The effect of inhaling mother’s breast milk odor on the behavioral responses to pain caused by hepatitis B vaccine in preterm infants: a randomized clinical trial

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Abstract

Background: Non-pharmacologic pain relief in preterm infant is an important measure. Familiar odors for neonates have soothing effects in some researches. The aim of this study was to compare the effect of maternal breast milk odor (MBMO) with that of another mother's breast milk odor (BMO) on the behavioral responses to pain caused by hepatitis B (HB) vaccine injection in preterm infants.

Methods: This single-blind randomized clinical trial was performed in the neonatal intensive care unit of Babol Rouhani Hospital, Iran from February 2019 to March 2020. Ninety preterm infants who must to receive the HB vaccine, were randomly assigned into three groups of MBMO (A), another mother's BMO (B) and control with distilled water(C). Oxygen saturation(SaO2), blood pressure(BP) and heart rate(HR) were recorded by electronic monitoring and premature infant pain profile (PIPP) were determined through video recording in tree groups during intervention. The chi-square, ANOVA and ANCOVA were used for analyzing data, and P<0.05 was considered significant.

Results: No significant differences were seen between tree groups in mean± SD of HR, BP, Sao2 before intervention, (P>0.05). After intervention, the mean heart rate in group A, B and C was 146. 6±14.3, 153.70±17.5 and 155.70±17.7 respectively, (P=0.01). There was no significant difference between groups in the mean of SaO2, systolic and diastolic blood pressure after intervention (P>0.05). The mean PIPP score in groups A, B and C was 6.6±1.3, 10 ±2 and 11.4±1.9 respectively, (P<0.001).

Conclusions: Stimulation with MBMO is effective in reducing the pain of preterm infants, so it can be used in less invasive procedures such as needling.

Trial registration: IRCT, IRCT20190220042771N1. Registered 18 May 2019- Retrospectively registered, https://en.irct.ir/trial/37646

Background

Many of the measures in the neonatal intensive care units (NICU) are accompanied for Preterm infants (1, 2). Neonatal pain in very preterm infants has the potential to adversely affect development in multiple domains, including nociceptive changes, altered brain development, stress systems and functional abilities. Prolonged exposure to pain in preterm infants has been associated with impaired brain development while infants are in the NICU (3). Pain assessment methods are performed using physiological (heart rate and respiratory rate) and behavioral criteria (crying time, changes in facial expression and limb movements) (4).

The premature infant pain profile (PIPP) is a set of measurable behavioral and physiological responses such as facial expression changes (squeezing eyes, raising eyebrows, wrinkling nasolabial groove) as well as changes in heart rate and decreased SaO2, intrauterine age and behavioral status of the infants which are definite reasons for their pain (5).

There is a strong tendency to use non-pharmacological interventions as a simple and safe way for infants’ pain relief. Several methods have been applied to relieve pain based on five senses(5). Among them, the smell sense is fully developed at birth (6) and affects the neonate's emotional relationship with his/her mother (7). Familiar odors such as maternal odor have soothing effects on newborn. The human infant has the ability to detect the mother's breast odor without experiencing breastfeeding at birth (8). The breast milk odor (BMO) enhances infant's sucking through the facial and trigeminal motor nerves in the brainstem, which, in turn, stabilizes the physiological state in the infant (9). In some cases, breastfeeding in human newborn infants completely eliminates pain responses animal models showed that the pain modulating effect of breastfeeding is likely mediated by opioid and non opioid mechanisms (10). Some studies have shown that fetal-maternal odors (mother's breast milk, body and amniotic fluid odors) can decrease stress responses including crying and motor activities in infants separated from breast milk or under painful interventions(11). In a study, it was suggested that the maternal breast milk odor (MBMO) had a soothing effect on preterm infants, and their pain score was lower than that in the infants exposed to formula odor (12). Nevertheless, the results of Alemdar et al. (2017) demonstrated that the BMO had no statistically significant difference in the
physiological and behavioral responses of MBMO group compared to other groups (amniotic fluid odor, maternal body odor and control groups) (13).

As the contradictory studies about the effect of MBMO and another mother’s BMO on preterm infants as well as importance of pain relief, this study was aimed to determine the effect of inhaling human milk on the behavioral responses of pain caused by HB vaccine in preterm infants.

**Methods**

**Study Design and setting:** This single-blind randomized clinical trial was done from February 2019 to March 2020 in a NICU of academic center (Rouhani Hospital, Babol, Iran).

**Participant:** Preterm infants 28-37 weeks of gestation, who have to be vaccinated for hepatitis B – zero turn the vaccine- were randomly assigned to three groups. The inclusion criteria were infants with no painful procedure and no feeding for up to one hour before the intervention, stability in vital signs, no head and skull abnormalities as well as no receiving painkillers, sedatives and anticonvulsants. The exclusion criteria were maternal withdrawal from the study and infant severe disease or death.

**Sample size:** Considering 80% power and 0.05 error probability in this study, the number of cases was determined to be at least 30 neonates in each group.

**Data collection and processing:** According to the ward's schedule during the first four days of life, injection of HB vaccine was done. Preterm neonate was placed on a warmer by servo control and skin temperature 36.5-37°C in quiet room. All conditions including room temperature (25°C), light, injection device were the same for all three groups as well as the vaccine administration was done by one person. The researcher and nurse did not use any aromatic substances in vaccination room during the study. The probe of monitoring was placed on the right wrist of baby without applying additional pressure. Heart rate, blood pressure and SaO2 of all preterm infants were recorded before starting the intervention as the initial time and immediately after completing vaccination (using the standard pulse oximeter and ECG monitoring of Saadat Company, Iran).

In both groups of A and B, the breast milk samples taken in the early morning before eating breakfast were used to stimulate the smell sense of neonates. Pouring 2 ml of maternal breast milk and another mother’s breast milk on a cotton swab was done as an intervention, and 2 ml of distilled water as control group (group C). Next, these swabs were placed three centimeters away from the baby’s nose. This process started 3 min before vaccination and continued until the vaccination was completed (2).

**Pain measurement**

The Premature Infant Pain Profile (PIPP) was used as the primary outcome variable. PIPP scores were recorded immediately before and after the vaccination for each infant. The PIPP is a behavioral measure of pain for premature infants. It includes seven indicators: 1) gestational age, 2) the behavioral state, 3) change in heart rate, 4) change in oxygen saturation, 5) brow bulge; 6) eyes squeeze and 7) nasolabial furrow. The total score is the summation of all seven indicators, with a minimum of 0 and maximum of 21; the higher the score, the greater the pain behavior(14). If the overall PIPP score is between 0 and 6 points, the pain level is mild; if between 7 and 12 points, it is moderate; and if between 13 and 21 points, it is severe (15)

The PIPP tool was revised and validated for use with preterm babies born at 26-37 weeks of gestation by Gibbins et al. in 2014 (16)

Video recording of behavioral responses was taken from the beginning to the end of the process by a trained nurse, and then PIPP scoring was performed through watching video by the first author. The scoring was done while the video viewer was unaware of the test group. Throughout the intervention, any actions on the neonates such as contact, movement and so on were avoided.
Data were collected by using the demographic questionnaire including: birth weight, current disease (respiratory distress syndrome, transient tachypnea of newborn, sepsis and very low birth weight), sex, gestational age, postnatal age, Apgar score and PIPP score.

**Data analysis:** Statistics advisor performed the data analysis blindly by using SPSS Version 18. Descriptive information was shown as frequency, percentage, mean and standard deviation. Chi-square test for the relationship between two qualitative variables (demographic and PIPP qualitative variables with group variable), ANOVA test for comparing quantitative variables at the levels of more than two variables (quantitative demographic variables with group variable) and ANCOVA test for comparing research outcomes (SBP, DBP, SaO2 and heart rate) were used to remove the pretest effect and a P value< 0.05 were considered significant.

**Ethical consideration**

The study protocol was approved by the Ethics Committees of Babol University of Medical Sciences (IR.MUBABOL.REC.1397.253). The trial is registered in the IRCT20190220042771N1 Before participation in the study, written informed consent was obtained from each child's primary guardian.

**Results**

**Study subjects**

ALL 90 preterm infants, who included, were completed the study. The infants of the three groups were not significantly different in terms of sex, age, infant's current disease (Spsis, Respiratory Distress Syndrome (RDS), Transient Tachypnea of Newborn (TTN), very low birth weight (VLBW), gestational age, weight and APGAR score (p>0.05) (table 1).

**Table 1. Comparison of demographic variables of preterm infants in three groups**
| Variable                        | MBMO(A) | Another mother BMO(B) | Control(C) | P value |
|--------------------------------|---------|-----------------------|------------|---------|
| Sex n(%)                       |         |                       |            | 0.56*   |
| male                           | 15(50)  | 16(53.3)              | 12(40)     |         |
| female                         | 15(50)  | 14(46.7)              | 18(60)     |         |
| Infant's age (hour) n(%)       |         |                       |            | 0.11*   |
| 24-48                          |         |                       |            |         |
| 48-96                          | 16(53.3)| 12(40)                | 20(66.7)   |         |
|                                | 14(46.7)| 18(60)                | 10(33.3)   |         |
| Infant's disease n(%)          |         |                       |            | 0.94*   |
| RDS, Sepsis                    | 3(10)   | 4(13.3)               | 3(10)      |         |
| VLBW                           | 22(73.3)| 23(67.7)              | 23(67.7)   |         |
| TTN                            | 5(16.7) | 3(10)                 | 4(13.4)    |         |
| Infant's gestational age (WK)  | 32.93±2.49| 31.53±2.17          | 32.53±2.44 | 0.07**  |
| (Mean ±SD)                     |         |                       |            |         |
| Infant's weight (g)            | 1806.33±553.72 | 1620±425.03   | 1688.83±404.02 | 0.29** |
| (Mean ±SD)                     |         |                       |            |         |
| Infant's Apgar score (Mean ±SD)| 7.83±1.08| 7.63±1.32           | 8.30±0.95  | 0.07**  |

*chi², **ANOVA

Table 2 shows variables including SBP, DBP, SaO2 and heart rate before and after the intervention by using ANCOVA test.

Table 2. Mean and standard deviation scores of SBP, DBP, SaO2 and heart rate in the studied groups pre and post intervention
As shown in table 2, by eliminating the effect of the pretest variable and use of ANCOVA test, there is no significant difference between the means± SD of SBP (p=0.48), DBP (p=0.34) and SaO2 (p=0.19) in terms of group membership. ANCOVA test showed that change in heart rate was significantly lower in group A (p=0.01).

PIPP score

The mean± SD of pain score in group A was 6.60±1.35, and 10.07±2.03 and 11.43±1.97 in groups B and C, respectively. The ANOVA test showed that there is a significant difference between groups (P<0.001), and the results of post-hoc Tukey’s test determined that this difference was between group A with groups B and C (fig.2).

Discussion

This study showed that the MBMO reduced heart rate and behavioral responses to pain score caused by HB vaccine in preterm infants rather than another mother’s BMO and control groups but there were no significant differences between tree groups in SBP, DBP and SaO2.

Zhang et al. (2018) conducted a systematic review study to investigate the analgesic effects of BMO on infants. Their results demonstrated that the heart rate changes and SaO2 pain scoring during and after blood sampling were lower in MBMO group than in control group (17), which are consistent with the finding of the current study, except for SaO2, so stimulation with MBMO has a soothing effect on the behavioral responses to pain and reduces infant’s pain.

The results of Sajjadi et al. (2016) illustrated that the mean scores of PIPP had a significant difference in MBMO group compared to control group. Moreover, a significant difference was found in heart rate and SaO2 after intervention. Their results are similar to those of the present study, except for change in SaO2. Alemdar et al. (2017) conducted a study on the effect of mother’s BMO, amniotic fluid odor and body odor on physiological and behavioral responses to heel stick pain in preterm infants. They expressed that there was no statistically significant difference between groups in terms of physiological and behavioral responses to pain such as heart rate, duration of crying and pain scale, although in the amniotic fluid odor group, the SaO2 was slightly different (12), which are inconsistent with the results of the present study. One of the possible reasons for this discrepancy is the difference between both studies in terms of methodology and intervention process. In their study, 5cc of mother's breast milk was poured on a sponge and placed five centimeters away from the neonate's nose for fifteen minutes before and after the intervention, while in our study, the cotton swab placed three centimeters away from the infant's nose. This process in the current study started 3 min before vaccination and continued until the vaccination was completed and attempts were made to minimize the effect of accustoming the sense of smell. Aziznejad et al. (2013) evaluated the
physiological indicators and concluded that there was a statistically significant difference only in the respiratory rate between intervention with sucrose and control groups immediately after the intervention, but there was no significant difference between four groups in other variables (duration of crying, heart rate and SaO2). (18).

In three studies (Zhang, Sajjadi and Alemdar), unlike the present study, changes in SaO2 were different in the intervention group and control groups, however, in the previous study of us (Aziznejad et al), which was performed in the same conditions, no difference in SaO2 was reported. One possible reason could be a difference in the equipment used.

**Conclusion**

On the basis of this research the MBMO can be used as a familiar smell to manage the preterm infant's pain before performing needling procedures such as vaccination.

**Limitations**

Due to the limited equipment, the use of special probes for infants during the study was provided by several companies. The difference in sensitivity of these probes may have caused the SaO2 changes to not be accurately determined.

**Abbreviations**

ANOVA: Analysis of Variance; MBMO: Maternal Breast Milk Odor; BMO: Another Mother's Breast Milk Odor; HBV: Hepatitis B Vaccine; HR: Heart Rate; NICU: Neonatal Intensive Care Unit; PIPP: Premature Infant Pain Profile; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SaO2: Arterial Oxygen Saturation; RDS: Respiratory Distress Syndrome; VLBW: very low birth weight; TTN: Transient Tachypnea of Newborn; ANCOVA: Analysis of Covariance; chi²: Chi-square; APGAR: Appearance, Pulse, Grimace, Activity, Respiration;

**Declarations**

**Ethics approval and consent to participate**

The study protocol was approved by the Ethics Committees of the Babol University of Medical Sciences (IR.MUBABOL.REC.1397.253). The trial is registered in the Iranian Registry of Clinical Trial (IRCT20190220042771N1). Before participation in the study, written informed consent was obtained from each child's primary guardian.

**Consent for publication**

The article does not contain any individual' details and consent for publication is not applicable.

**Availability of data and materials**

The datasets generated and analyzed during the current study are not publicly available due to an agreement with the participants on the confidentiality of the data but are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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Authors’ contributions

ASA Study conception/design, carried out the analysis, interpretation of the data and drafting the manuscript. PA First supervisor the study, Study conception/design, analysis, drafting of manuscript, critical revisions for important intellectual content, Administrative/ technical/ material support, Final revision. ZAR The second supervisor the study critically revised and checked closely the proposal, the analysis and interpretation of the data and design the article. The share of the first and third authors was equal. HGA performed the data analysis and ZV contributed the data collection. All authors read and approved the final manuscript.

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Figures

![Flowchart]

**Figure 1**
Figure 2

The PIPP score's changes in three groups (Note: the same letters indicate no significant difference at level 5%)

Supplementary Files

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- CONSORT2010Checklist.doc