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REGULAR ARTICLE

Maternal singing during kangaroo care led to autonomic stability in preterm infants and reduced maternal anxiety

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ABSTRACT

Aim: Kangaroo care (KC) and maternal singing benefit preterm infants, and we investigated whether combining these benefitted infants and mothers.

Methods: A prospective randomised, within-subject, crossover, repeated-measures study design was used, with participants acting as their own controls. We evaluated the heart rate variability (HRV) of stable preterm infants receiving KC, with and without maternal singing. This included low frequency (LF), high frequency (HF) and the LF/HF ratio during baseline (10 min), singing or quiet phases (20 min) and recovery (10 min). Physiological parameters, maternal anxiety and the infants' behavioural state were measured.

Results: We included 86 stable preterm infants, with a postmenstrual age of 32–36 weeks. A significant change in LF and HF, and lower LF/HF ratio, was observed during KC with maternal singing during the intervention and recovery phases, compared with just KC and baseline (all p-values <0.05). Maternal anxiety was lower during singing than just KC ($p = 0.04$). No differences in the infants' behavioural states or physiological parameters were found, with or without singing.

Conclusion: Maternal singing during KC reduces maternal anxiety and leads to autonomic stability in stable preterm infants. This effect is not detected in behavioural state or physiological parameters commonly used to monitor preterm infants.

INTRODUCTION

Mothers of preterm neonates are increasingly interested in how they can contribute to their infant's growth and comfort in the neonatal intensive care unit (NICU). Feldman and Eidelman reported improved neurobehavioral regulation as a result of early kangaroo care (KC) in the NICU (1). Music therapy, another method used to promote growth and to reduce stress has also been recommended as part of developmental care in the NICU (2).

Exposing infants to their mothers' voices in NICUs has been shown to increase oxygen saturation (3), decrease episodic apnoea and bradycardia (4) and improve weight gain (5) and feeding tolerance (6). In a large trial of 272 preterm infants ≥32 weeks of gestation, live music interventions applied by music therapist were shown to enhance quiet, alert and sleep states, improve suck response and oxygen saturation and reduce perceptions of fear and anxiety in parents (7). An intervention that played a recording of the mother's voice when the infant sucked on a pacifier was associated with decreased duration of tube feeding, without adverse effects on stress or growth (6).

Infants born prematurely spend their first weeks and even months of life in the NICU. During this time, they are deprived of the maternal sounds they would otherwise hear in utero. Evidence, primarily from animal studies, suggests that the functional development of the auditory system is largely influenced by environmental acoustic inputs early in life (8,9). Therefore, recorded maternal voices have been suggested as a supplement to maternal speech exposure, even when the mother was not physically present (10).

KC is an intervention that brings an infant in direct contact with the mother, skin-to-skin, and could be the ideal way to transmit maternal sounds, especially singing.

Abbreviations
HRV, Heart rate variability; HF, High frequency; LF, Low frequency; KC, Kangaroo care; NICU, Neonatal Intensive Care Unit; STAI, State-trait anxiety inventory.

Key notes
- The combination of kangaroo care and maternal singing has not been evaluated in preterm infants.
- Our study showed that combining maternal singing with kangaroo care resulted in better autonomic stability, as indicated by heart rate variability changes, in stable preterm infants of 32–36 weeks' postmenstrual age and reduced maternal anxiety.
- Combining kangaroo care and maternal singing is recommended for stable, preterm infants.

Authors who have tried to combine music and KC have failed to show marked benefit for preterm infants (11,12). However, in those studies, an external music source was used and not the mother's voice. In the current study, we hypothesised that hearing their mother's voice, together with KC, would be beneficial to the preterm infant. Given that markers such as heart rate, respiratory rate, oxygen saturation and observational parameters may not be sensitive enough to detect a change (12,13), we decided to add the measure of heart rate variability (HRV) power.

Heart rate, measured by counting heartbeats per minute, is the net effect of the decelerating influence of the vagal (parasympathetic) fibres and the accelerating influence of the sympathetic fibres on the inherent rhythmicity of the heart's sino-atrial node. HRV has been used to evaluate autonomic nervous system fluctuations in heart rate related to maturation in healthy infants and in infants with pathological conditions. The autonomic nervous system undergoes significant maturation between 31 and 38 weeks of gestation, represented by decreases in heart rate and increases in HRV (14). Decreases in heart rate that occur with maturation represent increasing parasympathetic influence (vagal tone) on heart rate and better sympatho-vagal balance and stability. This maturation provides a neural platform to support the increasing ability of the infant to engage objects and people in a dynamic environment (15). Greater maturation of the autonomic nervous system is related to better outcomes, including gross motor skills and mental processing in very low birth weight infants (16). Interventions in the neonatal period, such as KC, were shown to enhance autonomic nervous system development (1). KC during painful stimuli has been shown to cause changes in HRV that indicate more autonomic stability among preterm infants (17). This study evaluated the effect of KC in combination with maternal singing compared with KC alone on autonomic stability. Furthermore, we evaluated whether KC with maternal singing had an effect on maternal anxiety compared with KC alone.

METHODS

Participants

This study was performed in the NICU of the Meir Medical Center, Kfar Saba, Israel, from 1 October 2011 to 31 March 2012. Inclusion criteria were clinically stable infants at a postmenstrual age of 32–36 weeks, whose hearing was confirmed by distortion product oto-acoustic emissions. Exclusion criteria were a congenital anomaly that affected hearing and brain anomalies associated with neurological problems, such as grade 3–4 intraventricular haemorrhage and periventricular leukomalacia. Additional exclusion criteria were hyperalertness to maternal voice, defined as crying when the mother started singing and relaxing when the music stopped and medication that might have interfered with the reaction to musical stimuli, such as anticonvulsive or sedative drugs or oxygen, Of 116 preterm infants screened for the study, 10 were excluded for medical indications and 15 were excluded as the parents refused to participate. In another five dyads, data were incomplete. The study population comprised of 86 mother–infant dyads, belonging to 53 Jewish and 33 Arab families.

The study was approved by the Institutional Review Board of Meir Medical Center and conducted in line with the ethical and humane principles of research established by the Declaration of Helsinki (trial registration number NCT 01427894). Before enrolment, the parents received verbal information about the study objectives and design, and provided written informed consent.

Procedure

In this prospective, randomised intervention, a within-subject, crossover, repeated-measures design was used with participants acting as their own controls. Each therapy session started 30 min after completion of feeding. The sessions began with 10 min of KC therapy alone, followed by either KC alone or the intervention of KC and live maternal singing for 20 min. The sessions ended with another 10 min of KC therapy alone. The two therapies were performed (with mother–infant dyads) over 2 days, alternating according to the randomisation schedule. A table of random numbers was used, where value one was assigned to KC alone on the first day and KC with live singing on the second day, and the value zero was assigned to the opposite order. One investigator (GS) generated the random allocation sequence and put the numbers in sealed envelopes. Another investigator (SA) enrolled participants and a third investigator (CD) assigned participants to interventions after signed informed consent was obtained.

During KC, the mother sat in a chair, reclining at approximately a 40-degree angle beside the crib. The infant was placed upright, prone, in skin-to-skin contact with the mother, who laid a receiving blanket folded in quarters across the infant's back with her hospital gown over that. Body temperature was recorded continuously by a servo mechanism. Live maternal singing was performed during KC. The mother was instructed to sing with a repetitive, soothing tone, softly, simply and with slow tempo. She was asked to include lullabies, preferably those that she sang during pregnancy, in her preferred language. The mothers could see a sound analyser (Extech SL 130, Ectech instruments, Nashua, NH, USA) placed near their infant's ear and were instructed to sing at a sound level of between 60 and 70 dB. The ambient noise level was controlled to minimise its effect on mother–infant behaviour. Monitor alarms were silenced and the door was closed. As recommended, environmental sound levels during procedures did not exceed 45 dB (18).

Measures

Measurements were taken during all phases of the study, baseline, singing or quiet phases and recovery. The infants' and mothers' heart rates, oxygen saturation and respiratory rates were recorded every 2 min. A seven-point score, based on the criteria of Als et al. (19), was used to assess the infants' behavioural states: (i) deep sleep, (ii) light sleep, (iii) drowsy, (iv) quiet awake or alert, (v) actively awake and aroused, (vi) highly aroused, upset or crying and (vii) prolonged respiratory pause of more than eight-seconds. Maternal anxiety was measured before and after each session, using the state-trait anxiety inventory (STAI) form Y scale (20). This is a well-validated self-report questionnaire comprising 20 descriptive statements of anxiety symptoms. Higher scores indicate higher levels of anxiety, with a range of 20–80 points. We choose this questionnaire because the Hebrew version has high reliability and validity (21), it takes only a few minutes to complete and our team was familiar with it after using it in previous studies (12).

Data were recorded by a single physician (SA) and analysed in a blinded fashion by a second author (IL), who was unaware of treatment allocations. The electrocardiogram analogue signal from a cardiorespiratory monitor was fed into a computer containing the HRV software (ANS-R1000 system Ansar, Inc., Philadelphia, PA, USA). The analogue electrocardiogram signal was converted to digital values reflecting cyclic changes in the RR interval. The data were transformed into a waveform across a spectrum of various frequencies. Frequencies were measured in hertz (1 Hz = 1 cycle/sec). Applied to HRV, sympathetic influences appear at frequencies <0.15 Hz, the low frequency (LF) power spectrum. The high frequency (HF) power spectrum is in the frequency range of >0.15–1.80 Hz, which is predominately influenced by parasympathetic inputs. The HRV indices for LF, HF, LF/HF ratio and heart rate were summarised for KC with and without music. Given that the HRV indices represent changes in heart rate over time, the geometric mean was used for these data. Heart rate and respiratory activity graphs display the 128-second (512 points) segment required to produce the heart rate and respiratory activity spectra. Movement and artefacts were eliminated by comparing the amplitude (height) of the R-wave to be included with the amplitude for the last acceptable R-wave. Waves of more or <38% deviance from the previous wave were automatically eliminated.

Statistical analysis

The statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD or median (range) for continuous variables and numbers and percentages for nominal parameters. The normality of the data distribution was assessed by Kolmogorov-Smirnov test. The difference between groups was analysed using chi-square, *t*-test, Mann–Whitney, repeated-measures analysis of variance, or Friedman test, with Bonferroni correction, for paired data, each as appropriate. Pearson correlations were calculated to determine the relationship between infant characteristics (e.g. postmenstrual age and postnatal age) and LF/HF ratio. Differences were considered significant when $p < 0.05$.

Sample size calculations were based on 20% success in reducing LF/HF ratio (balance of sympathetic-parasympathetic activity) with KC alone (22) and a goal of 40% reduction in the LF/HF ratio for KC combined with maternal singing. Therefore, a sample size of 86 infants was determined to be sufficient to detect these differences.

RESULTS

The study population comprised 86 mother–infant dyads. Characteristics of the study population are shown in Table 1.

The mean sound level during KC with maternal singing was within the recommended range (50–65 dB). There was a significant difference between sound levels during KC alone (42.6 ± 5.6 dB) and KC combined with maternal singing (55.1 ± 8.9 dB) (p = 0.04). Table 2 lists the mean values of heart rate, oxygen saturation and respiratory rate of all study phases, of infants and mothers during KC alone and KC with maternal singing. There were no statistical or clinical differences in these parameters.

Compared with KC alone, KC with maternal singing had a significant beneficial effect on maternal anxiety: 56.2 ± 10.8 before and 42.8 ± 8 after KC alone (p = 0.09) versus 50.6 ± 12.3 before and 26.4 ± 8.2 after KC with maternal singing (p = 0.04). The mean difference in preintervention to postintervention STAI scores was significantly greater in KC with maternal singing compared with KC alone (Table 2) (p = 0.03). No difference in mean body temperature was recorded between KC and KC with singing (36.4 ± 0.4°C vs 36.6 ± 0.5°C; p = 0.18), respectively. The infants were in deep sleep during all phases of testing (Table 2). LF power was lower and HF power was higher in KC with maternal singing than with KC alone during singing and recovery phases (p = 0.026 and p = 0.04, respectively for LF, and p = 0.03 and p = 0.02 for HF, respectively) (Table 3). Hence, the LF/HF ratio was lower during singing and recovery phases compared with KC alone (p = 0.04). The LF/HF ratio was also significantly lower during singing and recovery compared with baseline phase (p = 0.04 and p = 0.01, respectively). No correlation was found between postmenstrual age (r = 0.24) or postnatal age (r = 0.13) and LF/HF ratio.

Table 1 Characteristics of study population (n = 86)*

Characteristic	Value
Ethnic origin	
Jewish/Arabic (no.)	53/33
Infants	
Male/Female (no.)	39/47
Postmenstrual age at birth (weeks)	31 (25–33)*
Postnatal age at study onset (days)	25 (14–61)
Birth weight (grams)	1411 (640–2512)
AGA/SGA (no.)	78/8
Weight at testing (grams)	1885 (1620–2780)
Bronchopulmonary dysplasia (no.)	1
Mothers	
Age (years)	27 (17–41)
Education (years)	12 (6–20)

AGA = appropriate for gestational age; SGA = small for gestational age.
*Data are presented as median (range).

Table 2 The effect of therapy on mother–infant dyads

Variable	Mode of Therapy: KC alone	KC with maternal singing	p-Value
Heart rate (BPM)*			
Infants	135 ± 8	127 ± 21	0.67
Mothers	79 ± 13	74 ± 15	0.21
O_2 saturation (%)*			
Infants	97 ± 3	96 ± 4	0.23
Mothers	97 ± 2	96 ± 2	0.18
Respiratory rate (min)*			
Infants	51 ± 16	47 ± 16	0.38
Mothers	24 ± 7	27 ± 5	0.31
Behavioral state*,†			
Infants	1	1	0.7
STAI score‡	12.8 ± 2.3	23.7 ± 5.4	0.03

BPM = beats per minute; KC = kangaroo care; min = minute; STAI = state-trait anxiety inventory.
*Mean value for all study phases (baseline, intervention and recovery).
†Infants were in deep sleep (behavioral score 1 on a 7-point score adopted from Als criteria (19) in both modes of therapy.
‡Mean difference from pre- to post-intervention.

DISCUSSION

KC and music are well established, safe, inexpensive and easily implemented methods that have been found to reduce infant stress and improve neurodevelopmental outcomes. In a previous study (12), live harp music during mother–infant KC significantly reduced maternal anxiety response but had no apparent effect on the infants' physiological parameters or behavioural states. Therefore, in this study, we combined KC with maternal singing. We found that HRV changed, indicated by increased HF power (indicative of parasympathetic tone) and decreased LF power (sympathetic tone) during maternal singing and during recovery. These changes in HRV indicated better autonomic stability (17). Therefore, we assume that the decreased LF/HF ratio found with maternal singing was related to a calming effect on the preterm infant.

No correlations were found between postmenstrual or postnatal ages and HRV indices. No changes in mean heart rate, respiratory rate, oxygen saturation or modified behavioural state criteria (19) were noted between KC with maternal singing and KC alone.

We also found that mothers who sang to their children during KC felt less anxiety, as demonstrated by the decrease in STAI questionnaire scores after maternal singing. It is well established that the mother's voice has an effect on a preterm or term infant, as the infant reacts to the unique changes in pitch and sound. Foetuses respond to their mother's voice with detectable changes in heart rate as early as 32 weeks of gestation (23) and can demonstrate a preference for it after birth (3,5,24). Filippa et al. (3) recently demonstrated the positive effect of maternal singing. They evaluated the effects of maternal singing and speaking to preterm infants who were in incubators and found that the infants had higher oxygen saturation and heart rate during intervention. Unlike, Filippa et al., we did not find a difference in mean heart rate or oxygen saturation in response to maternal voice. This might be explained by the fact that the infants in our cohort study were held in the KC position during both interventions. During KC, the mother's skin-to-skin contact with the preterm infant provides multisensory stimulation including emotional, tactile, proprioceptive, vestibular, olfactory, auditory, visual and thermal in a unique, interactive way that can significantly decrease or mask the effects of other stimuli (11,12,22). Thus, changes in heart rate and oxygen saturation related to critical events might be significantly diminished by KC even without maternal singing. Furthermore, in a study by Loewy et al. (7), the differences in heart rate, respiratory rate and oxygen saturation found after applying various live music stimuli to preterm infants were statistically different to no music, but biologically unimportant. Other studies did not find a difference in heart rate, respiratory rate or oxygen saturation when applying music to preterm infants (11–13). Therefore, we used HRV to measure the effect of singing on preterm infants.

Skin-to-skin care using KC, in a quiet room when the infant's ear rests directly on the mother's chest and sounds are transmitted through tissue as well as through air, might resemble the foetal auditory environment where the mother's voice is heard in utero. We were aware that because KC alone produced changes in behavioural state and HRV that indicated better autonomic stability, the maternal voice might not confer additional benefit for

Table 3 Heart rate variability spectral power in kangaroo care with and without maternal singing

Study phase	Kangaroo care (KC): LF power (ms^2/Hz)	HF power (ms^2/Hz)	LF/HF ratio	KC and maternal singing: LF power (ms^2/Hz)	HF power (ms^2/Hz)	LF/HF ratio
Baseline	30.5 ± 4.9	14.7 ± 2.6	2.1 ± 1.2	28.1 ± 7.3	6.8 ± 1.4	4.1 ± 1.7
Intervention	35.8 ± 6.5	10.5 ± 5.7	3.4 ± 1.6	16.3 ± 4.9*,#	16.8 ± 2.4#	1.0 ± 0.8*,#
Recovery	29.5 ± 8.2	14.2 ± 3.3	2.0 ± 1.9	10.4 ± 2.6*,#	26 ± 5.1*,#	0.4 ± 0.2*,#

HF = high frequency; LF = low frequency.
*$p < 0.05$ compared to KC alone.
#$p < 0.05$ compared to baseline.

the preterm infant. However, as in previous studies, we found that combined modalities derived from developmental care interventions have an additive effect. This bimodal exposure to sensory stimuli (auditory and tactile) was found to facilitate perceptual memory and learning in infants (25). Maternal heart sounds (26), vestibular movement (chest respiratory movement) (27), body scent (28) and voice (4) are all triggers for reducing stress and producing soothing effects on preterm infants during maternal singing.

In neonates, the spectrum for high frequency HRV is high, in the range of 0.15–1 Hz, corresponding to their faster respiratory rate of 24–60 breaths per minute. However, in a number of clinical situations such as neonatal sepsis, where both high and low frequency HRV are depressed with no change in the LF/HF ratio, infants treated with drugs that affect respiration (anticonvulsive drugs), or who have illnesses associated with high respiratory rates (28), make frequency-based HRV unreliable.

This cohort study included only stable preterm infants, of 32–36 weeks' postmenstrual age with no sepsis, no acute respiratory illness and no drugs that affect respiration. Therefore, the HRV results were not influenced by a specific clinical illness. A few factors unrelated to the effect of KC with maternal singing might have influenced the HRV results. As documented in previous KC studies, the mother's body heat can increase the infant's body temperature, which can affect HRV (29). However, we measured body temperature continually during KC and did not find any difference between groups. The change from a horizontal position in the open crib to a head-up position in KC may cause predominance of LF compared to HF (30). However, as our study was a within-subject, crossover, repeated-measures design, this effect was controlled between the two intervention modalities. We did not test the effect of maternal singing on HRV without KC, which might be beneficial as well. However, as KC is standard in our NICU, we feel that the proper way to deliver music stimuli is when the mother is with her infant during KC therapy. This approach of combining KC with maternal singing is supported by the observation that bimodal exposure to sensory stimuli (auditory and tactile) facilitated perceptual memory and learning in infants (25).

In conclusion, applying live maternal singing during KC therapy produced changes in HRV that indicated a better autonomic stability and had an apparent effect on the mothers' anxiety response as well. These safe, inexpensive and easily implemented therapies can be applied during daily neonatal care. They are especially advocated when singing might decrease an infant's restlessness and maternal anxiety associated with just providing KC. Given that maternal singing during KC improved the autonomic stability of the preterm infants during their stay in the NICU, further studies evaluating the long-term effects of this bimodal exposure, and whether continuing this therapy during infancy facilitates gross motor skills, mental processing, perceptual memory, social behaviour and learning, are warranted.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

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