***0.000****	***0.000****	***0.000****	***0.000****		
Bonferroni[1]	**d > a, b, c, e,f e > a, b, c, f**	**d > a, b, c, e,f e > a, b, c, f**	**d > a, b, c, e,f, e > a, b, c, f**	**d > a, b, c, e, f, e > a, b, c, f, f > a, b, c**		

$p < 0.05$, **One-way analysis of variance (ANOVA), ***Repeated measures analysis of variance (ANOVA).
Bonferroni[1]: a = Bp 5. min; b = Bp 2. min; c = Ibp); d = Iap e = Ap 2.min.; f = Ap 5.min,
Bonferroni[2]: 1 = MIG; 2 = NIG; 3 = BG; 4 = CG.
Pp: Pre-procedure, Ibp: Immediately before the procedure, Iap: Immediately after the procedure, Ap: After the procedure.
* Bonferroni was applied to find the group that made a difference in all groups.

Additionally, the HRP value at the 5th minute after the procedure was higher than the value at the 2nd minute before the procedure; the HRP value immediately after the procedure was higher than the value at the 5th minute after the procedure. In both the BG and CG groups, the HRP values measured immediately after the procedure were higher than all other time points.

Distribution and comparison of crying times

According to Table 6, there was a statistically significant difference in crying times among the groups ($p < 0.05$). Infants in the CG cried significantly longer after vaccination than those in the MIG and NIG ($p < 0.05$). Although the BG showed a tendency toward shorter crying time, the difference was not statistically significant compared to the CG.

Discussion

This study demonstrated that MSSM reduced procedural pain, increased SpO_2, and shortened crying durationcompared to the control and exclusive breastfeeding groups. There was no significant difference in pain between mother and nurse administration; a lower score tendency was observed in mother administration. Heart rate didn't differ significantlyacross groups.

Discussion of findings on the effect of MSSM on infant pain

In this study, pain scores in the groups receiving MSSM were found to be significantly lower than in the control group. In particular, the effect obtained by adding breast milk as a taste stimulus to MSSM is consistent with previous literature. Fitri et al. (2020) reported that the MSSM + sucrose and MSSM + breast milk groups were more effective than sucrose alone, with no difference between the two MSSM groups. In our study, pain scores were also lower in the MSSM + breast milk groups compared to the control group. This finding supports the pain-reducing effect of using breast milk as a taste and smell stimulus. Indeed, the literature predominantly reports the use of solutions such as sucrose, saccharose, glucose, and dextrose as taste stimuli (Fitri et al., 2020; Nieto García et al., 2019; Zeraati et al., 2015). However, breast

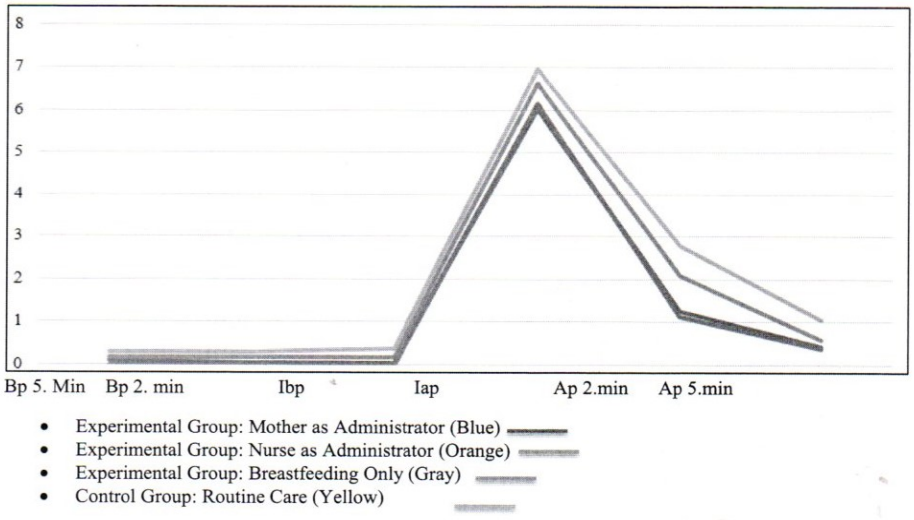


Fig. 3. Differences in FLACC Pain Scale Total Scores During Procedure Phases.

Table 3
Inter-Observer Agreement of Total FLACC Pain Scale Scores.

	1.Gözlemci	2.Gözlemci	r** / p*	ICC
	$\bar{x}\pm$ SS Med	$\bar{x}\pm$ **SS** Med		
Bp 5. min	0.00 ± 0.15 *0.35*	0.00 ± 0.14 *0.34*	0.958/0.000	0.978
Bp 2. min	0.00 ± 0.14 *0.34*	0.00 ± 0.13 *0.33*	0.955/0.000	0.977
Ibp	0.00 ± 0.15 *0.35*	0.00 ± 0.15 *0.35*	0.916/0.000	0.956
Iap	7.00 ± 6.46 *1.36*	7.00 ± 6.45 *1.38*	0.997/0.000	0.999
Ap 2. min	2.00 ± 1.82 *1.30*	2.00 ± 1.81 1.29	0.997/0.000	0.998
Ap 5. min	0.00 ± 0.60 *0.75*	0.00 ± 0.60 *0.75*	0.981/0.000	0.990

*p < 0.05, **Pearson korelasyon, ICC: Intraclass Correlation Coefficient.

milk is recommended as a non-pharmacological method and has been found to be more effective than sucrose in some studies (Fitri et al., 2020; Lan et al., 2021). In our study, the use of breastfeeding as a taste stimulus made MSSM completely non-pharmacological. These findings support H1.

In the current study, the pain scores in the breastfeeding group (BG) were significantly lower than in the control group. Nieto García et al. (2019) demonstrated that breastfeeding is more effective than non-nutritive sucking and dextrose + non-nutritive sucking; Lan et al. (2021) reported that adding breastfeeding as a taste and smell stimulus reduces pain. Our findings are consistent with these studies. The effect of breastfeeding can be explained by the infant's direct exposure to the taste and smell of breast milk. The increase in beta-endorphin levels by tryptophan in breast milk, as a precursor to melatonin, may be a mechanism that suppresses nociception (Fitri et al., 2020). The decrease in pain scores with breastfeeding in our study is consistent with the literature and supports H1.

In the present study wefound no statistically significant difference between MSSM and breastfeeding groups. This finding differs from the results of Karimi et al. (2022). While Karimi et al. found MSSM to be more effective than breastfeeding, our study found no significant difference between the two methods. This may be due to the difference in the stimuli used. Karimi et al. used dextrose as a taste stimulus and lavender oil as an odour stimulus, whereas our study used direct breast milk and natural maternal odour. Furthermore, MSSM was applied for a longer duration in our study (starting 5 min before the procedure and continuing for 5 min afterwards). These differences may explain the inconsistency in the findings. Accordingly, our H2 hypothesis was not supported due to the lack of difference in effectiveness between MSSM and breastfeeding.

In this study, there was no statistical difference between the MSSM application performed by the mother and the nurse. This finding is consistent with the results of Bellieni et al. (2007) and Zeraati et al. (2015). However, in our study, pain scores tended to be lower in the MSSM group applied by the mother. Scatliffe et al. report that the mother's presence and physical contact trigger oxytocin release, thereby soothing the infant (Scatliffe et al., 2019). Accordingly, the absence of a statistically significant difference does not fully support H5. However, the lower scores in the mother-administered group may be clinically meaningful.

In our study, MSSM was applied not only before and during the procedure but also for 5 min afterwards. This extended application reduced pain scores more rapidly and effectively. In contrast, in the study by Zeraati et al. (2015), MSSM was applied only before and during the

Table 4
Distribution and Comparison of SpO_2 Measurements ($n = 96$).

Time of Measurement	Groups				Test Value *p*	Bonferroni[2]
	MIG	NIG	BG	CG		
	$\underline{X}\pm$SS *Med*	$\underline{X}\pm$SS *Med*	$\underline{X}\pm$SS *Med*	$\underline{X}\pm$SS *Med*		
Bp 5. min	96.63 ± 2.81 *96.00*	96.33 ± 2.14 *96.50*	95.88 ± 2.91 *96.00*	94.92 ± 2.28 *95.00*	2.050** *0.112*	–
Bp 2. min	96.29 ± 2.51 *96.00*	96.88 ± 2.46 *97.00*	95.50 ± 2.64 *95.50*	94.46 ± 2.50 *95.00*	4.116** ***0.009****	**2 > 4**
Ibp	96.29 ± 2.42 *96.00*	96.63 ± 2.08 *96.00*	96.04 ± 2.29 *96.00*	94.67 ± 2.62 *95.00*	3.186** ***0.027****	**2 > 4**
Iap	92.79 ± 3.92 93.00	91.46 ± 3.24 *91.50*	91.08 ± 3.34 *92.00*	89.17 ± 3.14 *89.00*	4.587** ***0.005****	**1 > 4**
Ap 2. min	95.71 ± 3.64 *96.00*	94.83 ± 3.36 *95.50*	93.42 ± 3.23 *92.00*	92.58 ± 2.62 *92.00*	4.504** ***0.005****	**1 > 4**
Ap 5. min	96.38 ± 2.45 *96.50*	95.46 ± 2.87 *96.00*	94.92 ± 2.65 *95.00*	93.00 ± 2.04 *93.00*	7.659** ***0.000****	**1 > 4,** ***2 > 4***
Test Value	17.957***	30.022***	20.603***	29.509***		
p	***0.000****	***0.000****	***0.000****	***0.000****		
Bonferroni[1]	***a > d, b > d, c > d,*** **e > d, f > d**	***a > d, b > d, e, c > d,*** **e > d, f > d**	***a > d, e, b > d, c > d, c > e,*** **e > d, f > d, e**	***a > d, e, f, b > d, e, b, f, c > d, e, f,*** **e > d, f > d**		

*p < 0.05, **One-way analysis of variance (ANOVA), ***Repeated measures analysis of variance (ANOVA).
Bonferroni[1]: a = Bp 5. min; b = Bp 2. min; c = Ibp; d = Iap; e = Ap 2.min.; f = Ap 5.min.
Bonferroni[2]: 1 = MIG; 2 = NIG; 3 = BG; 4 = CG.
* Bonferroni was applied to find the group that made a difference in all groups.

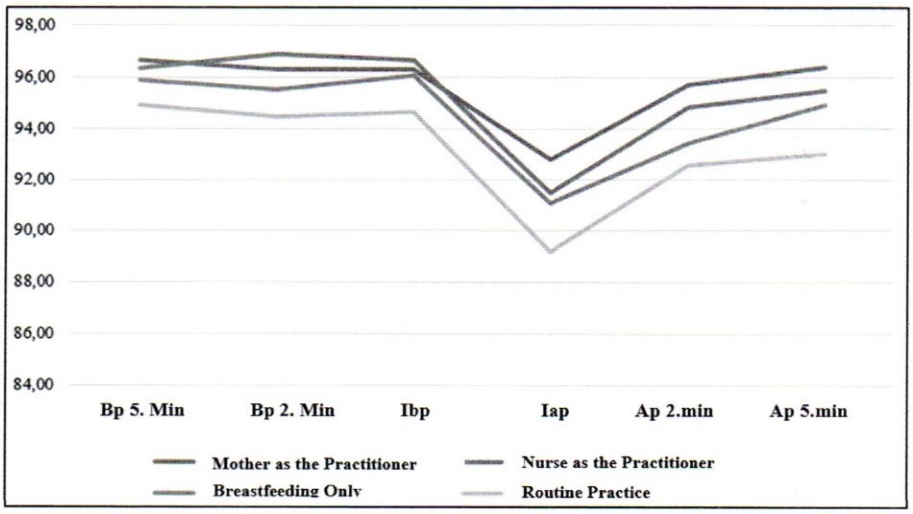


Fig. 4. Comparison of SpO_2 Measurements.

procedure, and the post-procedure period was not evaluated. These findings suggest that extending MSSM into the post-operative period may provide additional clinical benefit and support our H3.

Discussion of findings on the effect of MSSM on infants' SpO_2 levels

Infants are frequently subjected to numerous painful procedures, and this situation affects heart rate, respiratory rate, blood pressure, intracranial pressure, and hormonal and metabolic changes. Pain responses may cause increases or decreases in heart rate, but SpO_2 generally declines(Cowen et al., 2015; Eriksson & Campbell-Yeo, 2019). In our study, statistically significant differences were found between the groups in the SpO_2 values measured during the procedure ($p < 0.05$). Specifically, SpO_2 values after the procedure were found to be higher in the MSSM group administered by the mother, while SpO_2 values pre-procedural were higher in the MSSM group administered by the nurse, compared to the control group.

Zeraati et al. (2015) investigated the effect of MSSM on SpO_2 during eye examinations in premature infants and reportedthat the values measured during the procedure were higher in the group receiving MSSM compared to the control group. Similarly, in this study, SpO_2 levels were found to be higher in the groups receiving MSSM before, during, and after the procedure compared to the group receiving routine care. Furthermore, the literature reports that touch, one of the components of MSSM, contributes to maintaining more stable SpO_2 levels in newborns (Jones & Santamaria, 2018). It is considered that MSSM, applied for 10 min in this study, supported this physiological stability.

Overall, higher SpO_2 in both the mother and nurse administered groups versus controls supports H2. We also found a significant intra-procedural difference between MIG and NIG ($p < 0.05$), suggesting practitioner-related variation in SpO_2 response and supporting H6.

Discussion of findings on the effect of MSSM on infants' HRP values

(HRP) is considered an important physiological indicator in assessing pain in infants, and changes in HRP may indicate pain (Faye et al., 2010). In this study, we observed lower HRP fluctuations in the

Table 5
Distribution and Comparison of HRP Values ($n = 96$).

Time of Measurement	Groups				Test Value
	MIG	NIG	BG	CG	*p*
	$\underline{X}$±SS *Med*	$\underline{X}$±SS Med	$\underline{X}$±SS Med	$\underline{X}$±SS Med	
Bp 5. min	142.83 ± 9.89 *142.00*	142.13 ± 9.52 *139.00*	143.83 ± 8.66 *143.50*	142.42 ± 10.35 *142.00*	0.144** *0.933*
Bp 2. min	142.79 ± 9.21 *141.00*	141.58 ± 9.55 *141.00*	144.50 ± 10.07 *145.50*	143.29 ± 10.74 *141.50*	0.357** *0.784*
Ibp	143.79 ± 8.81 *143.50*	143.58 ± 9.46 *141.50*	144.79 ± 10.40 *143.50*	143.13 ± 10.55 *141.00*	0.123** *0.946*
Iap	147.83 ± 11.79 *150.00*	151.63 ± 10.80 *153.00*	153.75 ± 9.76 *157.00*	153.71 ± 10.55 *154.00*	1.606** *0.193*
Ap 2. min	144.00 ± 9.80 *144.00*	146.46 ± 9.03 *144.50*	146.88 ± 9.84 *147.00*	147.21 ± 12.35 *145.50*	0.477** *0.699*
Ap 5. min	143.79 ± 9.50 *143.50*	144.33 ± 9.23 *142.50*	145.50 ± 8.62 *144.00*	145.71 ± 10.66 *144.50*	0.224** *0.879*
Test Value	5.369***	15.702***	25.321***	15.384***	
p	***0.000****	***0.000****	***0.000****	***0.000****	
Bonferroni[1]	**d > a**	**d > a, b, c, f, e > a, b, c, f > b,**	**d > a, b, c, e, f,**	**d > a, b, c, f,**	

*p < 0.05, **One-way analysis of variance (ANOVA), ***Repeated measures analysis of variance (ANOVA).
Bonferroni[1]: a = Bp 5. min; b = Bp 2. min; c = Ibp; d = Iap; e = Ap 2.min.; f = Ap 5.min,
Bonferroni[2]: 1 = MIG; 2 = NIG; 3 = BG; 4 = CG.
* Bonferroni was applied to find the group that made a difference in all groups.

Table 6
Distribution and Comparison of Crying Times ($n = 96$).

Groups	$\bar{X}$±SS *Med*	Test Value *p*	***Bonferroni
MIG	26.79 ± 10.57 *22.00*	5.743** ***0.001****	**4 > 1, 2**
NIG	27.54 ± 15.02 *22.50*		
BG	36.08 ± 14.41 *38.00*		
CG	43.17 ± 21.47 *38.00*		

*p < 0.05, **One-way analysis of variance (ANOVA).
***Benforroni: 1 = MIG; 2 = NIG; 3 = BG; 4 = CG.
Crying duration measured in seconds.

groups receiving MSSM, although the difference was not statistically significant.

The literature reports that touch balances HRP by regulating stress hormones (Feldman et al., 2010), while maternal voice and breastfeeding slow heart rate and reduce pain responses (Shah et al., 2023; Yu et al., 2019). Zeraati et al. (2015) also reported that MSSM was effective on HRP in preterm infants and that higher HRP was observed in the control group. These findings were directionally consistent but did not reach statistical significance. In conclusion, MSSM was found to prevent large fluctuations in HRP values but did not create a significant difference. Therefore, this results don't support H7.

Discussion of findings on the effect of MSSM on infants' crying duration

This study identified a significant difference in crying duration between groups during the procedure. The crying duration of infants receiving MSSM from their mothers (MIG) and nurses (NIG) was significantly shorter than that of the control group, while the duration was lower in the breastfeeding group (BG), but the difference was not statistically significant. Karimi et al. (2022) showed that crying duration and behavioral pain responses were shorter in the MSSM group after vaccination compared to the breastfeeding group, while Nieto García et al. (2019) reported that crying duration was shorter in infants receiving MSSM + breastfeeding compared to non-nutritive sucking or dextrose groups. Our study supports these results. It is thought that MSSM soothes infants through touch, visual, and auditory stimuli (Erkut & Yildiz, 2017) and that the analgesic effect of breastfeeding (Shah et al., 2023) contributes to this effect. In conclusion, the shorter crying duration observed in the MSSM-applied groups supports H4 and H8.

Strengths, limitations and challenges

This is one of the few studies comparing the effects of MSSM when applied by both the mother and the nurse. In this regard, it is among the first to evaluate both the implementer differences and the simultaneous effect of breastfeeding. MSSM is an easily applicable, low-cost intervention that requires no additional materials. Involving the mother in the process supports the family-centered care approach.

The study was conducted in a single center, which may limit generalizability. Only healthy infants were included, which restricts applicability to other populations. The duration of the intervention (~25 min) may not be feasible in all clinical settings. Since one of the observers was also a researcher, there is a risk of observer bias during the evaluation process. Olfactory stimuli were not used, which means all sensory modalities were not stimulated equally. Long-term effects (e.g., fear of vaccination, behavioral responses) were not assessed.

Implications for practice

The MSSM method can be integrated into clinical protocols for use during vaccinations and similar invasive procedures. Continuing sensory stimuli for at least 5 min after the procedure may contribute to pain reduction. Training programs can be developed according to whether the implementer is the mother or the nurse. More randomized controlled trials should be conducted to determine the optimal duration, content, and age-specific effectiveness of MSSM.

Conclusion

According to the results of this study, MSSM was found to be effective in reducing pain experienced by infants during the vaccination process. Infants in the MIG had lower pain scores compared to the other groups. However, no significant difference was found between the mother and nurse intervention groups, indicating that both implementers are effective. MSSM was found to increase SpO_2 levels, although it did not have a significant effect on Heart Rate Peak (HRP). Post-vaccination crying duration was reduced in all intervention groups, with the shortest durations observed in the MIG and NIG groups. While there are various studies in the literature regarding method, duration, and implementer differences, this study offers a unique contribution by extending the MSSM application into the post-procedure period and by comparing different implementers. The intervention can be recommended as a practical and effective method for nurses to use during painful procedures such as vaccinations. In this study, MSSM was continued not only before and during the procedure but also for 5 min after the procedure. This extended implementation was observed to continue reducing pain scores, which stands out as a strong, practice-oriented recommendation that is rarely addressed in the literature. Previous immunization experiences of the infants aged 4 and 6 months included in the study may have influenced their pain sensitivity. Further studies should examine the effect of MSSM in different age groups and during various painful procedures (e.g., blood sampling, injections). In particular, more data should be gathered to standardize the duration of the application and determine the optimal continuation period after the procedure. Multisensory approaches that actively include the olfactory sense should also be explored.

Research data

Research data can be made available upon request from the author.

CRediT authorship contribution statement

Kadriye Şahin: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Suzan Yıldız:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Emel Gür:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

Statement: During the preparation of this work the authors used ChatGPT-4o in order to improve to check grammar, spelling. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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