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ORIGINAL ARTICLE

Effects of Breast Milk and Vanilla Odors on Premature Neonate's Heart Rate and Blood Oxygen Saturation During and After Venipuncture

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heart rate;
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vanilla odor

Background: Different studies have shown that the use of olfactory stimuli during painful medical procedures reduces infants' response to pain. The main purpose of the current study was to investigate the effect of breast milk odor and vanilla odor on premature infants' vital signs including heart rate and blood oxygen saturation during and after venipuncture.
Methods: A total of 135 preterm infants were randomly selected and divided into three groups of control, vanilla odor, and breast milk odor. Infants in the breast milk group and the vanilla group were exposed to breast milk odor and vanilla odor from 5 minutes prior to sampling until 30 seconds after sampling.
Results: The results showed that breast milk odor has a significant effect on the changes of neonatal heart rate and blood oxygen saturation during and after venipuncture and decreased the variability of premature infants' heart rate and blood oxygen saturation. Vanilla odor has no significant effect on premature infants' heart rate and blood oxygen saturation.

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Conclusion: Breast milk odor can decrease the variability of premature infants' heart rate and blood oxygen saturation during and after venipuncture.

1. Introduction

The previous general belief was that premature infants cannot feel and sense pain; but nowadays, there is proof that premature infants can feel pain during surgical procedures. Recent studies show that infants can feel pain after the 26th week of gestational age.[1–4] Premature infants are frequently exposed to painful procedures during the first days of their life in neonatal intensive care units (NICU); hence, proposing methods that calm newborn infants is necessary.[5,6]

There are some studies that focused on the methods of calming newborn infants during painful procedures. The calming methods for infants can be divided into two main groups: pharmaceutical and nonpharmaceutical methods.[7] Because of the absence of biological harm, nonpharmaceutical methods are frequently discussed by researchers. Different methods based on infants' sense of taste,[8–10] sense of smell,[11] sense of hearing,[12] sense of touch,[13,14] and sense of sight have been frequently used in the literature. Previously published studies showed that the sense of smell is more mature than the other senses at birth. Newborn infants can detect the odor of their mother's nipples and get breast milk within the first days of their life.[15] The effects of breast milk odor[11,15] and vanilla odor[3,16,17] on infants' calming were discussed previously. The cited studies used different situations such as the infants' gestational age, sampling methods, and pain measurement scales. Some studies have discussed the calming effects of different odors on term infants during painful procedures; however, there are no studies about preterm infants. Some researchers believe that premature infants cannot recognize maternal breast milk odor because they are not fed by their mother's milk, but nowadays mother's milk is used for premature infants' feeding in NICU in many countries.

One of the main studies on the calming effects of different odors on preterm infants was conducted by Goubet et al,[3] who studied the effects of familiar and nonfamiliar vanilla odor on response to pain in preterm infants during venipuncture and heel stick. The sample size used in their study was small, and each group consisted of eight or nine individuals. Crying time and grimacing quality were used for investigation of odor calming effects. Results showed that infants who were exposed to a familiar vanilla odor had no significant increase in crying and grimacing during venipuncture. Infants in nonfamiliar odor group and control group showed a significant increase in crying time during venipuncture. Badiee et al[11,18] focused on the effect of breast milk odor and formula milk odor on preterm infants' responses to pain during and after heel lancing. They used premature infant pain profile (PIPP) scale for pain score measurement. Results showed that after the heel lancing, the PIPP score was significantly lower in the breast milk group than in the formula milk group. PIPP is a scale for pain score measurement including seven indicators. Three indicators are for facial actions, two are for heart rate and blood oxygen saturation, one is for gestational age, and one is for behavioral states. There are other pain assessment tools for premature infants[19–21] that use different indicators for pain measurement. Even though using different parameters can lead to an accurate evaluation of the pain that infants suffer during painful procedures, it also leads to limited information about variation in vital signs. However, it should be noted that rapid variations in infants' vital signs may cause lower brain development.[22] Therefore, studying the effects of pain on infants' vital signs is necessary.

The main purpose of this study is to report the effectiveness of breast milk odor and familiar vanilla odor on premature infants' vital signs during and after venipuncture. Specifically, we focused on the infants' heart rate and blood oxygen saturation. It is hypothesized that smelling breast milk odor during and after sampling decreases variations in infants' heart rate and blood oxygen saturation. A similar hypothesis is considered for the effect of familiar vanilla odor.

2. Methods

The current study was conducted at the NICU of Al-Zahra Hospital, which is affiliated to Tabriz University of Medical Sciences, Tabriz, Iran. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences, and parents' consent was obtained. Sample size was determined using the Pocock sample size formula. The level of statistical significance was set at 0.05. Blood oxygen saturation is considered as the main outcome of the study, and the difference between the estimated proportion of study outcome in the exposed and unexposed group is considered 1. As a result of the calculations, a sample size of 135 infants was required. The sample population was divided into three groups with 45 infants each. Infants who participated in the current study were premature infants with a gestational age of 28–34 weeks and postnatal age of 3–28 days. They were breast-fed and had no congenital or systemic abnormalities. They had a previous venipuncture experience and had physiological stability. Their Apgar scores were higher than 7 at 5 minutes after birth. Lack of intraventricular hemorrhage, lack of periventricular leukomalacia, no need for surgery, and not receiving analgesic were the other requirements. Infants who were eligible for inclusion in the study were randomly assigned into three groups using the Rand List software. Each group had 45 members, and the infants were not fed 30 minutes prior to venipuncture; their weights were measured prior to sampling. Infants in the breast milk group and the vanilla group were exposed to breast milk odor and familiar vanilla odor, respectively, from 5 minutes prior to the start of sampling to 30 seconds after the end of sampling. Infants in the control group were exposed to smell placebo. For exposing infants to the odors, a clean cotton ball dipped in breast milk or vanilla or placebo was placed 1 mm far from their noise. Infants of the vanilla group were familiarized with vanilla odor by a 10-g clean cotton ball dipped in 10 drops of vanilla solution. The vanilla solution had 0.64% vanilla (by mass), and for its preparation, vanilla was mixed with glycerin using a hot plate stirrer at 300 rpm [the hot plate stirrer was obtained from Heidolph Group (model MR 3001 K; Schwabach, Germany); vanilla and glycerin were obtained from Merck KGaA Chemical Company (Darmstadt, Germany)]. The cotton balls were placed in incubators 10 cm from the infants' nose 12 hours prior to sampling. Sampling was obtained from 7:30 AM to 8:30 AM. Previous studies showed that the time of painful procedures may affect the intensity of pain,[23] so the sampling time was similar for all infants. During the procedure, all infants were placed in a 37°C warmer. The heart rate and blood

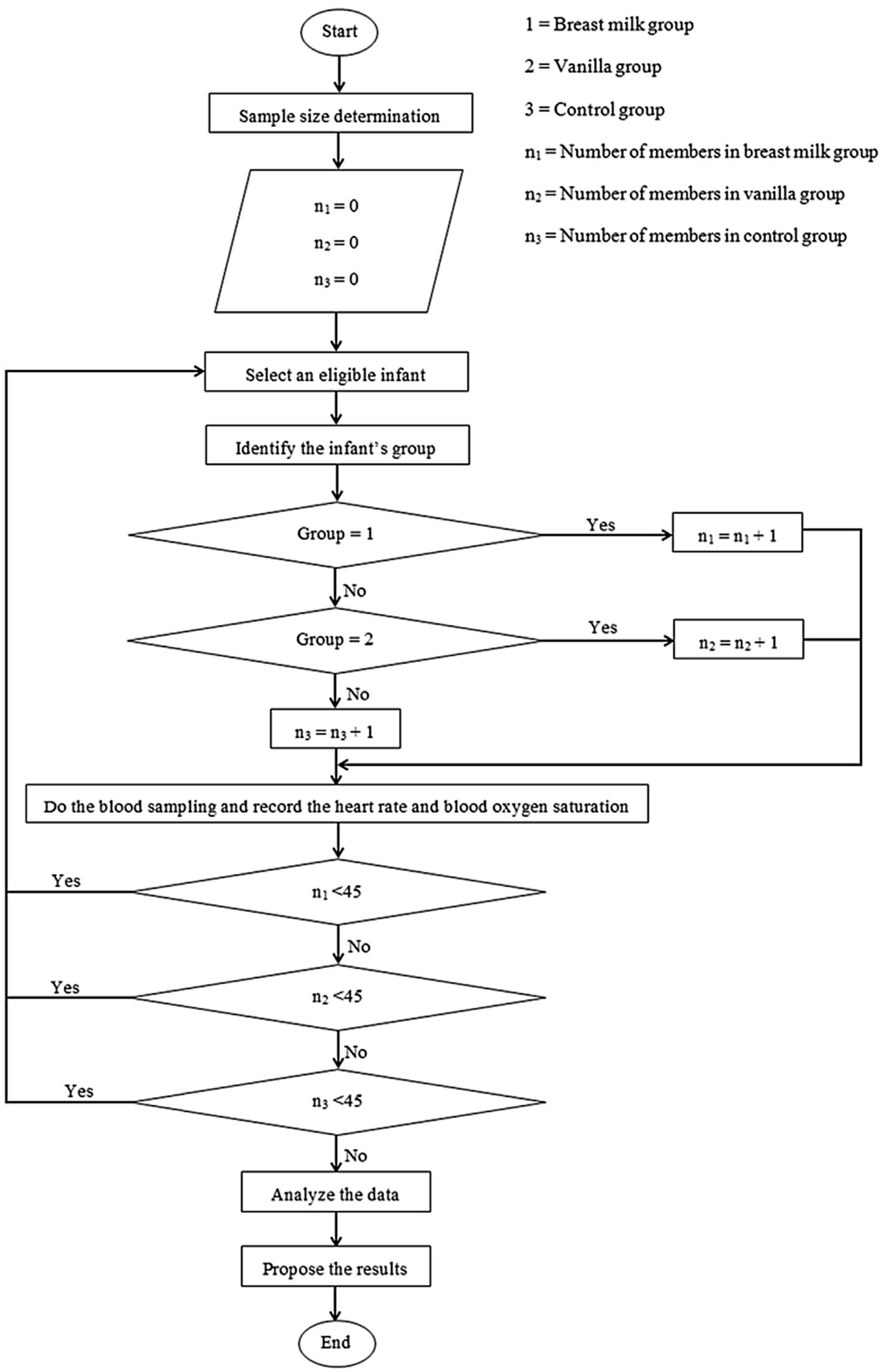

Figure 1 Data collection and problem-solving algorithm.

oxygen saturation were measured using a calibrated pulse oximeter (Novametrix Medical Systems Inc., Wallingford, CT, USA). Heart rate, blood oxygen saturation, and sampling period were registered by two cameras (Olympus, Center Valley, PA, USA). Heart rate and blood oxygen saturation were recorded 30 seconds prior to sampling, 30 seconds after the start of sampling, and 30 seconds after the end of sampling. Statistical analyses were performed on obtained data using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA), and $p < 0.05$ was considered statistically significant. Analysis of variance (ANOVA) was used to study demographic characteristics, heart rate, and blood oxygen saturation. For all variables, first, a three-group analysis was performed. If the p value of ANOVA was >0.05, another analysis was performed; but if the p value of three-group analysis was <0.05, Tukey's honest significant difference *post hoc* analysis was performed for comparing pairs of groups. Repeated-measures ANOVA was used for analyzing heart rate and blood oxygen saturation changes versus time. Using this option, heart rate and blood oxygen saturation at each time (prior to or during or after sampling) were compared to heart rate and blood oxygen saturation at other times. Similar statistical methods were used as described in previously published study.[15]

Figure 1 shows the algorithm of data collection and problem solving for current study. As shown, sampling was continued until the number of infants for every group reached 45.

3. Results

Repeated-measures ANOVA showed that the heart rate during sampling is significantly higher than the heart rate prior to the start of sampling for all groups ($p < 0.001$). A similar analysis showed that during and after sampling, blood oxygen saturation is significantly lower than the blood oxygen saturation prior to the start of sampling for all groups ($p < 0.001$). This shows that using breast milk odor and vanilla odor could not completely eliminate the effects of a painful procedure on vital signs.

Table 1 shows the demographic characteristics of infants in the three groups. It can be seen that there are no significant differences between the three groups in demographic characteristics.

Another important factor that might affect the results of the study was the infants' behavioral state prior to the sampling. Each state is organized into a pattern that differs from the other states. States are divided into four groups: sleep-active, sleep-quiet, awake-quiet, and awake-active. The Chi-square test shows that there is no significant difference between the behavioral states of infants in different groups ($p = 0.36$). Another important parameter is the infants' baseline heart rate and blood oxygen saturation. ANOVA showed that there was no significant difference between the infants' baseline heart rates in different groups ($p = 0.45$). Prior to sampling, the average heart rate was 151.31 ± 11.37 beats/min in the breast milk group, 157.1 ± 7.77 beats/min in the vanilla group, and 142.31 ± 11.53 beats/min in the control group. There were similar results for blood oxygen saturation. The statistical analysis showed that the infants' baseline blood oxygen saturation was not significantly different in different groups ($p = 0.62$). Prior to the start of sampling, the average of blood oxygen saturation was 91 ± 8.33 in the breast milk group, 88 ± 3.81 for the vanilla group, and 93 ± 6.03 for the control group.

Another parameter that can affect the heart rate and blood oxygen saturation during and after sampling is the sampling duration. Duration of sampling is the time (in seconds) elapsed between two times. The first one is the time it takes for the needle to be inserted into the vein, and the second one is the time it takes for the needle to be withdrawn from the vein. ANOVA showed that there is no significant difference between the sampling durations in the three groups ($p = 0.847$).

Figure 2 shows the variation in infants' heart rate during and after venipuncture. The figure shows that most of the infants in all groups have a heart rate increase of 0–14 beats/min. The distribution of decreased blood oxygen saturation is indicated in Figure 3. This figure shows that similar to heart rate, variation in blood oxygen saturation was not zero in breast milk and vanilla groups.

Table 2 shows the estimated mean and standard deviation of the variations in heart rate and blood oxygen saturation during and after sampling. The statistical analysis showed that variation in infants' heart rate in the breast milk group was significantly lower than the variation in infants' heart rate in the vanilla group ($p = 0.029$) during sampling. Moreover, there was a significant difference in the infants' heart rate variation between the breast milk group and the control group ($p = 0.021$), but there was no significant difference in the heart rate variation between the vanilla group and the control group ($p = 0.23$) during sampling. A similar trend was observed for blood oxygen saturation. Changes in blood oxygen saturation in the

Table 1 Demographic characteristics of infants in breast milk odor, vanilla odor, and control groups.

Group		Breast milk odor	Vanilla odor	Control	p
Sex	Girl	18 (40)	30 (66.7)	25 (55.4)	0.16
	Boy	27 (60)	15 (33.3)	20 (44.6)	
Gestational age (wk)		31.64 ± 2.1	30.93 ± 2	31.46 ± 1.96	0.22
Age (d)		10.03 ± 7.08	11.38 ± 7.06	10.60 ± 7.89	0.89
Birth weight (g)		1566.9 ± 414.89	1505.3 ± 409.12	1569.8 ± 405.93	0.17
Weight in sampling day (g)		1575.6 ± 322.93	1587.8 ± 365.84	1578.4 ± 354.63	0.175

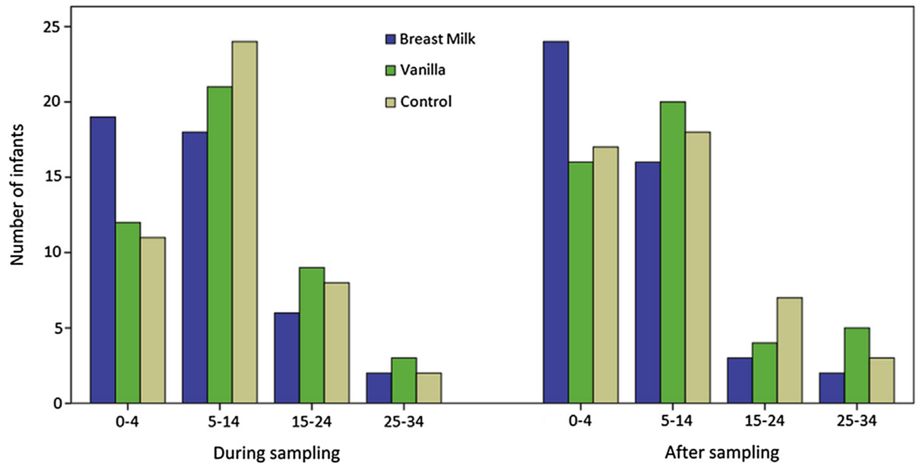

Figure 2 Heart rate variations during and after sampling (*X* axis shows changes in heart beats/min and *Y* axis shows number of infants).

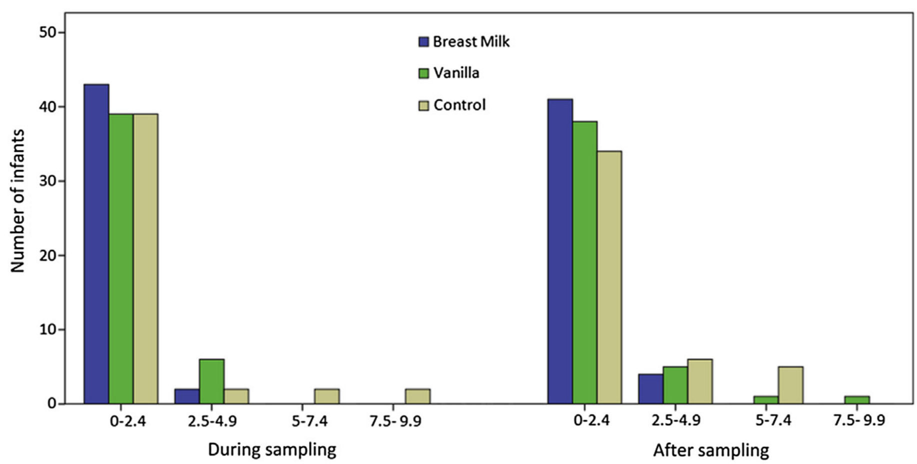

Figure 3 Blood oxygen saturation variations during and after sampling (*X* axis shows changes in blood oxygen saturation and *Y* axis shows number of infants).

breast milk group were significantly lower than the changes in blood oxygen saturation in the vanilla group ($p = 0.031$) and the control group ($p = 0.014$) during sampling. There was no significant difference in blood oxygen saturation changes between the vanilla group and the control group ($p = 0.16$) during sampling.

The results in Table 2 show that infants who were exposed to the breast milk odor had a significantly lower increase in heart rate and lower decrease in blood oxygen saturation compared to the vanilla group ($p = 0.018$) and the control group ($p = 0.011$) after the end of sampling. After the end of sampling, breast milk odor also affected the blood oxygen saturation. Blood oxygen saturation changes in the breast milk group were significantly different from the blood oxygen saturation changes in the vanilla group ($p = 0.048$) and in the control group ($p = 0.04$). Compared to the control

Table 2 Changes in heart rate and blood oxygen saturation of infants in breast milk odor, vanilla odor, and control groups during and after sampling.

Group	During sampling		After sampling	
	Heart rate changes (beats/min)	Blood oxygen saturation changes (%)	Heart rate changes (beats/min)	Blood oxygen saturation changes (%)
Breast milk odor	5.9111 ± 0.4871	0.3578 ± 0.07334	4.7556 ± 0.2963	0.5111 ± 0.09606
Vanilla odor	9.4444 ± 0.5334	0.9178 ± 0.2696	9.0444 ± 0.15742	1.0689 ± 0.09296
Control	9.6222 ± 1.0171	1.1422 ± 0.28232	8.8667 ± 0.9447	1.489 ± 0.1163
p	0.021	0.014	0.011	0.04

group, vanilla odor did not have any significant effect on heart rate ($p = 0.37$) and blood oxygen saturation ($p = 0.44$) after the end of sampling.

4. Discussion

The most important finding of this study is that breast milk odor can decrease the effects of painful procedures on infants' heart rate and blood oxygen saturation during and after blood sampling. Although there is no published research about the effects of breast milk and vanilla odors on premature infants' vital signs, there are several studies that have focused on the effects of premature infants' responses to pain. Nishitani et al[15] showed that smelling the breast milk reduces the pain felt during heel prick blood sampling. They had studied the term infants and had used salivary cortisol as an index of biochemical response to pain.[15] Similarly, Rattaz et al[17] showed that the duration of crying, head movements, and facial changes in infants who were exposed to breast milk odor were significantly lower than in other infants. Even though their study was conducted on term infants, the results showed that breast milk odor has a calming effect during painful procedures.[17] Badiee et al[18] studied the effect of breast milk odor on response to pain in premature infants. They used PIPP for pain measurement. PIPP reduction owing to breast milk odor smelling was the main result of their study.[18] All the studies have focused on the calming effects of breast milk, and variations in vital signs were not discussed in detail.

Another finding is that vanilla odor has no significant effect on heart rate and blood oxygen saturation. Goubet et al[3] showed that premature infants who were exposed to familiar vanilla odor had decreased crying duration and facial actions. Goubet et al[3] studied only the effects of vanilla on crying duration and facial actions, which can be affected by baseline behavioral state and gestational age.

Results showed that breast milk odor caused lower heart rate and blood oxygen saturation after sampling in comparison to heart rate and blood oxygen saturation prior to the start of sampling. Breast milk decreases the impact of pain in neonates and calms them during and after a painful procedure. Previous studies showed that breast milk odor has a calming effect on infants after the end of sampling.[15,17]

It is concluded that breast milk odor can be used as a nonpharmaceutical sedative in NICUs for calming premature infants during venipuncture.

5. Conclusion

The purpose of this study was to investigate the effectiveness of breast milk odor and familiar vanilla odor on premature infants' heart rate and blood oxygen saturation during venipuncture. The findings showed that venipuncture caused higher heart rate and lower blood oxygen saturation, and breast milk odor leads to lower variations in heart rate and oxygen saturation during venipuncture.

Many preterm infants are exposed to venipuncture blood sampling as a part of their medical care in the NICU. The current study indicates that having infants exposed to breast milk odor can decrease heart rate and blood oxygen saturation changes during and after sampling. Breast milk odor can be a good alternative in the management of vital signs fluctuation, and it can be easily used by nurses in NICUs during and after venipuncture.

Conflicts of interests

There were no conflicts of interests.

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References

1. Giannakoulopoulos X, Sepulveda W, Kourtis P, Glover V, Fisk NM. Fetal plasma cortisol and β-endorphin response to intrauterine needling. *Lancet* 1994;**344**:77—81.
2. Gupta R, Kilby M, Cooper G. Fetal surgery and anaesthetic implications. Continuing education in anaesthesia. *Crit Care Pain* 2008;**8**:71—5.
3. Goubet N, Rattaz C, Pierrat V, Bullinger A, Lequien P. Olfactory experience mediates response to pain in preterm newborns. *Dev Psychobiol* 2003;**42**:171—80.
4. Glover V. The fetus may feel pain from 20 weeks. *Conscience* 2004;**25**:35—7.
5. Craig KD, Whitfield MF, Grunau RV, Linton J, Hadjistavropoulos H. Pain in the preterm neonate: behavioral and physiological indices. *Pain* 1993;**52**:287—99.
6. Grunau R, Holsti L, Haley DW, Oberlander T, Weinberg J, Solimano A, et al. Neonatal procedural pain exposure predicts lower cortisol and behavioral reactivity in preterm infants in the NICU. *Pain* 2005;**113**:293—300.
7. Johnston CC, Fernandes AM, Campbell-Yeo H. Pain in neonates is different. *Pain* 2011;**152**:S65—73.
8. Bilgen H, Ozek E, Cebeci D, Ors R. Comparison of sucrose, expressed breast milk, and breast-feeding on the neonatal response to heel prick. *J Pain* 2001;**2**:301—5.
9. Stevens B, Yamada J, Lee GY, Ohlsson A. Sucrose for analgesia in newborn infants undergoing painful procedures. *Cochrane Database Syst Rev* 2013;**1**:CD001069.
10. Stevens B, Yamada J, Ohlsson A. Sucrose for analgesia in newborn infants undergoing painful procedures. *Cochrane Database Syst Rev* 2004;**3**:CD001069.
11. Badiee Z, Asghari M, Mohammadizadeh M. The calming effect of maternal breast milk odor on premature infants. *Pediatr Neonatol* 2013;**54**:322—5.
12. Standley JM. Meta-analysis of the efficacy of music therapy for premature infants. *J Pediatr Nurs* 2002;**7**:107—13.
13. Gray L, Watt L, Blass E. Skin-to-skin contact is analgesic in healthy newborns. *Pediatrics* 2000;**105**:e14.
14. Parry S. Acute pain management in the neonate. *Anaesth Intensive Care Med* 2011;**12**:121—5.
15. Nishitani S, Miyamura T, Tagawa M, Sumi M, Takase R, Doi H, et al. The calming effect of a maternal breast milk odor on the human newborn infant. *Neurosci Res* 2009;**63**:66—71.
16. Goubet N, Strasbaugh K, Chesney J. Familiarity breeds content? Soothing effect of a familiar odor on full-term newborns. *J Dev Behav Pediatr* 2007;**28**:189—94.
17. Rattaz C, Goubet N, Bullinger A. The calming effect of a familiar odor on full-term newborns. *J Dev Behav Pediatr* 2005; **26**:86—92.

18. Badiee Z, Nassiri Z, Armanian A. Cobedding of twin premature infants: calming effects on pain responses. *Pediatr Neonatol* 2014;**55**:262—8.
19. Grunau R, Craig KD. Pain expression in neonates: facial action and cry. *Pain* 1987;**28**:395—410.
20. Cignacco E, Mueller R, Hamers JP, Gessler P. Pain assessment in the neonate using the Bernese Pain Scale for Neonates. *Early Hum Dev* 2004;**78**:125—31.
21. Hummel P, Puchalski M, Creech SD, Weiss MG. Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain. *J Perinatol* 2008;**28**: 55—60.
22. van der Kolk BA. The neurobiology of childhood trauma and abuse. *Child Adolesc Psychiatr Clin N Am* 2003;**12**:293—317.
23. Hall JE. *Guyton and Hall textbook of medical physiology*. 12th ed. Elsevier; 2011.