The Effect of the Smell of Breast Milk and Non-Nutritious Sucking on Pain Behavioral Response and to First-Time Hepatitis B Vaccine in Term Newborns

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Abstract:
Background: The issue of Pain Management finds special significance in infants who are unable to verbally express pain. Studies have shown that the use of non-pharmacological pain control techniques can be effective in reducing neonatal pain. The aim of this study was to compare the effects of olfactory stimulation (with breast milk) and non-nutritive sucking (with a pacifier) on the physiological and behavioral responses in term neonates to the hepatitis B vaccine.

Methods: In this clinical trial, which was done in 2015 at the Nohom-e Dey Hospital of Torbat Heidariyeh, 90 eligible infants were randomly selected and divided into two intervention and one control groups. In the breast-milk odor group (n = 30), the neonates were exposed to the mother's odor during vaccination. In the non-nutritive sucking group (n = 30), a standard soft pacifier was used, whereas, in the control group (n = 30), no intervention was carried out. Data collection tools included demographic information forms and the Neonatal Pain Response Scale. Data were edited and analyzed using SPSS 20 software.

Results: This study showed that there was a statistically significant difference between the mean scores of neonatal behavioral responses after intervention in the three groups (p <0.05). The mean behavioral response was 0.73 lower in the breast-milk odor group than in the control group, and the mean behavioral response in the non-nutritive sucking group was 0.6 lower than that of the control group.

Conclusion: The results of the study showed that both olfactory stimulations with breast milk and non-nutritive sucking have a positive impact on neonatal pain reduction, nearly equally.

Keywords: Breast milk odor, Non-nutritive sucking, Behavioral responses, Pain, Term baby, Physiological reactions.

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1. INTRODUCTION
The International Association for Pain considers pain a hidden feeling and emotional experience associated with acute or potential tissue damage. This definition emphasizes pain as a bio-psychological experience and a sign of tissue destruction [1]. In the past, it was commonly thought that infants did not feel pain. For this reason, pain relief was not considered. But recent research has shown that term infants are susceptible to pain, just like in infants and children, and that premature infant may be even more susceptible to pain and its harmful effects [2]. Since infants are not able to verbally express their pain, they respond to painful stimuli in visible and measurable behavioral and physiological reactions such as through changes in facial expressions, raising of the eyebrows, squeezing of the eyes, lips, etc. in the former, and increased heart rate and
decreased arterial oxygen saturation in the latter; all this being due to painful actions producing a stress response in the neonatal [3]. Accordingly, pain control plays an important role in preventing adverse physical and psychological effects [4]. The American Pain Association has named it the fifth vital sign to emphasize its importance and raise awareness among health team members about its control [5]. Pharmacological and non-pharmacological measures are among the methods of pain control in neonates [6]. In infancy, pain medications are rarely used to reduce the effects of pain during painful procedures because central painkillers can cause harmful side effects such as rash, hives, reddening of the skin, etc. Therefore, paying attention to non-pharmacological methods effective in reducing neonatal pain is very important [7]. Non-pharmacological methods for mild to moderate pain are practiced by nurses and do not require a physician’s instructions.

Non-pharmacological methods for controlling neonatal pain can include hugging, distraction, non-nutritional sucking, shaking the baby, breast-feeding, skin-to-skin contact, and swaddling [6]. These methods are likely to alter pain sensation and pain response by shifting attention and reducing pain perception [8]. Two examples of non-pharmacologic pain management techniques, which appear to be effective in reducing neonatal pain, are non-nutritious sucking and stimulation of the olfactory system. Although, the mechanism of pain control by the non-nutritious sucking method is unknown, sucking triggers the release of serotonin in the brain, which reduces pain [9]. Research has shown that non-nutritious sucking reduces behavioral distress and restlessness and appears to modulate the transmission or the process of pain perception by the internal non-opioid system [10]. In a study by Liu et al., on neonatal pain control, infants who were allowed non-nutritious sucking during painful blood sampling procedures showed less pain responses [11]. Another study by Badiee et al. in 2013 aimed at comparing the effect of breast milk and formula odor on infant relaxation, found that breast milk odor had a pain-reducing effect in infants and could be used as a non-pharmacological pain-reduction method [12].

Since a healthy baby is exposed to many painful measures in the first few days after birth, pain control is necessary to mitigate its deleterious effects. Since non-pharmacological methods control the pain with different mechanisms and provide a wide range of treatments, it is necessary to select those methods that have the highest impact on reducing neonatal pain.

Studies show that non-nutritious sucking and breast milk odor can both be effective in reducing neonatal pain, but the superiority of either has not been studied. The aim of this study was to compare the two non-pharmacological methods of non-nutritious sucking and breast milk odor on the behavioral index of pain induced by first-time hepatitis B vaccine – as one of the first painful experiences in healthy term neonates. We hope that the utilization of the results of our study can be an effective step in the management of neonatal pain, which has become a topic of great interest to neonatal health-care providers today.

2. MATERIALS AND METHODS

In this study, a randomized controlled clinical trial was conducted on three groups of healthy and term infants who were referred to Nohome Dey Hospital of Torbat-e-Heydariyeh for their first hepatitis B vaccine that was administered to them between 7 am and 9 am. To do this study, the design was first approved by the Ethics Committee (IR.GMU.REC.1394.22) and then it was registered in the Iranian Registry of Clinical Trials (IRCT) with code (IRCT2015072423323N1) and a written letter submitted to the Deputy of Education of Torbat Heydariyeh University of Medical Sciences, after which sample collection started. The study population consisted of infants aged 1-3 days old when receiving their first dose of the hepatitis vaccine during the study. The inclusion criteria for the infants under study included a written, informed consent letter from the parents of the infants for participation in the study; term and healthy infants with gestational age between 37 and 42 weeks; absence of congenital malformations and diseases; no sleep medications 48 hours prior to vaccination; an Apgar score greater than 8 at birth; and weight between 2500 to 3500 grams. Whereas, the exclusion criteria for neonates included any respiratory and gastrointestinal problems during the intervention, and parents unwilling to go on with the research and/or not ready to manipulate or shake the baby during the intervention. Based on the data from the neonates’ records, infants who met the inclusion criteria were selected by convenience sampling and were randomly assigned to permissive blocks of 30 individuals in each group.

In order to collect data, a person was needed as a research assistant. In addition, a nursing staff in the neonatal department was required to assist the research by carrying out the procedures. A meeting was set with the chosen persons and after introduction, adequate training on correct vaccinator practices and methodology of research was given by the main researcher, without presenting the research goals. In addition, necessary training on how to use the behavioral response scale and how to rate it, was imparted to the research assistant, too.

The vaccination room was prepared and the infant bed was heated daily by a Tucson warmer to reach 37°C. A thermometer was used on the bed for this purpose. To control the ambient temperature, a thermometer was also installed in the room, and the ambient temperature was maintained at 25°C. Syringes that were used for all the specimens were 2cc syringes with No. 23 needles.

To gain similar behavioral conditions as the basic behavioral condition before intervention in all three groups, the restless infants were first calmed and then gently put on the cot. Behavioral pain responses were assessed from the beginning to the end of the vaccination by the researcher assistant. All the actions were performed in tandem without interruption. Any interruption, shaking, making noise or touching the baby, and or any other action was strictly avoided throughout the intervention. The duration of the injection was the same for all units and no one except the research team was allowed to enter into the vaccination room during the interventions.

In the breast milk odor group, 2 ml of breast milk was needed to stimulate the baby’s olfactory senses. Before taking
the baby to the vaccination room, the mother was asked to remove the baby’s clothes and place the infant on a dry bedsheet. Then, it was taken to the vaccination room and placed on a preheated bed. The Pulse oximeter sensor was slowly closed around the baby’s wrist. Immediately before the start of the intervention, the number of breaths per minute was recorded using watch and pulse and the percentage of saturation of arterial oxygen was noted via the pulse oximeter. Next, a piece of gauze that had been prepared beforehand and had no odor or color and was completely impregnated with 2 cc of breast milk, was placed three centimeters from the infant's nose using tweezers. This continued for up to three minutes simultaneously, measurements were taken with a chronometer. The vaccinator then injected the vaccine into a third of the femoral lateral muscle by gently grasping the leg. Immediately after removing the gauze soaked in breast milk from the baby's nose, the percentage of oxygen saturation and pulse was read on the pulse oximeter and recorded again, along with the number of breaths which were counted in one full minute. Behavioral responses to pain were also scored from the beginning to the end of the vaccination with the help of a researcher. All the above actions were applied consecutively and without interruption.

In the second group, the non-nutritious sucking one, a standard, small, short, latex-type pacifier, brand name Camro No. 1, for infants aged 0 to 6 months was used for all the infants. The pacifier was held in the baby's mouth with gentle pressure. This was continued for up to 3 minutes, then the vaccine was administered with the same technique holding the infant’s leg gently, and immediately after removing the needle and taking out the pacifier from the baby's mouth, its breathing, oxygen saturation and pulse were recorded again. Behavioral pain responses were also scored during the vaccination.

There was no intervention for infants in the third group, the control group.

Data were analyzed using SPSS software version 20 and described through descriptive statistics (frequency tables, mean and standard deviation); for analysis of data, inferential statistics were used. As the data was normal data based on Kolmogrov Smirnov test, Paired t-test, one-way ANOVA and Tukey questionnaire were used to analyze physiological and pain indicators (inter- and intra-group). A meaningful level of less than 0.05 was considered for data analysis.

Data gathering tools included a demographic checklist and the infant pain behavioral response scale.

2.1. Demographic Checklist

This checklist includes the baby's weekly gestational age, sex, birth weight, type of delivery (cesarean section, normal) and the baby’s Apgar score at birth.

2.2. Modified Behavioral Pain Scale

Neonatal pain response scales were used to determine the behavioral responses to pain following vaccination; this scale was developed by Tadio et al. and measures three behavioral parameters (facial expression, crying and baby movements) in the infant. After assigning a score to each of the modes, the scores were finally aggregated, and an overall score of behavioral responses was obtained, with the lowest score being zero and the highest score being ten. This tool has been used repeatedly in various studies and its validity and reliability have been confirmed [13, 14].

3. RESULTS

The results of the analysis of variance test, according to Table 1, showed that in terms of the gestational age of the research units, there was no significant statistical difference between the three studied groups, and in that sense, the groups were homogeneous (p=0.42). The first minute Apgar score, according to the records of birth for all babies in the study group was 9.

Based on the findings of Table 2, between the three groups studied in terms of weight of the units, there was no statistically significant difference and so, the groups were homogeneous (p=0.84).

According to Table 3, the majority of research units (55.5%) were boys, and there was no significant statistically significant difference between the three groups and thus, the groups were homogeneous (p= 0.24).

Table 1. Comparison of the mean gestational age of research units per week in the three groups studied.

| Studied groups          | Number | Mean ± SD   | Analysis of variance |
|-------------------------|--------|-------------|----------------------|
| Breast Milk Odor        | 30     | 38.89 ± 1.343 | F = 0.87, df= 2.85, p= 0.42 |
| Non-nutritious sucking  | 30     | 38.73 ± 1.048 |                       |
| Control                 | 30     | 39.13 ± 1.137 |                       |
| Total                   | 90     | 38.92 ± 1.17  |                       |

Table 2. Comparison of average weight of research units in grams in the three groups studied.

| Studied groups          | Number | Mean ± SD       | Analysis of variance |
|-------------------------|--------|----------------|----------------------|
| Breast Milk Odor        | 30     | 3098.62 ± 342.665 | F = 0.17, df= 2.86, p= 0.84 |
| Non-nutritious sucking  | 30     | 3091.00 ± 230.552 |                       |
| Control                 | 30     | 3134.67 ± 332.988 |                       |
| Total                   | 90     | 3108.20 ± 302.96 |                       |
Table 3. Frequency distribution of research units in three study groups.

| Group       | Sex     | Breast Milk Odor Number (%) | Non-nutritious sucking Number (%) | Control Number (%) | Total Number (%) | Kai-Square test result |
|-------------|---------|------------------------------|-----------------------------------|-------------------|-----------------|------------------------|
|             | Girl    | 12(40)                       | 17(56.7)                          | 11(36.7)          | 40(44.5)        | $X^2 = 2.79$            |
|             | Boy     | 18(60)                       | 13(43.3)                          | 19(63.3)          | 50(55.5)        | df=2                   |
|             | Total   | 30(100)                      | 30(100)                           | 30(100)           | 90(100)         | $P = 0.24$             |

Table 4. Frequency distribution of delivery type of research units in three study groups.

| Group Type of Delivery | Breast Milk Odor Number (%) | Non-nutritious sucking Number (%) | Control Number (%) | Total Number (%) | Kai-Square test result |
|------------------------|-----------------------------|----------------------------------|--------------------|-----------------|------------------------|
| Normal                 | 18(60)                      | 14(46.6)                         | 20(66.6)           | 52(57.7)        | $X^2 = 2.55$            |
| Caesarean section      | 12(40)                      | 16(53.4)                         | 10(33.4)           | 38(42.3)        | df=2                   |
| Total                  | 30(100)                     | 30(100)                          | 30(100)            | 90(100)         | $P = 0.27$             |

Table 5. Comparison of Neonatal Behavioral Response Scores in the three study groups after the intervention.

| Studied groups       | Number | Mean ± SD | Analysis of variance |
|----------------------|--------|-----------|----------------------|
| Breast milk odor     | 30     | 8.00±0.172| F = 5.15             |
| Non-nutritious sucking | 30     | 8.13±0.172| df² = 2.86           |
| Control              | 30     | 8.73±0.172| $P = 0.008$          |

Based on Table 4, the type of delivery of most research units was of a normal delivery (57.7%), and in this regard, there was no significant statistical difference between the groups ($p=0.27$).

The results showed that there was no significant difference between the three groups in terms of gestational age and type of delivery, and gender and neonatal age ($p>0.05$). (Table 5)

According to the results of the analysis of variance, comparison of the two groups - breastfeeding odor and non-nutritious sucking with the control group after the intervention - showed a significant difference between the mean scores of neonatal behavioral responses ($p = 0.008$). Moreover, the results of the Tukey post hoc test showed that there was a significant difference between the behavioral responses in the two groups of control and breast milk odor ($p = 0.009$). The mean behavioral response was 0.73 lower in the breast milk smell group than in the control group (Table 5).

There was a statistically significant difference between the behavioral responses in the control and non-nutrient sucking groups ($p = 0.04$). The mean behavioral responses in the non-nutrient sucking group were 0.6 lower than the control group. There was no statistically significant difference in behavioral responses between the two groups of breast milk odor and non-nutrient sucking ($p = 0.8$) (Table 5).

Pre-intervention analysis of variance (ANOVA) to check the percentage of blood oxygen saturation showed that there was no significant difference between groups ($p = 0.35$). But after intervention between the intervention groups, a significant difference ($p = 0.001$) was observed. According to the paired t-test results, the mean percentage of blood oxygen saturation in the non-nutrient sucking and control groups after the intervention was significantly different ($P<0.05$) (Table 6).

Table 6. Comparison of blood oxygen saturation percentage, pulse rate and respiratory rate mean in the three groups before and after the intervention.

| Studied groups     | Physiologic Response | Before Intervention | After Intervention | Paired t-test results |
|--------------------|----------------------|---------------------|--------------------|-----------------------|
|                    |                      | Mean ± SD           | Mean ± SD          |                       |
| Breast milk odor   | Blood oxygen pulse   | 92.63±3.20          | 5.08±1.72          | $t = 1.43, (P = 0.16)$ |
|                    | respiratory rate     | 19.5±141.5          | 2.96±143.3         | $t = 2.45, (P = 0.02)$|
|                    |                      | 19.5±141.5          | 10.85±49.0         | $t = -2.75, (P = 0.01)$|
| Non-nutritious sucking | Blood oxygen pulse | 3.05±93.83          | 3.80±90.0          | $t = 4.77, (P = 0.000)$|
|                    | respiratory rate     | 13.6±137.37         | 2.96±142.33        | $t = 2.56, (P = 0.01)$|
|                    |                      | 11.28±44.1          | 11.76±47.83        | $t = -2.11, (P = 0.43)$|
| Control            | Blood oxygen pulse   | 93.43±3.46          | 5.67±86.37         | $t = 6.42, (P = 0.000)$|
|                    | respiratory rate     | 17.98±141.96        | 2.96±153.73        | $t = -6.36, (P = 0.000)$|
|                    |                      | 11.36±41.67         | 10.17±47.87        | $t = -3.44, (P = 0.002)$|

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Results of the analysis of variance before the intervention to evaluate the mean pulse rate per minute indicated that there was no significant difference between groups (p = 0.3). But after the intervention, there was a significant difference between the intervention groups (p = 0.01). According to the results of the paired t-test, the mean pulse rate per minute in all three groups was significantly different after the intervention (p <0.05). Results of the analysis of variance before the intervention in order to evaluate the average respiratory rate per minute showed that there was no significant difference between the groups (p = 0.6). Also, there was no significant difference between intervention groups after the intervention (p = 0.8) (Table 6).

4. DISCUSSION

The aim of this study was to investigate the effect of breastfeeding odor and non-nutritional sucking on behavioral responses to first-time hepatitis B vaccine in term neonates. The results related to the purpose of determining and comparing pain behavioral responses for hepatitis B vaccine in the three study groups after intervention showed that the mean scores of post-vaccination behavioral responses in the breastfeeding and non-nutritious sucking groups were statistically significant. The mean score of behavioral responses in both groups was lower than the control group. A comparison of each of the two groups showed that in the breast milk odor group, the mean behavioral response was lower than the control group. However, there was a significant difference between the two groups in terms of behavioral response score and it showed the positive effect of olfactory stimulation with breast milk during vaccination and attenuation of these responses compared to the control group. A study by Ratz et al., which examined the effect of olfactory stimulation with breast milk and vanilla odor (as odor previously known to neonates) on neonatal behavioral modalities during blood sampling, showed that neonatal restlessness and crying when exposed to olfactory stimulation was significantly lower than that in the control group [15]. In a study by Nishani et al., olfactory stimulation with breast milk significantly reduced the duration of neonatal crying during heel blood sampling compared to the control group [16]. In addition, in the Varendy study, stimulation of infants after birth by the amniotic fluid as the odor that infants are familiar with, significantly reduced their behavioral responses to crying and restlessness after birth [17]. These results are in consistent with the results of the present study in the breast milk odor group in terms of pain behavioral responses. According to research in this area, olfactory stimuli can be effective contextual keys for retrieving memories [18]. Accordingly, it seems that in the present study, olfactory stimulation with breast milk restored the memory associated with the relaxation of the mother to the infant, and thus alleviated pain-related behavioral reactions – similar to previous studies where olfactory stimulation with familiar smells had a calming effect on behavioral responses.

There was also a statistically significant difference between the behavioral responses in the control and non-nutritious sucking groups. The mean score of behavioral responses in the non-nutritious sucking group was 0.6% lower than the control group. This indicates a calming effect of non-nutritional sucking during painful vaccination and relief of pain behavioral indicators in this group compared to the control group.

Among the studies conducted in this area, few studies have examined the effects of non-nutritive sucking on pain behavioral indicators separately. Most studies have used pain scores, which are a set of behavioral and physiological indicators. In the present study, however, the effect of this intervention on behavioral and physiological dimensions has been investigated separately that includes the following.

In a study by Sue et al., non-nutritive sucking significantly reduced the behavioral responses to pain and the duration of crying after the injection [19]. In the Mirzarahimi et al. study, pain scores in the non-nutritive sucking group during heel blood sampling were significantly lower than in the control group [10]. In the Gibbons and Stevens study, non-nutritive sucking significantly reduced the pain score in neonates during blood sampling compared to the control group [20]. In another study, non-nutritive sucking reduced pain behavioral responses during painful heel blood sampling [21]. These results are consistent with the findings of the present study. The mechanism underlying the physiology of this reflex effect on infant relaxation is still unknown, but it is suggested that stimulation of sucking reflex via the release of serotonin may play a role in infant relaxation [10]. Also, according to the most common theory of neonatal pain, namely the gate control theory, accuracy and distraction, can be effective in blocking or reducing pain messages. It may also be that in the present study, the significant reduction in behavioral responses to pain when using non-nutritive sucking was due to distraction.

A comparison of behavioral response scores in the two intervention groups showed that the mean score of behavioral responses in the breast milk smell group was lower than that of the control group, and the non-food sucking group was lower than the control group. However, there was no statistically significant difference between the two groups (breast milk odor and non-nutritious sucking). And it can be said that the effect of the two interventions on neonatal pain behavioral indicators is almost the same. Therefore, the hypothesis of the present study that the effect of the two interventions (olfactory stimulation with breast milk and non-nutritious sucking on a pacifier) on behavioral responses are different in neonates is rejected.
The major limitations of this study were the inability to control all the factors involved in the intervention, such as unifying the condition of the neonates for several hours before the intervention and the small number of samples.

CONCLUSION

The results of this study showed that the non-pharmacological intervention of breastfeeding odor and non-nutritive sucking during vaccination can moderate pain behavioral responses. Therefore, both methods can be effective in controlling neonatal pain and can be used as effective methods for alleviating mild to moderate pain, such as vaccination pain.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee Gonabad University of Medical Sciences (No. IR.GMU.REC.1394.22) and registered in Clinical Trial Registration Center with code (IRCT2015072423323N1).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this research are available from corresponding author [H.Y] upon request with permission from Ethics Committee of GMU.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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