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Effect of Expressed Breast Milk on Neonates' Pain During Intramuscular Injection of Hepatitis B Vaccine

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ABSTRACT

Evidence suggests that neonates exhibit heightened pain perception and increased sensitivity to its long-term effects compared to older infants. This study aimed to compare the effects of expressed breast milk and formula on behavioral and physiological responses, as well as crying duration, during the intramuscular administration of the Hepatitis B vaccine in newborns. In this randomized clinical trial, 60 healthy term neonates were allocated into three groups and evaluated during the intramuscular injection. The control group received injections following the standard ward procedure. In the first intervention group, neonates were administered 2cc of breast milk, while in the second intervention group, they received 2cc of formula via a syringe two minutes prior to the injection. Behavioral and physiological responses, along with crying durations, were documented using video recording and pulse oximetry. Data analysis was conducted using ANOVA, Kruskal-Wallis, Mann-Whitney, and chi-square tests. A significant difference was observed in the duration of crying ($p = 0.03$) and behavioral responses to pain during the injection ($p = 0.04$) among the groups. Expressed breast milk was found to reduce behavioral measures and crying duration during intramuscular injection in term neonates. Consequently, this simple, effective, accessible, and cost-free method can be employed to mitigate the adverse effects of pain in neonates.

Keywords: Expressed Breast Milk; Neonates; Pain; Intramuscular Injection

Introduction

In the early years of life, immunization is the most common painful procedure, which causes repeated injections in infants [1]. Traditionally, pain in neonates has been undermanaged due to limited knowledge about pain processing and the difficulty of conducting studies on the effects of pain on neonates [2]. Recent studies have shown that pain has short-term effects on neonates, such as increased heart rate, respiratory rate, skin blood flow, sweating, secretion of stress hormones, blood pressure alterations, intravascular hemorrhage, and hypoxemia [3, 4]. Additionally, repeated pain experiences can alter neonates' development, behavior, and pain processing in adulthood [5, 6]. Therefore, the prevention and management of pain are crucial in neonatal care [7]. Due to the dubious efficacy and potential side effects of pharmacological interventions, non-pharmacological methods are increasingly recommended [8, 9, 10]. It has been shown that sucrose (12% to 50%) and other sweeteners are effective in reducing procedural pain in neonates [4, 11, 12]. Breast milk, with 7% lactose, can be a suitable physiological substitute for sucrose. However, the efficacy of breast milk in reducing procedural pain in neonates is controversial. Upadhyay reported that breast milk is effective in relieving neonatal procedural pain [13]. Peng et al. reported that formula milk and its fat and

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protein constituents reduced crying during blood collection in neonates, while neither water nor lactose was effective [14]. Bilgen et al. compared direct breastfeeding, EBM, and sucrose during heel pricks and found that sucrose was more effective than both breastfeeding and EBM, while the latter two were comparable [15]. Other studies did not show a statistically significant decrease in pain with the use of breast milk [10, 16, 17, 18]. The aim of the present study was to compare the effect of expressed breast milk with formula on behavioral and physiological responses and crying time during intramuscular injection of the Hepatitis B vaccine in newborns.

Materials and Methods

This study was a prospective randomized controlled trial conducted in the Post-natal Care Ward of Asali Teaching Hospital in Khorramabad. The subjects were 65 term neonates who underwent intramuscular injection for routine Hepatitis B vaccination. Term babies born via normal vaginal delivery between 38 and 42 weeks of gestational age, with Apgar scores greater than 7 at the 5th minute, birth weight over 2500 grams, and older than four hours of life, and who had been breastfed at least once, were included. Sick babies with unstable vital signs, requiring oxygen or any drugs, resuscitation at birth, neurological abnormalities, or major congenital defects were excluded.

Mothers of all eligible newborns were informed about the study's aim, risks, and benefits. Those who agreed to participate and provided consent were included. The protocol was reviewed and approved by the institutional ethical committee of Lorestan University of Medical Sciences (2004/4/7).

Using a random number table and sealed envelopes, subjects were allocated to three groups. In the first intervention group (n=22), infants were fed 2ml of their mothers' expressed breast milk (EBM) two minutes before immunization [19, 20]. In the second intervention group (n=22), infants were fed 2ml of a standard formula for term neonates two minutes before immunization. In the control group (n=21), the injection was carried out using the routine ward procedure (no analgesic technique).

The infants were brought to a quiet room between 10-12 a.m., at least 30 minutes after their last meal. A pulse oximeter probe was attached firmly to the first toe of the right foot (to allow adaptation). The nurse prepared the formula or obtained expressed breast milk from the baby's mother in a disposable cup, following health principles. The nurse then administered 2ml of the assigned test solution via a syringe on the anterior part of the tongue. All babies were awake during the procedure, clothed in nappies and a light dress, and wrapped in a blanket. The mother was instructed to hold the infant across her lap in a cross-cradle position, ensuring that the infant's thighs were accessible throughout the procedure. Each mother was encouraged not to soothe the infant vocally, through tactile touch, or by rocking before, during, or after the injection. Two minutes after receiving the solution, a trained nurse slightly pressed on, cleaned, and disinfected the injection site with alcohol and administered a 0.5^{cc} dose of the Hepatitis B vaccine intramuscularly in the vastus lateralis muscle of the thigh at a 90º angle to the skin using a 26-G 1.59-cm needle. After removing the needle, the nurse applied a dry cotton swab. All vaccinations were performed by the same experienced nurse. The injection site, technique, duration, and needle length were the same for all subjects.

Demographic characteristics were collected and recorded on prepared forms for all subjects. Three pain scales were used:

1. Physiological signs: Heart rate (HR) and oxygen saturation (O_2Sat) levels were measured by one of the researcher's assistants using a pulse oximeter two minutes before the vaccination procedure, during the injection, and two minutes after the needle was removed. Data were recorded at 10-second intervals.

2. Behavioral scale of pain: This tool consists of three categories (facial expression, cry intensity, and calming state), each scored 0-2, with a total score of 0-6. The validity and

reliability of the scale were confirmed in a study on 80 newborns [20]. The procedures were filmed with a video camera. The videotapes were scored by two independent observers who were blind to the infant's group assignment. The results of the two observers were compared, and if there was a discrepancy, the situation was reassessed until the two observers reached an agreement.

3. Duration of crying (in seconds): Measured from the moment of needle insertion until cessation, using an electronic timer.

Frequency distributions and cross-tabulations of variables of interest were obtained. The mean HR and O_2Sat values were calculated by summing the values two minutes before, during, and two minutes after the procedure. Birth weight, age, heart rate, and oxygen saturation levels were analyzed using ANOVA. Sex was analyzed using the chi-square test. The duration of crying and behavioral responses were analyzed using the Kruskal-Wallis and Mann-Whitney U tests.

Results

Five neonates (2 from the EBM group, 2 from the formula group, and 1 from the control group) were excluded from the study due to irritability caused by attaching the pulse oximeter probe, crying, or mothers rocking the babies after the injection. There were no significant differences between the groups in demographic data (Table 1).

The baseline, during, and post-vaccination HR were similar across the groups. Similar results were observed for O2Sat levels (Table 2). There were no significant differences between the groups in behavioral signs of pain 2 minutes before the injection ($\chi^2=0$, $p=1$). There was a significant difference between the groups in the duration of crying and behavioral responses to pain during the injection (Table 3). Further analysis showed that the duration of crying in the EBM and formula groups was different from the control group, but there was no significant difference between the EBM and formula groups. Analysis of behavioral pain responses showed that behavioral responses to pain during and after the injection were different between the EBM and control groups. However, the formula group did not show any significant difference from the EBM or control group (Table 4).

Table 1. Demographic characteristic of the study population

Parameter	Age(h)	Weight(g)	Sex *N* (%)	
	M ± SD	M ± SD	Male	female
EBM	13.53±6.88	3352. 5±382.6	11(55)	9(45)
Formula	11.74±7.98	3340±328	10(50)	10(50)
Control	11.82±7.88	3302±301.96	13(65)	7(35)
F*	1.07	0.146	**0.95	
P value	0.357	0.87	0.62	

*ANOVA; ** Chi square

Table2. Physiological signs (Heart rate, Spo2) before, during and after vaccination in study groups

	Heart rate (M ± SD)			Spo2(M ± SD)		
	Before	During	After	Before	During	After
EBM	132.86±19.99	135.24±18.01	147.54±17.75	96.13±1.86	95.70±1.98	95.46±2.28
Formula	138.54±17.84	138.63±15.89	139.95±16.47	94.68±2.41	94.27±1.95	94.40±2.38
Control	133.09±15.54	130.55±26	137.75±14.36	96.64±3.44	96.05±2.56	94.83±3.08
F*	.646	1.129	.273	2.935	3.703	.830
P value	.528	.331	.762	.061	.061	.441

*ANOVA

Table3: Crying time and behavioral pain responses during and after vaccination in the study groups

Variable	Group	Mean Rank	χ^{*2}	P
After injection Crying	Control	38.85	7.044	0.03
	EBM	25.20		
	Formula	27.45		
Pain during injection	Control	38.30	6.56	0.04
	EBM	25.05		
	Formula	28.15		
Pain after injection	Control	37.20	4.81	0.09
	EBM	26.28		
	Formula	28.02		

* Kruskal –wallis

Table4: Crying time and behavioral pain responses, during and after vaccination in study groups

Variable	Group	Mean Rank	Z*	P
Crying. after injection	Control	25.18	-2.53	0.01
	EBM	15.82		
Crying after injection	Control	24.18	-1.99	0.04
	Formula	16.82		
Crying after injection	EBM	19.88	-0.339	0.73
	Formula	21.12		
Behavioral Pain during injection	Control	25.10	-2.54	0.01
	EBM	15.90		
Behavioral Pain after injection	Control	24.42	-2.23	0.03
	EBM	16.58		
Behavioral Pain during injection	Control	23.70	-1.77	0.08
	Formula	17.30		
Behavioral Pain after injection	Control	23.28	-1.54	0.12
	Formula	17.72		
Behavioral Pain during injection	EBM	19.65	-.47	0.64
	Formula	21.35		
Behavioral Pain after injection	EBM	20.20	-0.17	0.87
	Formula	20.80		

* Mann-Whitney U

Discussion

Based on the results of this randomized controlled study, administering 2ml of breast milk two minutes before vaccination significantly reduced crying time and behavioral responses to pain during and after the injection compared to the control group. However, administering 2ml of formula milk only reduced crying time compared to the control group. No significant differences were found between breast milk and formula milk in these results. Some recent studies have suggested that breast milk administered before acute, short-lasting, and repeated painful procedures in term newborns may have analgesic effects. Bembich et al. reported that feeding 5ml of EBM before venipuncture effectively reduced pain symptoms in term neonates [13]. Örs et al. found that a 25%

sucrose solution had superior pain-reducing effects compared to breast milk, with no difference between human milk and placebo [18]. Similarly, Stevens et al. reported that strong sweet solutions, such as sucrose and 30% glucose, successfully relieved pain, whereas breast milk did not [17]. Bucher et al. showed that an artificial sweetener reduced pain reactions to heel pricks in term neonates, while glycine increased pain reactions, and breast milk had no effect [16]. Mathai et al. found that rocking or giving a pacifier was more effective as non-pharmacological analgesics than EBM, distilled water, sucrose, or massage for heel prick pain in neonates [10]. Uyan et al. showed that although hind milk had a minor advantage in crying time, duration of the first cry, and percent change in heart rate during heel pricks, it did not reach statistical significance. They concluded that neither foremilk nor hind milk was superior to placebo in relieving pain [21].

These differences may be related to the type of procedure—intramuscular injection in our study versus heel pricks in most other studies—which produce different levels of pain severity. This suggests that breast milk intervention may only be effective in cases of mild to moderate pain. Alternatively, the failure to demonstrate an analgesic effect of breast milk could be related to the lower sugar content of breast milk (7%) compared to other sweet solutions used in these studies.

In this study, the administration of EBM before vaccination did not relieve physiological signs of pain, such as HR and SaO2. However, Bembich et al. showed that administering EBM before venipuncture resulted in a shorter duration of crying and fewer changes in facial expressions, HR, and SaO2 [13]. This difference could be due to the higher volume of milk used in their study (5ml compared to 2 ml in our study). A dose effect has been demonstrated in studies using sucrose or glucose. Stevens et al. showed a significant reduction in pain perception with the use of 30% glucose compared to 10% [17]. Similarly, studies showed that 24% sucrose was more effective than 12% in providing analgesia after venipuncture [1]. However, sucrose solutions have high osmolality, which poses a risk for necrotizing enterocolitis. Additionally, sucrose, which contains glucose and fructose, may have adverse effects in infants with fructose intolerance [2]. Frequent use of sucrose may also increase the risk of tooth decay [16].

The rationale for using EBM as an analgesic is that babies associate EBM with breastfeeding, recognizing its taste and relating it to the comforting experience of breastfeeding. Such a pleasant association would not exist with other sweet solutions [13]. Studies have shown that certain tastes and flavors reduce newborn pain. As little as 2ml of milk, with its fat and protein content, can have an analgesic effect [22, 23]. In rats, the mechanisms underlying taste-induced analgesia are opioid-mediated and block pain afferents at the level of the spinal cord [24-27]. Familiar odors may also stimulate the release of cholecystokinin [9], an opioid-modulating substance that promotes stressor adaptability and can achieve an analgesic effect by potentiating opioid activity [28]. Furthermore, infants are responsive to olfactory cues from their mothers' nipple regions [29]. Recent studies have found an analgesic effect of direct breastfeeding. Dilli et al. and Efe et al. found that breastfeeding reduced vaccination-related pain in infants [30, 31]. Gray et al. showed a significant reduction in crying, grimacing, and HR during heel lance procedures while the baby was breastfeeding [32]. Kumari et al. found reduced pain scores after venipuncture during direct breastfeeding [4]. Given that breast milk delivered via syringe in most studies was not an effective oral analgesic, it may be that skin-to-skin contact and sucking during breastfeeding have an analgesic effect unrelated to the breast milk itself [16]. However, direct breastfeeding during painful procedures may have some drawbacks. Some mothers may feel uncomfortable breastfeeding in front of others, especially male caregivers, and some may prefer not to be present during painful procedures. Additionally, associating breastfeeding with painful stimuli may create negative memories, and although no studies have reported it, breastfeeding may increase

the risk of aspiration during painful experiences.

In conclusion, feeding EBM is a simple, inexpensive, non-invasive, and natural method for reducing pain during minor invasive procedures. It is convenient, easy to use, can be repeated without risk, and is easy for nurses to supervise. Therefore, this pain-reduction approach can be easily adopted as part of standard immunization injections, and its use should be more widely promoted. Breast milk feeding is recommended for its proven health, nutritional, immunological, developmental, psychological, social, economic, and environmental benefits. We found that the components of breast milk during immunization for newborns reduced behavioral signs of pain and crying duration due to pain. The present findings add the benefit of analgesia to the other effects of breast milk. However, it is important to emphasize that while breast milk may have an analgesic effect, it should not be considered suitable for major analgesia or as a substitute for properly planned analgesia in procedures. It cannot replace pharmacological treatment in cases of severe and chronic pain. Future research should address whether breast milk is an effective measure of pain relief among sick preterm and term neonates suffering from chronic pain.

This study had several strengths, including randomization, blinding, and the use of three instruments for pain assessment. However, there were some limitations. First, the sample size was small. Definitive studies with larger sample sizes are needed to confirm that breast milk minimizes the pain experience. The generalizability of this study's findings is limited by the use of a single injection. The efficacy of this approach needs to be evaluated in cases of multiple and repeated injections, which young infants typically receive during vaccination visits.

Conclusion

The findings demonstrated that expressed breast milk significantly reduced crying time and behavioral responses to pain during intramuscular injection in term neonates. Therefore, this simple, useful, available, and free approach can be recommended to lessen the adverse effects of pain in neonates.

Conflict of Interests

The authors declare that they do not have any conflict of interests.

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