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Research paper

Effects of breast milk on pain severity during muscular injection of hepatitis B vaccine in neonates in a teaching hospital in Iran

Z. Hatami Bavarsad^a, K. Hemati^b, K. Sayehmiri^c, P. Asadollahi^d, G. Abangah^e, M. Azizi^f, K. Asadollahi^{g,*}

^a Department of Social Medicine, Faculty of Medicine, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran

^b Department of Anaesthesiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

^c Department of Epidemiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

^d Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

^e Department of Gastroenterology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

^f Department of Anatomy, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

^g Department of Social medicine, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

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ABSTRACT

Introduction and aims: Human breast milk is a natural pain reliever that contains endorphins. The aim of this study was to compare the effects of breast milk and powdered milk on pain severity after a muscular injection in 1-day-old neonates.

Materials and methods: One hundred neonates admitted to a teaching hospital in Ilam city, Iran, participated in a randomized clinical trial in 2016. One-day-old neonates were divided into four equal groups including: the control group (no feeding); the breastfed group; the bottle-fed mother's milk group and the powdered formula group. All infants received the hepatitis B vaccine by muscle injection in the same position of the thigh. The severity and duration of pain were compared among all groups during and after injection using the DAN scoring method (evaluation behavioral scale of acute pain in newborn infant).

Results: One hundred neonates (57% boys) participated in this study. The mean \pm SD age and weight for participants were 39.15 ± 0.05 weeks and 3016 ± 28 g, respectively. Crying duration either during or after the injection in breastfed infants was significantly shorter compared to the control and powdered formula groups (9.2 ± 3.9 and 16 ± 4.6 s vs. 38.2 ± 8.9 and 30.0 ± 4.4 s, respectively, during injection, $P < 0.003$); (11.8 ± 3.4 and 20.6 ± 5.1 s vs. 56.2 ± 6.5 and 49.8 ± 9.6 s, respectively, after injection, $P < 0.006$). There was also a significant relationship between behavioral variations and pain during injection ($P < 0.0001$).

Conclusions: The results of this study showed that breastfeeding decreases pain severity during painful experiences in neonates, which is in accordance with other reports. Based on this finding, neonates are advised to be breastfed if a painful intervention such as vaccination is needed. The pain-relieving effect of breast milk could also be added to its other suitable effects.

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1. Introduction

Pain is not only a symptom of discomfort, but it could be considered a major health problem with certain consequences, particularly during childhood [1]. Pain is an experience with either sensational or emotional aspects that is related to both actual and potential damage to body tissues [2]. Until 1980, it was believed

that neonates did not feel pain, although research in 1987 evaluated neonatal surgery in the absence of analgesics or anesthetics [3,4]. Later, research revealed that the physiological components of pain sensation are formed at the early stages of the fetal period and the rate of pain sensation at the nerve ends of neonates is similar or even greater than adults [5,6]. It might be assumed that pain is felt by neonates only during painful experiences and it is forgotten afterwards; however, chronic consequences will appear throughout life [7]. Painful experiences cause immediate, short-term and long-term effects such as fear, irritability, sleep disturbances,

* Corresponding author.

E-mail address: K.Asadollahi@masoud_1241@yahoo.co.uk (K. Asadollahi).

innutrition, lengthening of wound healing time, alteration of immunological activity, damage to emotional relations, delayed development, and different unexpected responses to painful experiences [8,9]. Many side effects associated with pain include increased serum levels of glucose, ketone particles, lactates, pyruvates and nonsteroidal acids, and catecholamine, as well as increased pulse rate, blood pressure, and intracranial pressure. Pain also causes a decline in blood oxygen, acidosis, irregular respiration, and pneumothorax [10,11]. The American Society of Pain considered pain as the fifth vital sign and suggested that healthcare providers monitor pain as they do other vital signs [12]. Iatrogenic pain due to medical interventions is one of the main problems in pediatrics and lack of associated pain-control procedures can result in long-term consequences and emotional stress at different levels [13–15].

Pain is one of the common experiences in hospital environments, even for healthy neonates who undergo medical procedures such as screening tests, vaccination, and circumcision. [16,17]. Painful medical interventions such as blood taking, catheterization, and application of intentional medications commonly occur in hospitalized neonates, which are accompanied by short- and long-term effects such as tachycardia, tachypnea, increased metabolic needs, an effect on myelination of nerves, particularly those associated with pain, altered responses to pain, and emotional reactions [18]. It is believed that breastfeeding, as a nonmedication method, can relieve pain, and a number of recent studies have reported that breastfeeding has considerably decreased the pain responses among full-term neonates [19]. Based on reports, mother's milk, particularly colostrum, includes a concentration of beta-endorphins twice as high as their rate in plasma, and the pain-relieving effects of milk via various mechanisms could be achieved, even in the absence of the infant's mother [20].

A number of physiological recommendations have been suggested by Canadian clinical practice to reduce pain during vaccination such as rapid vaccination, no aspiration, the most painful vaccine last, the least painful brand, positioning, tactile stimulation near the vaccination site, breastfeeding, and sugar water administration [21].

The aim of this study was to compare the effects of breast milk and powdered formula on pain severity after a muscular injection in 1-day-old neonates.

2. Materials and methods

2.1. Study design

One hundred neonates admitted to Taleghani Teaching Hospital in Ilam city, Iran, participated in a randomized clinical trial in 2016. The ethics committee of Ilam University of Medical Sciences (IUMS) approved this study and the neonates' parents completed an informed consent form before starting the project. One hundred 1-day-old neonates were divided into four equal groups including:

- the control group (no feeding);
- the breastfed group;
- the bottle-fed mother's milk group;
- powdered formula group.

The criteria for participation included full-term neonates, weighing ≥ 2500 g, born via vaginal delivery, having an Apgar score > 7 , lacking any disease or congenital disorder, breastfed at least once, at least 2 h after birth, and having a consent form completed. Some participants' data such as the mother's name, gestational age at birth, neonatal weight at birth, Apgar score,

and type of intervention were obtained from neonatal documents in the hospital. All infants received the hepatitis B vaccine by muscle injection in the same position of the thigh. The severity and duration of pain were recorded and compared among all groups during and after injection using the DAN scoring system (evaluation behavioral scale of acute pain in newborn infant) [22]. The hepatitis B vaccine was injected in neonates in the control group routinely with no feeding. However, the vaccine was injected in other three groups during oral feeding in different conditions: feeding from the mother's breast, bottle feeding of mother's milk, and feeding of powdered formula. A similar method was considered for three feeding groups, i.e., neonates in a calm environment started feeding for 2 min and were still feeding during the injection for at least 2 min.

All neonates including control groups (without feeding) were kept in their mothers' arms before, during, and after injection. Neonates in the control group were also maintained in a calm position for 2 min by their mothers but without feeding and promptly were returned to their previous conditions after injection without analgesia.

Maternal milk was taken from the mother's breast in a healthy condition by mothers and powdered formula was prepared based on instructions by mothers; all these procedures were carried out under supervision of neonatal ward nurses. Maternal milk via bottle and powdered formula were prepared and ready for use before the injection and at both times (before and after injection) were administrated similar to the breastfed group, in their mother's arms.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Data collection

Data associated with pain were collected by a prepared checklist in three sections:

- crying duration was recorded via a chronometer with 0.1 s accuracy;
- data associated with sound, face, and limb variations were recorded and physiological criteria such as pulse rate and oxygen saturation were measured by pulse oximeter;
- relevant scores were allocated to different responses of the neonate to pain, during and after injection, according to the DAN scoring method.

The rationale for the selection of hepatitis B vaccine in this study was due to the necessity and routine administration of this vaccine on the 1st days after birth, and because before this period neonates had not experienced any painful conditions.

Hepatitis B vaccination is performed routinely for all newborns before their discharge from the hospital. Among those nurses working in the vaccination unit, we selected one expert individual and during the study this person performed all vaccinations with similar criteria described for each group. Two nurses, who were blinded to the aim and methods of the study, were trained and they measured the scores separately; the mean scores measured by these individuals was recorded as the final result.

2.3. Statistical analysis

Data analysis was performed using SPSS 20 (SPSS Inc, Chicago, IL, USA) software. The results were expressed as mean \pm SD for

quantitative variables and/or frequencies for qualitative variables. The *t*-test and χ^2 test were applied for comparison of the results accordingly. A *P*-value equal to or less than 0.05 was considered statistically significant for all variables.

3. Results

One hundred neonates (57% boys) were randomly allocated into four equal groups. The mean \pm SD age and weight for participants were 39.15 ± 0.05 weeks and 3016 ± 28 g, respectively. Table 1 shows the comparison of the neonates' general characteristics: age at birth (39–39.3 weeks), birth-weight (2972–3202 g), and weight at vaccination (2928–3110 g) in the different groups. The results revealed that there was no significant difference between the four groups compared at the time of study for these variables ($P > 0.05$).

Table 2 shows the comparison of pulse rate, arterial O₂ saturation, and crying duration during and after injection in the different feeding groups. The results showed that only crying duration, during ($P < 0.003$) and after ($P < 0.006$) the injection, was significantly shorter in breastfed infants compared to the control and powdered formula groups.

Fig. 1 compares the mean score of behavioral variations due to pain during injection in the different neonate feeding groups based on the DAN scoring method. The results showed that the mean score of all behavioral variations including face grimaces, limb movement, and vocal responses during injection were significantly ($P < 0.0001$) lower in breastfed infants compared to the control, bottle-fed mother's milk, and powdered formula groups.

Fig. 2 compares the mean score of behavioral variations due to pain after injection in the different feeding groups based on the DAN scoring method. The results show that the mean score of all behavioral variations including face grimaces, limb movement, and vocal responses after injection were significantly ($P < 0.0001$) lower in breastfed infants compared to the control, bottle-fed mother's milk, and powdered formula groups.

Table 3 compares the behavioral responses due to pain during and after injection in the different feeding groups based

on the DAN scoring system. The results showed that the mean scores of face grimaces, limb movement, and vocal responses were significantly lower in breastfed infants compared to the control, bottle-fed mother's milk, and powdered formula groups ($P < 0.0001$).

4. Discussion

The aim of this study was to investigate the effects of neonatal feeding, in different methods and conditions, on pain severity after a muscular injection (hepatitis vaccination) in 1-day-old neonates based on a standard scoring system. The results showed that crying duration and behavioral responses such as face grimaces, limb movement, and vocal responses during and after injection were significantly lower in breastfed neonates compared to the other groups.

Evaluation of methods to prevent or treat the pain in neonates has interested academic and clinical media in recent decades. Because of the harmful effects of pain over the short- and long-term, including fear, irritability, sleep disturbances, innutrition, lengthening of wound healing time, alteration of immunological activity, damage to emotional relations, delayed development, and different unexpected responses to painful experiences, finding a method to reduce or eliminate pain experience in neonates has been sought by researchers, parents, and health policy makers. The effects of sugar solutions on pain relief has been approved by different studies; however, the pain-relieving effects of breast milk containing 7% glucose, commonly accessible to neonates, remain controversial [23]. Based on the results of this study, maternal milk, particularly breastfeeding, significantly decreased the crying duration during and after muscular injection compared with the other groups. Bottle-fed mother's milk compared to the powdered formula and control groups reduced the crying duration but not as much as breastfeeding. The duration of crying was also reduced nonsignificantly in the powdered formula group compared to the control group, but much less than that in the breastfed infants and those bottle-fed

Table 1
General characteristics of neonates in the different groups.

Variable	Group	Number	Mean \pm SD	<i>P</i> -value
Neonatal age at birth, (weeks)	Control, (no feeding)	25	39.1 \pm 0.6	0.14
	Breastfeeding	25	39.3 \pm 0.6	
	Bottle feeding mother's milk	25	39.2 \pm 0.5	
	Bottle feeding powdered formula	25	39 \pm 0.4	
Neonatal weight at birth, (g)	Control, (no feeding)	25	3202 \pm 221	0.113
	Breastfeeding	25	3000 \pm 351	
	Bottle feeding mother's milk	25	2972 \pm 384	
	Bottle feeding powdered formula	25	3032 \pm 285	
Neonatal weight at vaccination, (g)	Control, (no feeding)	25	3110 \pm 661	0.114
	Breastfeeding	25	2928 \pm 332	
	Bottle feeding mother's milk	25	3004 \pm 442	
	Bottle feeding powdered formula	25	3005 \pm 287	

Table 2
Comparison of some variables during and after injection in different neonate feeding groups.

Group	Pulse rate, (beats per minute)		Arterial O ₂ saturation, (mg/dL)		Crying duration, (s)	
	During injection	After injection	During injection	After injection	During injection	After injection
Control, (no feeding)	150.6 \pm 8.6	158.2 \pm 7	91.9 \pm 1.3	89.04 \pm 6.07	38.2 \pm 8.9	56.2 \pm 6.5
Breastfeeding	123.3 \pm 6.8	128 \pm 6	93.6 \pm 2.6	96.08 \pm 0.09	9.2 \pm 3.9	11.8 \pm 3.4
Bottle feeding mother's milk	144.4 \pm 9.9	146.9 \pm 1	94.4 \pm 1.3	93.5 \pm 1.5	16 \pm 4.3	20.6 \pm 5.1
Bottle feeding powdered formula	93.5 \pm 2	157.4 \pm 9.7	93.5 \pm 1.5	92 \pm 1.8	30.0 \pm 4.4	49.8 \pm 9.6
<i>P</i> -value	0.63	0.63	0.08	0.1	0.003	0.006

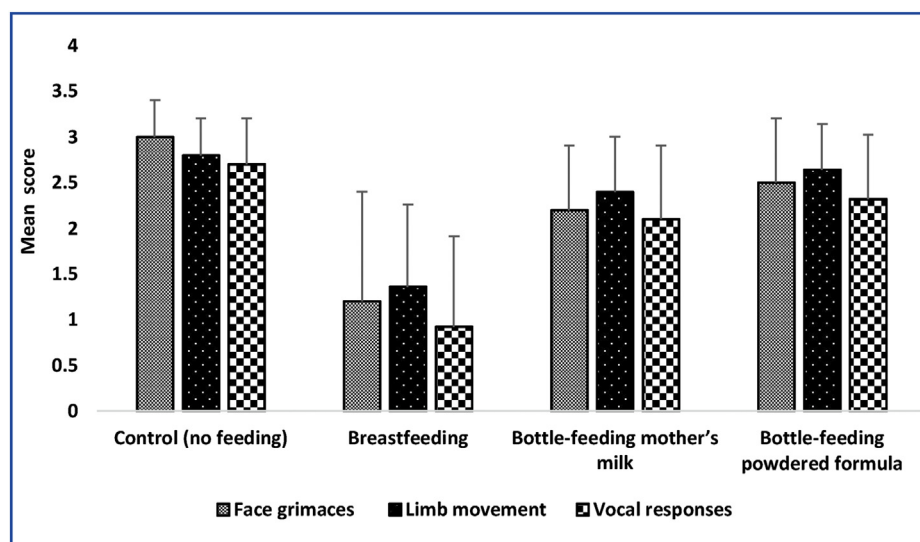


Fig. 1. Comparison of the mean \pm SD score for behavioral variations due to pain during injection in different feeding groups of neonates based on the DAN scoring method.

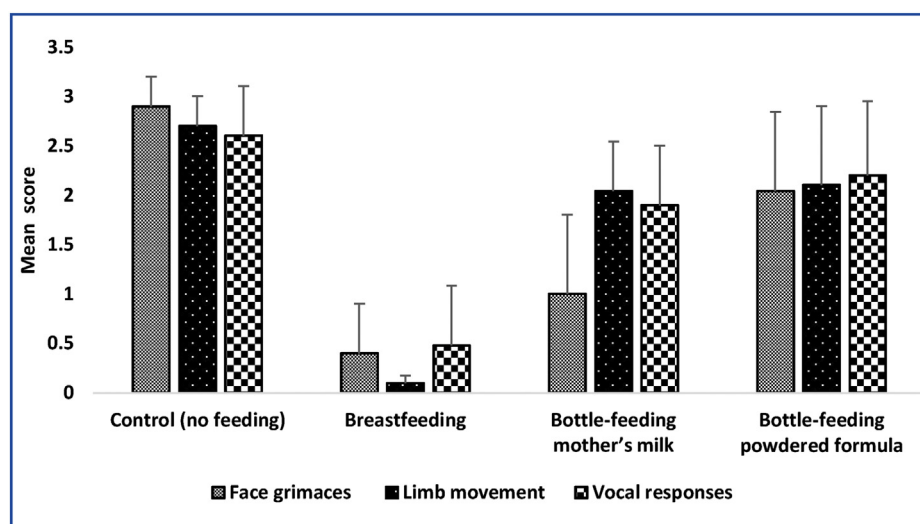


Fig. 2. Comparison of the mean \pm SD score for behavioural variations due to pain after injection in different feeding groups of neonates based on the DAN scoring method.

Table 3

Comparison of the behavioral responses due to pain during and after injection in different feeding groups of neonates according to the DAN scoring system.

Group	Face grimaces, (mean \pm SD)		Limb movement, (mean \pm SD)		Vocal responses, (mean \pm SD)	
	During injection	After injection	During injection	After injection	During injection	After injection
Control, (no feeding)	3	2.92 \pm 0.276	2.84 \pm 0.374	2.72 \pm 0.458	2.72 \pm 0.458	2.56 \pm 0.506
Breastfeeding	1.24 \pm 1.16	0.44 \pm 0.51	1.36 \pm 0.86	0	0.92 \pm 0.996	0.48 \pm 0.585
Bottle feeding mother's milk	2.24 \pm 0.723	1.76 \pm 0.597	2.4 \pm 0.577	2.04 \pm 0.538	2.12 \pm 0.832	1.93 \pm 0.64
Bottle feeding powdered formula	2.48 \pm 0.714	2.03 \pm 0.789	2.64 \pm 0.489	2.12 \pm 0.781	2.32 \pm 0.69	2.16 \pm 0.746
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

SD: standard deviation.

with breastmilk. If the difference between crying duration in the breastfed group and other groups was associated with mother's milk alone, it would be expected that parallel to the breastfed group the crying duration in infants bottle-fed mother's milk would be significantly lower than the control and powdered formula groups, but the duration was closer to the control and powdered formula groups than the breastfed group. Therefore, other confounders such as direct skin-to-skin contact between

mother and neonate, suckling the mother's breast, better adaptation of breast and nipple, sensation factors such as the mother's odor or the milk temperature should be considered for this discrepancy.

Based on the logistic regression analysis, there was no significant difference between the different groups for mean arterial oxygen saturation either during or after injection except for crying duration. This result was in accordance with the reports

by Modarres et al. [23], Badiie et al. [24], Carbajal et al. [25], Ghasemi et al. [26] and Phillips et al. [27]. Based on the results obtained herein, feeding the infant mother's milk during muscular injection, particularly breastfeeding, reduces all behavioral pain reactions and crying duration during and after injection in neonates. The Modarres et al. study showed that breastfeeding and skin contact between mother and neonate caused a reduction in face grimaces and crying duration in neonates who had blood taken from their foot [23].

In the current study, comparison of the face grimace, limb movements, and vocal responses in neonates showed that 93.3% of those with maternal milk feeding had calm faces, 89.3% had calm limb movement, and 93.3% had no vocal response during injection. These findings may indicate the comforting effects of breastfeeding during injection or a pain experience in neonates.

The mean scores of face grimaces, limb movement, and vocal responses based on the DAN scoring system, either during or after injection, were significantly lower in breastfed infants compared to the control, bottle-fed mother's milk, and powdered formula groups. A study by Badiie et al. compared pain severity in neonates who had blood samples taken from their foot, and showed lower pain severity in the breastfed group compared to the powdered formula group [24]. Another study by Carbajal and colleagues in 2003 revealed that breastfeeding during blood sampling significantly decreased the behavioral indices of pain and crying duration in neonates compared to the placebo group (feeding on distilled water), 30% glucose group, and 30% glucose + pacifier sucking group [28]. A study reported by Ghasemi and co-workers indicated that the severity and duration of crying as well as face grimaces, during and after vaccination, were significantly lower among breastfed neonates or infants bottle-fed mother's milk compared to those fed on powdered milk [26]. Phillips et al.'s study reported that neonates having a pacifier while being held by their mother indicated less painful reactions compared to those using a pacifier while being held by other individuals, and the most pain feeling control was in neonates held by their mothers and breastfed [27]. These reports are in accordance with our findings showing that maternal milk has a greater pain-relieving effect than other feeding types such as powdered formula, distilled water, 30% glucose, and pacifier sucking in neonates, and it is more effective when the child is breastfed, but this may be associated with other confounding factors.

According to the findings of the current study, maternal milk, even bottle-fed (nonsignificantly), decreased the mean scores of behavioral variations in neonates and therefore it could be assumed that maternal milk contains some pain-calming compounds. In addition, some studies have reported that the density of beta-endorphins in mother's milk, particularly in colostrum, is twice as high as its rate in plasma [20], and therefore more investigations in this field are suggested. Further studies are needed to explain the difference between pain indices, based on the DAN scoring system, between breastfeeding and bottle feeding mother's milk.

The group of infants who were breastfed had significant differences in pain severity compared with the bottle-fed mother's milk, powdered formula, and control groups. One of the limitations of this study was the lack of another control group using non-nutritive sucking to sort out the sucking effects on pain as a possible confounder; this should be considered in similar further studies.

5. Conclusion

The results of this study demonstrated that breastfeeding decreases pain severity during painful experiences in neonates,

which is in accordance with other reports. Based on these findings, it is recommended that neonates be breastfed during a painful intervention such as vaccination. The pain-relieving effects of breast milk could also be added to its other suitable effects. Some mothers and even healthcare workers may be worried about neonatal aspiration, vomiting, cyanosis, or breath variations if they are feeding during injections or painful experiences; however, the current study and similar studies have shown that these phenomena do not occur and breastfeeding neonates in these conditions could be advised.

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Disclosure of interest

The authors declare that they have no competing interest.

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