The Effect of Kangaroo Care on Pain and Physiological Parameters in Preterm Infants on Heel-stick Procedure: A Randomized Controlled, Cross-over Study

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Abstract

Background: Due to the higher risk of pain in preterm infants, pain management is important for such infants. The present study aimed to determine the effectiveness of kangaroo care on the severity of pain and physiological parameters after heel-stick in preterm infants. Materials and Methods: This was a prospective and randomized controlled cross-over study. Twenty preterm infants allocated into an intervention (30 minutes of kangaroo care before and throughout heel-stick) and a control condition (standard incubator care during heel-stick). The severity of pain and the physiological parameters (heart rate and oxygen saturation) were collected after the heel-stick procedure. Results: The pain mean scores in the intervention and control conditions were respectively 4.9 (SD=2.91) and 11.00 (SD=4.21) and a significant difference was found between the two conditions regarding the pain score 30 seconds after the heel-stick [\(t=5.33, P<0.001\)]. Moreover, a significant difference between the two conditions concerning the infants’ heart rate (F=3.73, P<0.001) and oxygen saturation (F=1.84, P<0.001) was demonstrated during the three study periods. Conclusion: In order to relieve the preterm infants’ pain and prevent quick acceleration and deceleration of heart rate and oxygen saturation in the intervention condition, the intervention is recommended to be done in a nursery. [GMJ. 2013;2(4):157-68]

Keywords: Heel stick; Kangaroo Care; Pain; Premature Infant

Introduction

The number of the preterm infants admitted to Neonatal Intensive Care Units (NICU) has increased through the recent years. In order to maintain survival, these infants may have more than 10 painful procedures every day during their hospital stay [1]. It has been reported that 55\%-86\% of these procedures are related to heel-sticks [1-3]. In comparison to a full-term infant, a preterm one is at a higher risk for pain because of the
immaturity of the descending pathway to inhibit or dampen nociception at birth, resulting in hypersensitivity to pain [4,5]. Pain related to heel-sticks is a complex and multi-dimensional experience [6] which is life threatening, as well [7,8]. Moreover, it can have long-term cumulative behavioral consequences, such as changes in pain sensitivity [9,10], disturbance in structural and functional organization of the development of the brain [11], and reaction to stress [12,13].

Considering the complications of painful procedures in preterm infants, systematic pain management is a central issue in neonatal care to promote the well-being and unimpeded development of the neonate [14]. In the recent years, some pharmacological and non-pharmacological interventions have been introduced to treat and reduce the procedural pain. Pharmacological pain management for preterm infants might not be acceptable to most clinicians because of the side effects and complications of the medications [15]. The most attractive, safe, and easy non-pharmacological intervention enabling parental participation in preterm infants care is Kangaroo Care (KC) or skin-to-skin contacts. In a study, it was demonstrated that preterm infants in maternal KC showed lower pain mean scores in comparison to the paternal KC condition [16]. The kangaroo mother care has considerable advantages of reducing the expenditures, increasing the chances of survival because of better thermal control, promoting breastfeeding [17,18], and improving well-being and bonding [19].

The KC has been demonstrated to decrease the physiological and behavioral reactions to pain in preterm infants [20,21]. Castral et al. reported that skin-to-skin contact led to a decrease in the behavioral measures and less physiological increase during the procedure [22]. Physiological changes include a reduction of variation in heart rate [6,21,23,24], a diminished elevation in heart rate [20,22,25], an enhanced level of oxygenation [23], increased stability in oxygen saturation [21,24,25] and respiratory rate [6], decreased central venous pressures [23], and a shortened recovery time as denoted by return to baseline physiological values [20,23].

In Middle Eastern cultures, emotional relationship between the mothers and their infants is particularly strong. According to our own experiences of preterm infants, mother–infant separation after birth within the NICU is a known source of stress which might have adverse effects on the health status, development and function of the infants’ immune system, and neuro-cognitive development of the infant [26]. Although KC has been studied in preterm infants’ pain [16,22,27,28], this was limited in the Middle East [22,29] and it is not routinely used in Iran yet. Therefore, the present study aimed to determine the effect of the KC on the severity of pain and physiological parameters (heart rate and oxygen saturation) in a heel-stick procedure. It was hypothesized that being in the KC condition will decrease the pain severity response and variation of physiologic response in preterm infants. This study provides additional evidence-based findings in the Middle East.

Materials and Methods

Study population

The study population was selected from the NICU in Nemazi Hospital, Shiraz University of Medical Sciences (SUMS), Shiraz, Iran, between February 1st 2010 and January 31th 2011. The study protocol was approved by the Ethics Committee of SUMS, Shiraz, Iran. Eligible participants were all the infants who were between 27 and 36 weeks of gestational age and were 3–28 days old, were cared for in an incubator, were in a stable respiratory condition, had Apgar scores >6 at 5 minutes, whose mothers were >18 years old and Persian speaking, and had informed parental consent. On the other hand, the subjects with congenital, chromosomal, or neurological anomalies, intra ventricular hemorrhage, seizures, sepsis, surgery, and metabolic conditions as well as those who required pharmacological treatments, such as sedatives, skeletal muscle relaxants, and vasopressors, were excluded from the study.

Using a pilot study (n=6) with comparisons of the pain mean scores between the KC (5.83, SD=3.55) and incubator care (11.67, SD=3.98) conditions, power of 0.8, and
α=0.05, a 16-subject sample size was selected for the study (8 preterm infants in each condition). The sample size was increased to 20 infants to allow dropping-out. During the study, no subjects were withdrawn in both conditions and all the infants finished the study. The CONSORT diagram of enrollment, allocation, follow-up, and data analysis is shown in Figure-1.

Study design
The present randomized, controlled, double-blind, cross-over study was conducted on two conditions in order to determine the effects of the KC condition on pain 30 seconds after the heel-stick and also the physiological responses before, during, and after the heel-stick procedures in preterm infants. The preterm infants were selected through convenience sampling and were then allocated to intervention (KC) and control conditions (incubator care) by block randomization. In this cross-over study, some preterm infants were randomly assigned to “sequence A” and received the KC on the first day and routine incubator care on the second day. The other infants were randomly assigned to “sequence B” and received the routine incubator care on the first day of the study and the KC on the second day. Although cross-over designs are extremely powerful, such as controlling the intra- and inter-subject variability and providing the highest possible equivalence among the subjects exposed to both conditions, carry-over effects cause one condition to influence the second condition due to the experience of the first one [30]. Therefore, a washed period (24 h) was applied between the two conditions in order to remove the carry-over effects.

Figure 1. Effect of Kangaroo Care on Preterm Infant’s Pain; Diagram of Enrollment, Allocation, Follow Up and Data Analysis Based on a Cross Over Design. *KC: kangaroo care, IC: incubator care
Sequence A: the study condition was KC, then after 24 h IC; sequence B: the study condition was IC, then after 24 h KC
Outcome variables included the preterm infants’ pain score 30 seconds after the heel-stick and physiological parameters (heart rate and oxygen saturation) 2 minutes before (baseline), during, and 3 minutes after the heel-stick in the intervention and control conditions.

The Premature Infant Pain Profile was used for assessment of pain in preterm and full term infants (28–42 weeks of gestational age). This instrument evaluates the severity of pain by seven indicators: three behavioral (facial expressions: brow bulge, eyes compress, and nasolabial furrow), two physiological (heart rate and oxygen saturation), and two contextual (gestational age and sleep/wake state) [31-34]. Pain was scored on a four-point scale (0–3) for each of the seven indicators. The total score of this instrument ranges from 18 to 21 depending on the infant’s gestational age. Pain score <6 indicates non–painful events, score ≥6 suggests pain occurrence, and pain score ≥12 demonstrates moderate to severe pain [32].

Reliability and validity of “premature infant pain profile” for pain evaluation has been shown in many studies [32-34]. They reported the inter-rater reliability of the instrument as 0.85–0.95 (0.85 before and 0.90 during the heel-stick, 0.89 for the first three minutes after the heel-stick, and 0.90 for the 10th minute after the heel-stick) [35]. The instrument has also been used in more recent studies [35,20]. In our study, inter-rater reliability of the instrument 30 seconds after the heel-stick was 0.86. In addition, two individuals observed the same video-recordings of the heel-stick procedures (with a Kappa Coefficient of 0.90). The behavioral (facial expressions) and sleep/wake state of the “premature infant pain profile” were continuously recorded by a quiet, non-colorful, digital video camera (Canon IXY, 400F) with high quality facial images. The photos were taken by a professional photographer who was blind to the aim of this study. The photographs were taken from a close distance and focused on the infant’s face with very little surrounding area, while rotated to an angle in the kangaroo condition in order to decrease the probability of unblinding by the investigator assistants who scored the videotapes. A laptop computer with Media Player software which allowed viewing of the camera’s default screen was applied for viewing each recording session. The videotapes were coded based on the facial actions (brow bulge, eye squeeze, and nasolabial furrow) by another research assistant who was blinded to the aim of the study. Consistency and accuracy of the data were maintained by scoring the videotapes in a quiet room.

Physiological parameters (heart rate and oxygen saturation) were measured using a standard portable noninvasive, digital, electronic pulse Oximeter (Delphi model, Salran Inc). The heart rate and oxygen saturation were continuously recorded on a computer and professional technicians regularly calibrated the pulse oximeter monitor. The heart rate and oxygen saturation were measured according to the following procedure: The probe of the pulse oximeter monitor was placed on the left foot of the infant and connected to the data collection system. It was attached to the infant’s left foot from 5 minutes before, until 10 minutes after the heel stick. These two physiological indicators were recorded from the pulse oximeter for three episodes: two minutes before, during, and three minutes after the heel-stick.

**Intervention and Procedures**

The intervention in both conditions was as follows: In the intervention condition (the KC), the infants were undressed and only wore a diaper, a cap, and socks. The mothers also put on a gown leaving the chest area open and the infant was placed between the mother’s breasts with head upright at an angle of approximately 60°, providing maximal skin-to-skin contact between the infant and mother. To prevent heat loss, the infant was covered with a blanket. Throughout the procedure, the mother held her hands clasped behind the infant’s back. However, the touch did not have to be used and restrict the infant’s activity. The KC intervention was started 30 minutes before the heel-stick procedure and continued throughout the heel-stick and blood collection and 10 min after it. The mothers were ascertained that there was no audio recording in the room; therefore, they were asked to speak to
their infants during the procedure. The mother was bedded in a completely comfortable and relaxing position in an armchair in a quiet and semi–dark room in the NICU. This room was silent with a stable temperature of 28-30°C. On the other hand, the preterm infants in the control condition received standard NICU care but not the KC. In this condition, the diaper-clad infants were placed in a prone position with a head side–lying condition and supported with nested rolls around their body in the incubator at 30°C. The prone position was chosen since it controls the frontal pressure component of KC, enabling us to assess the maternal proximity component as well as the fact that it is suggested for preterm infants [20]. The preterm infants were enclosed with a blanket since at least 30 minutes prior to the lancing procedure until the end of data collection. In this period, the incubator was placed in another room with the same noise, light, and temperature levels as the intervention condition.

Initially, the parents of the infants who had the inclusion criteria received a pamphlet which briefly described the present study. More details of the study were provided for the interested parents and, subsequently, parental informed consents were obtained from them.

The procedure of the heel-stick was performed between 8-10 A.M. as follows:

In both conditions, the infants remained undisturbed for 30 min prior to the heel-stick procedure until 10 min after blood collection. During the 30 min before the heel-stick, the infant’s right foot was covered with a blanket in both conditions. The blood samples were collected at the 30th minute of the KC as well as the control condition for routine laboratory examination (blood glucose). During the heel-stick procedure, the infant’s right foot was pulled out from the blanket. Blood-Lancets (BLUTLANZETTEN, Item-No. 211 01 01) with a blade length of 2 mm were used for the heel-stick procedure and the samples were obtained from the outer regions of the heel. A standardized protocol was used for heel-stick procedure [31] by the clinical lab technician who was blind to the study objectives and was trained by the author to a) Grasp the foot firmly and select the site, b) Release the foot and allow the first drips of blood to form, c) Wipe away the first drop of blood and avoid excessive squeezing, d) Hold the foot firmly and collect the blood from free-flowing drops at puncture site, and e) Release the foot and allow the infant to kick from time to time as this will increase the blood flow. When blood collection was complete, cotton ball and bandages were held on the lancet site and mild pressure was applied. It was held until the bleeding stopped and homeostasis occurred [31]. After that, the infant’s foot was covered with the blanket. The infants in the intervention and the control condition stayed in their position continuously for 10 minutes after blood sampling. The mean duration of the heel-stick procedure was 3.45 and 3.50 seconds in the intervention and the control condition, respectively. It should be noted that in the incubator care condition, the heel-stick was conducted in the incubator with nested rolls around the body of the preterm infants.

**Ethical considerations**

The infants were recruited into the study by one of the researchers and written informed consents were taken from all the preterm infants’ parents. The parents were free to stop participation in the study at any time. Besides, they were informed that participation/nonparticipation in this study would not influence the care their infants would receive.

**Statistical analyses**

The data were analyzed using the SPSS statistical software (v. 14.0). Descriptive statistics (mean, SD, frequency, and percentage for category data) were used. Independent Student t-test was used in order to compare the mean of pain score in KC and incubator care conditions 30 seconds after the heel-stick procedure. Moreover, repeated measures analysis of variance (RM-ANOVA) was utilized for comparing the preterm infants’ heart rate and oxygen saturation in the three times in two conditions. P<0.05 was considered as statistically significant.

**Results**

Demographic characteristics of the mothers
and their infants are shown in Table-1. The majority of the infants had been delivered by cesarean section (70%) and most of the infants were male (60%). Gestational age ranged from 27 to 36 weeks, with a mean of 31.85 (SD=3.11) weeks and the infants’ mean age was 13.40 days old (SD=9.21 days) on the first day of the study. In addition, the newborns had a mean birth weight of 1,755 g (SD=20.52 g) ranging from 860 to 2600 g. The mean of the infants’ mothers’ age was 28.90 (SD=5.33) years old and 5–min Apgar score was obtained as 8.35 (SD=0.87, range 6 to 9). No significant difference was found between the infants who had KC on the first day and those who had KC on the second day regarding their demographic characteristics. The mean scores of pain 30 seconds after the heel-stick in the intervention and control conditions were 4.9 (SD=2.91) and 11.00 (SD=4.21), respectively. A significant difference was observed between the two conditions regarding pain score 30 seconds after the heel-stick (CI: 3.78–8.41, t = 5.33, P<0.001). The mean of heart rate (beat per minute, bpm) was not different between the intervention (145.65, SD=16.05) and the control (144.45, SD=15.24) conditions at the baseline. However, the mean of heart rate during the heel-stick was significantly different between the two conditions (t=-2.75, P=0.009). During the heel-stick, the heart rate in the control condition increased by a greater extent compared to the intervention condition (P=0.009). Nevertheless, no difference was found between the two conditions 3 minutes after the heel-stick (t=0.65, P=0.52). Table-2 shows the mean of heart rate in both conditions before, during, and after the heel-stick procedure.

As can be seen in Figure-2 and Table-2, RM-ANOVA demonstrated a significant difference between the intervention and control conditions regarding the infants’ heart rate during the three study periods (F=3.73, P<0.001).

As shown in Table-2, before the heel-stick procedure, the oxygen saturation parameters for the intervention and control conditions were 93.5 (SD=4.62) and 95.00 (SD=3.19), respectively. No significant difference was observed between the two conditions regarding the mean of oxygen saturation two minutes before the invasive procedure (t=1.20, P=0.23).

The mean of oxygen saturation decreased to 93.00 (SD=5.54) in the intervention condition and 90.05 (SD=7.45) in the control condition during the heel-stick. Therefore, oxygen saturation in both conditions decreased between the first and second oxygen saturation assessments, but the magnitude of the decrease was greater in the control condition. However, the difference between the two conditions in the second measurement of oxygen saturation was not significant (t=1.42, P=0.16) (Table-2). The oxygen saturation indicators in the 3rd minute of the heel-stick technique were 93.85 (SD=3.40) and 93.50 (SD=4.62) in the intervention and control conditions, respectively. The mean of oxygen saturation in the 3rd minute of the heel-stick procedure was not different between the two conditions (t=0.27, P=0.78), (Table-2).

As Figure-3 depicts, the changes of oxygen

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N (%)</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers’ age</td>
<td>28.90 (5.33)</td>
<td>21–36</td>
<td></td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>12 (60%)</td>
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<tr>
<td>female</td>
<td>8 (40%)</td>
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<td>Birth type</td>
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<tr>
<td>Vaginal</td>
<td>6 (30%)</td>
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<tr>
<td>Cesarean section</td>
<td>14 (70%)</td>
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<td>Gestational age</td>
<td></td>
<td></td>
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<tr>
<td>27-30</td>
<td>7(35%)</td>
<td>31.85 (3.11)</td>
<td>27–36</td>
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<tr>
<td>31-33</td>
<td>7(35%)</td>
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<tr>
<td>34-36</td>
<td>6(30%)</td>
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<tr>
<td>APGAR score 5 min</td>
<td>8.35 (0.87)</td>
<td>6–9</td>
<td></td>
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<tr>
<td>Birth weight (g)</td>
<td>1755 (20.52)</td>
<td>860–2600</td>
<td></td>
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<tr>
<td>Postnatal age (day)</td>
<td>13.40 (9.21)</td>
<td>3–28</td>
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Table 2. Effect of Kangaroo Care on Preterm Infant’s Pain: Mean of heart rate and oxygen saturation parameters in Kangaroo care and incubator care condition in preterm infants through 2 min before, during, and 3 min after heel-stick procedure.

| Variable       | Condition        | 2 min before (Mean (SD)) | During (Mean (SD)) | 3 min after (Mean (SD)) | RM-ANOVA
df | Within-Subjects | Between groups |
<table>
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</thead>
<tbody>
<tr>
<td>Heart rate [bpm]</td>
<td>Kangaroo care</td>
<td>144.45 (15.24)</td>
<td>145.80 (17.93)</td>
<td>143.50 (17.66)</td>
<td>F=13.11</td>
<td>P &lt; 0.001</td>
<td>F=3.73</td>
</tr>
<tr>
<td></td>
<td>Incubator care</td>
<td>145.65 (16.05)</td>
<td>158.20 (16.29)</td>
<td>146.95 (15.77)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>t=0.24, P=0.80</td>
<td>t= -2.75, P=0.009</td>
<td>t=0.65, P=0.52</td>
<td></td>
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<tr>
<td>Oxygen saturation</td>
<td>Kangaroo care</td>
<td>93.50 (4.62)</td>
<td>93.00 (5.54)</td>
<td>93.85 (3.40)</td>
<td>F=8.61</td>
<td>P = 0.01</td>
<td>F=1.84</td>
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<tr>
<td></td>
<td>Incubator care</td>
<td>95.00 (3.19)</td>
<td>90.05 (7.45)</td>
<td>93.50 (4.62)</td>
<td></td>
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<td></td>
<td></td>
<td>CI: -4.04–1.04</td>
<td>CI: -1.25–7.15</td>
<td>CI: -2.25–2.95</td>
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<tr>
<td></td>
<td></td>
<td>t=1.20, P=0.23</td>
<td>t=1.42, P=0.16</td>
<td>t=0.27 , P=0.78</td>
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</table>

‡ RM-ANOVA= repeated measures analysis of variance  
* P-values refer to level of significance of the time × group interaction of RM-ANOVA with time (2 min before, during, 3 min after intervention) between groups (Kangaroo care vs. incubator care condition)

Figure 2. Effect of Kangaroo Care on Preterm Infant’s Pain; the Infants’ Heart Rate Repeated Measures Analysis of Variance (RM-ANOVA) Between the Kangaroo Care and Incubator Care Conditions Across the Three Study Periods

saturation across the phases of the procedure had the same patterns in both conditions. The oxygen saturation dropped from the baseline during the heel-stick procedure and increased 3 minutes after the procedure. Moreover, RM-ANOVA showed a significant difference between the two conditions regarding the infants’ mean scores of oxygen saturation during the three study periods (2 min before, during, and 3 min after the heel-stick) (F=1.84, P<0.001), (Table-2).
Discussion

The present study aimed to investigate the effects of KC on pain severity and physiological parameters of preterm infants requiring the heel-stick. The study results revealed a significant difference in pain severity in preterm infants 30 seconds after the heel-stick procedure. In addition, a significant difference was found between the intervention and control conditions regarding the infants' heart rate and oxygen saturation during the three study periods.

This study indicated that the mean of pain score 30 seconds after the heel-stick was significantly lower in the intervention condition compared to the control condition. These findings are consistent with other studies conducted on the issue [20,25,36]. Furthermore, it was revealed that the pain scores of the preterm infants were significantly lower at the 1st minute after the invasive procedure on the KC condition [28], while the infants in the control condition, who did not get the KC, had severe pain during the painful situation [28]. Researchers compared the pain response in infants during heel blood sampling situation in KC for 10–15 minutes prior to the invasive procedure with newborns who were swaddled. In their study, a significant decrease of severity of pain was detected in the KC compared to the swaddling infants [37]. In a systematic review, it was found that there was evidence that maternal KC could significantly diminish pain from a single pain situation in full-term infants as well as stable preterm infants (>26 week of gestational age) [38]. The KC could have an analgesic impact in various paths. First, KC’s continuous tactile arousal may perform as a pain inhibitory system by activating endogenous pain-modulating systems. Second, KC’s analgesic impacts is thought to be due to the blocking nociceptive transmission of afferent pathways or activating the descending inhibitory pathways [39,40]. Third, KC’s analgesic impact may be due to the effects of oxytocin. During skin-to-skin contact, the release of oxytocin increases in both infants and their mothers [41]. Oxytocin reduces the maternal anxiety and increases the calmness as well as social responsiveness [42]. Fourth, KC may indirectly decrease the neonatal pain by reduction of the total amount of noxious stimuli to which neonates are exposed to [39,40]. During KC, infants fall deeply asleep and are less prone to excitement due to the environment happenings in comparison to the time they are in incubators [43]. A final mechanism may be the maternal attendance which is related to the presence of maternal odor [39]. It was demonstrated that the infants who sense the familiar maternal odor during a heel-stick show no enhancement in crying compared with baseline crying levels [44]. The KC re-

Figure 3. Effect of Kangaroo Care on Preterm Infant’s Pain; the Infants’ Oxygen Saturation Repeated Measures Analysis of Variance (RM-ANOVA) Between the Kangaroo Care and Incubator Care Conditions Across the Three Study Periods
laxes infants, reduces the level of stress [45], and decreases the behavioral signs of discomfort [39]. The results of this study showed a significant difference between the intervention and control conditions regarding the infants’ heart rate during the three study periods, which is in line with other studies [20,21]. Johnston et al. demonstrated that the mean of heart rate was significantly lower at 30, 60, and 90 seconds after lance sampling in very preterm neonates [20]. In a longitudinal study for 90 min (15 min before, 60 min in KC and 15 min after) in preterm infants, differences were observed between these periods regarding heart rate [21]. In our study, during the heel-stick, more heart rate acceleration was seen in the incubator care condition compared to the KC state. Castral et al. [22] revealed that both groups of infants indicated enhanced heart rate during the puncture and heel squeeze although the changes in these parameters were less for the skin-to-skin contact condition [22]. Similar to our study, Warnock et al. observed that the infants had biphasic response to heel-stick and more heart rate deceleration in the incubator care in comparison to the KC condition [38]. The biphasic response pattern is related to the fear paralysis reflex, described by a sympathetic inhibition together with a vagal bradycardia, which can be activated by sudden noise and pain stimuli in infants. The sharp fall in heart rate can cause decreased cerebral blood flow and even fainting or death [27,46]. The findings of the current study revealed a significant difference between the intervention and control conditions regarding the infants’ oxygen saturation across the three study periods and following the heel-stick, the oxygen saturation decreased in both groups. However, a higher drop was seen in the incubator care condition and the infants in the KC situation had approximately stable oxygen saturation. Researchers have shown average oxygen saturation levels being significantly higher at 60 and 90 seconds post-heel lance in the KC compared to the incubator care [20]. The KC appears to be advantageous regardless of the infant’s oxygen requirement and during KC, oxygen demand may decrease or just become stable [47,48]. It was reported that the upright position of KC enhanced the efficiency of the diaphragm and pulmonary function, improving oxygenation by promoting cardio-respiratory stabilization. In addition, oxygen saturation is also enhanced due to the relationship between KC and motor regulation. This also diminishes agitation and, consequently, oxygen is saved and is not used up in excessive movements [49]. Limitations and implications This study had some limitations. The number of participants in each condition was too low to determine whether the intervention was effective in each phase or not. In addition, the pain score was only measured 30 seconds after the heel-stick. Also, the study participants included only the infants who were stable and with gestational age ≥ 27. Despite trying to provide blinding in each part of this research, one of the limitations was lack of blinding in the photographs of the infants’ faces immediately after the heel-stick. The Mother’s breathing revealed the fact that the infant was in the KC condition; therefore, the score of facial expression cloud not be blinded to the incubator and the KC conditions. Another limitation of the study was using the manual lancet. Therefore, for prevention of variability in heel-stick, future studies are recommended to use a standard automatic lancet device. Moreover, future studies with larger sample sizes are needed to measure the pain score over time; i.e., before, during, and after the invasive procedure. Other studies should also be conducted in order to investigate the infants who are not stable, those being cared on a ventilator, severely ill infants, and the ones with gestational age<27 weeks. Future studies could evaluate the effects of KC on neuro-developement or biological outcome of infants, such as salivary cortisol levels, energy consuming, sleep disturbance, tissue oxygenation index, and infant’s growth and development. The effects of the KC on mother’s psychological and physiological parameters should also be evaluated in future studies. Conclusions The finding of this study helps nurses and
health care assistants to provide effective non-pharmacological interventions for pain management and relieving the pain immediately after the painful procedures. With regard to relieving the preterm infants’ pain and prevention of quick acceleration and deceleration of heart rate and oxygen saturation in the KC condition, it is suggested that this intervention be done in nursery and its effects on mediating the autonomic nervous system’s responses to pain, such as the heart rate and oxygen saturation, should be highlighted for the parents, nurses, and physicians. To improve evidence-based nursing, it is recommended that further studies be conducted on the effects of this intervention on decreasing stress and the infants’ growth and development.

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