RESEARCH PAPER

Assisted Physical Exercise and Stress in Preterm Neonates

SUBHASH CHANDRA SHAW, M JEEVA SANKAR, ANU THUKRAL, RAMESH AGARWAL, ASHOK K DEORARI AND VINOD K PAUL

From Division of Neonatology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India.

Correspondence to: Dr M Jeeva Sankar, Division of Neonatology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029, India. jeevasankar@gmail.com
Received: September 25, 2017; Initial review: February 19, 2018; Accepted: May 09, 2018.

Objective: To evaluate the markers of stress before and after a session of assisted physical exercise in infants born before 35 weeks' gestation. **Methods:** 25 infants born at $28^{0/7}$ to $34^{6/7}$ weeks were subjected to assisted physical exercise daily for about 10-15 min at one week of postnatal age or 33 weeks of post menstrual age, whichever was later. Primary outcome was salivary cortisol and secondary outcome was Premature infant pain profile (PIPP) score. Outcomes were measured, on day 5 (±1) of exercise. **Results:** There was no difference in salivary cortisol between baseline and immediately after (*P*=0.16), at 90 min (*P*=0.6) or 120 min (*P*=0.7) after exercise. Salivary cortisol was lower at 30 min after exercise as compared to baseline (mean difference -0.08 µg/dL; 95% CI -0.16 to -0.002; *P*=0.04). The median (IQR) PIPP score was significantly higher at 5 min into exercise (4 (3-6) *vs* 4 (3-5); *P*=0.04) and at completion of exercise 6 (4-8) *vs* 4 (3-5); *P*<0.01), as compared to baseline. **Conclusion:** Assisted physical exercise does not seem to result in stress in premature infants.

Keywords: *Cortisol, PIPP score, Prematurity*.

Regular assisted physical exercise in the form of passive range-of-motion exercises has shown short term improvement in bone mineral content and skeletal growth in preterm infants [1], but whether this is stressful to the preterm infants or not, is not known. Stressful experience causes rise in cortisol and the peak cortisol response to any stressful or painful stimuli is thought to occur 20 to 30 minutes post-stimuli [2-4]. Physiological and behavioral manifestations of stress can also be measured by pain scores like Premature Infant Pain Profile (PIPP), which is a seven-item, four-point scale for assessment of pain in premature neonates [5]. As there is paucity of literature available on the potential risk of stress experienced by the preterm infants while undergoing assisted physical exercise, we conducted a pilot study in preterm infants born at less than 35 weeks, with measurement of salivary cortisol levels before and after a session of physical exercise. We also evaluated PIPP scores, heart rate and oxygen saturation before, during and after a session of assisted physical exercise.

METHODS

We conducted this before-and-after study in a tertiary neonatal unit in Northern India between January, 2013 and March, 2013. All intramural infants born at or after 28 weeks and up to 34^{+6} weeks were considered eligible for enrolment at one week of postnatal age or 33 weeks of post menstrual age (PMA), whichever was later. Infants with major malformations, intraventricular hemorrhage grade 3 or more, and requirement of inotropes or steroids, continuous positive airway pressure (CPAP) or mechanical ventilation, any injectable or any form of sedation were excluded. Gestational age (GA) was ascertained from the first day of the last menstrual period or by first trimester ultrasound or by the Expanded New Ballard Score (ENBS) [6] performed within 24 hours of birth in that order of preference. Informed written consent was obtained from one of the parents and the study was approved by the Institute Ethics Committee.

The assisted physical exercise was based on the protocol suggested by Moyer-Mileur, *et al*. [7]. Range-of-motion exercise with gentle compression, extension and flexion of both upper and lower extremities with each movement was performed five times at each joint (wrist, elbow, shoulder, ankle, knee, and hip, in this order) in one session every day. Physical exercise was started once the infant reached 33 weeks PMA or second week of postnatal age, whichever was later. Physical exercise was executed daily by principal investigator or designated nurse who was earlier trained by a certified physiotherapist. After 5±1 days of daily exercise, the outcomes were measured with video recording of whole session of physical exercise by a digital camcorder (Sony handicam DCR-PJ16E, Japan).

The primary outcome was salivary cortisol level. Saliva samples were collected with a Salivette (Sarstedt), with an insert containing a sterile polyester swab for collection of the saliva, yielding a clear and particle free sample. The prerequisite for collection of the saliva was that the infant should have been fed at least 1 hour prior to collection of the salivary sample. Salivette was frequently rubbed on the inside of the cheeks and once the inside end was visibly wet, it was cut transversely by a sterile blade. The cut piece was placed inside the salivette container and the rest of salivette was placed again in the mouth of the infant and the same procedure was repeated for a total of approximately 20 minutes for each salivette. The samples were collected as baseline before exercise, immediately after physical exercise and again at 30 min, 90 min and 120 min, respectively after a session of physical exercise between 2 to 5 PM. At baseline, two successive saliva samples were collected and the sample immediately before exercise was considered final if both samples were adequate. The samples were immediately centrifuged at 3000 rpm for 10 minutes. The clear filtrate was transferred to a separate tube and was stored at 2-8ºC for maximum of 24 hours. The day following collection of saliva, salivary cortisol was measured by electrochemiluminescence immunoassay (ECLIA) on a Roche ELECSYS e411 autoanalyser (Cobas; Roche diagnostics, Canada).

The secondary outcome was PIPP score, and was recorded before exercise, every 5 min during exercise, and at the end of a session of physical exercise. Exercise of wrists, elbows and shoulders of both upper limbs were done for approximately 5 min, then paused for 30 sec to record PIPP score. For next 5 min, exercise of ankles and knees were done and again paused for about 30 sec to record PIPP. Exercise of hips were carried out next and at the end PIPP score was recorded again. Continuous heart rate and oxygen saturation was recorded using a pulse oximeter (Masimo SET Radical 7, Masimo, Irvine, CA, USA). These video recordings were periodically reviewed by physiotherapist and scorings were checked by an independent clinician not involved with the study.

Sample size was calculated using the data from a previous study on changes in plasma cortisol in response to massage in preterm infants [8]. Assuming a mean difference in serum cortisol of 35.8 nmol/L between the baseline and at the end of study, common standard deviation of 78.9, one and four measurements, respectively before and after intervention, correlation between baseline and follow-up (r) of 0.75, power 80%, and type 1 error of 5%, we needed to enroll 26 infants.

Statistical analysis: Analysis was performed using Stata 11.2 (StataCorp, College station, Texas, US). Paired t test was used to analyze continuous variables with normal distribution, while Wilcoxon sign rank test was used for continuous variables with skewed distribution. Generalized estimating equation (GEE) model was used to evaluate the effects of exercise over a period of time as compared to the baseline. P value of <0.05 was taken as significant.

RESULTS

A total of 29 potentially eligible infants were born during the study period. After excluding 4 infants based on pre-specified criteria, we enrolled 25 infants. The mean (SD) gestation and birth weight of the study population was 32 (2) weeks and 1492 (414) g, respectively. Mothers of 14 (56%) infants received complete dose of antenatal steroids before delivery. The mean (SD) postnatal age of outcome measurement was 15.3 (7.4) days.

There was no difference in mean salivary cortisol level between baseline and immediately after exercise, at 90 min after exercise or at 120 min after exercise (***Table* I**). However, salivary cortisol was significantly lower at 30 min after exercise as compared to baseline with a mean difference of -0.08 mcg/dL (95% CI -0.16 to -0.002; *P*=0.04).

The median (IQR) PIPP score was significantly higher at 5 min into exercise (4 (3-6) *vs* 4 (3-5); *P*=0.04) and at completion of exercise (6 (4-8) *vs* 4 (3-5); *P*<0.01), as compared to baseline (***Table* II**). However, the proportion of infants experiencing severe pain requiring pharmaco-logical interventions (PIPP score ≥13) was not different between the end of exercise and baseline (8% *vs* 0; *P*=0.16).

DISCUSSION

The present study did not find any difference in salivary cortisol level before and after exercise (except rather a decrease in salivary cortisol at 30 min after exercise) possibly suggesting no stress caused due to exercise. The maximum PIPP score was reassuring too, as the pain reactivity concerns later neuromotor development [8]. Though PIPP scores were different statistically at 5 min of undergoing exercise and again at the end of exercise, the maximum PIPP score was still suggestive of minimal pain only (score of up to 6 is considered minimal pain) [5], requiring comfort measures only and no pharmaceutical intervention.

The major limitation of the study was that the priming of each infant took place for 5 days and the study was executed on day 5 of initiation of daily exercise. Thus, it may not reflect true stress that the infant might have

WHAT THIS STUDY ADDS?

- Assisted physical exercise does not seem to cause stress, as measured by salivary cortisol, in premature infants.

TABLE I SALIVARY CORTISOL LEVEL CHANGES IN PRETERM NEONATES (*N*=25)

Time point	*Salivary cortisol (µg/dL)*	*Adjusted difference between means (95% CI)*	*P value*
Baseline	0.368 (0.181)	–	–
Immediately after exercise	0.314 (0.099)	-0.056 (-0.13 to 0.02)	0.16
30 min after exercise	0.280 (0.129)	-0.080 (-0.16 to -0.002)	0.04
90 min after exercise	0.347 (0.193)	-0.021 (-0.1 to 0.06)	0.60
120 min after exercise	0.360 (0.173)	-0.016 (-0.09 to 0.06)	0.70

TABLE II SECONDARY OUTCOMES IN PRETERM NEONATES (*N*=25) AFTER EXERCISE

Time point	*Before exercise*	*At 5 min into exercise*	*At 10 min into exercise*	*At completion of exercise*	*P value*
PIPP[#]	4 (3,5)	4 (3, 6)	4.5 (2.5, 6.5)	6 (4,8)	<0.01
Heart rate (beats per min)	151 (19)	152 (17)	150 (18)	162 (20)	<0.01
Oxygen saturation (%)	97 (4)	97 (3)	97 (3)	96 (3)	0.66

Values expressed as mean (SD) or [#]median (IQR).

undergone on earlier days. Cortisol or behavioral/ physiological response might have been subdued due to habituation.

Prior studies pertaining to daily massage in preterm infants have shown variable results. Our results support the findings of Acolet D, *et al.* [9] , showing similar decrease in plasma cortisol after one hour of massage in preterm infants at 23 to 34 weeks' gestation. However, another crossover trial found variable change in salivary cortisol in preterm infants at 25 to 35 weeks [10]. Similarly, Modi and Glover [11] too found inconclusive results with increase in salivary cortisol in response to massage in some infants, and decrease in others, both postulating the role of change in behavioural state of the infants. The fetal hypothalamic-pituitary-adrenal system, which is responsible for release of cortisol, is functional from the beginning of second trimester and increases during the last 10 weeks of gestation. Cortisol levels peak shortly after birth and return to baseline by day of life 3 to 5 [12]. To circumvent any possible diurnal change in cortisol level, collection time of saliva was kept the same on all days of study as studies had shown earlier that the neuro-anatomical pathways including the hypothalamic nuclei of the cortisol circadian rhythm generating system are sufficiently developed at 31 to 34 post-conceptional weeks [13].

To conclude, assisted physical exercise was well tolerated in preterm infants born at 28 to 34 weeks' gestation. The study has implications for developing countries as we have a substantial load of prematurity and regular assisted physical exercise have shown promise in improvement in bone mineral content and skeletal growth in these preterm infants [1].

Acknowledgements: Dr Nandita Gupta, for assay of salivary cortisol samples and Mrs Sumita Gupta, physiotherapist for training of the investigator.
Contributors: SCS: recruited patients, collected and analyzed the data, and drafted the initial manuscript; MJS, RA: supervised data collection and analysis of data and did critical revision and finalization of the manuscript; AT: helped in data collection and analysis; AKD, VKP: contributed to the study design, data analysis and interpretation.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Schulzke SM, Kaempfen S, Trachsel D, Patole SK. Physical activity programs for promoting bone mineralization and growth in preterm infants. Cochrane Database Syst Rev. 2014;4:CD005387.
2. Morelius E, Nelson N, Theodorsson E. Salivary cortisol and administration of concentrated oral glucose in newborn infants: improved detection limit and smaller sample volumes without glucose interference. Scand J Clin lab

Invest. 2004;64:113-8.
3. Gunnar MR, Talge NM, Herrera A. Stressor paradigms in developmental studies: what does and does not work to produce mean increases in salivary cortisol. Psychoneuroendocrinology. 2009;34:953-67.
4. Herrington CJ, Olomu IN, Geller SM. Salivary cortisol as indicators of pain in preterm infants: A pilot study. Clin Nurs Res. 2004;13: 53-68.
5. Stevens B, Johnston C, Petryshen P, Taddio A. Premature infant pain profile: development and initial validation. Clin J Pain. 1996;12:13-22.
6. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. J Pediatr. 1991;119:417-23.
7. Moyer-Mileur L, Luetkemeier M, Boomer L, Chan GM. Effect of physical activity on bone mineralization in premature infants. J Pediatr. 1995;127:620-5.
8. Grunau RE, Whitfield MF, Fay T, Holsti L, Oberlander T, Rogers ML. Biobehavioural reactivity to pain in preterm infants: A marker of neuromotor development. Dev Med Child Neurol. 2006;48:471-6.
9. Acolet D, Modi N, Giannakoulopoulos X, Bond C, Weg W, Clow A, *et al*. Changes in plasma cortisol and catecholamine concentrations in response to massage in preterm infants. Arch Dis Child. 1993;68:29-31.
10. Gitau R, Modi N, Giannakoulopoulos X, Bond C, Glover V, Stevenson J. Acute effects of maternal skin to skin contact and massage on salivary cortisol in preterm babies. J Reprod Infant Psychol. 2002;20;83-8.
11. Modi N, Glover V. Non-pharmacological reduction of hypercortisolemia in preterm infants. Infant Behav Dev. 1998;21(Suppl.):86.
12. Giannakoulopoulos X, Sepulveda W, Kourtis P, Glover V, Fisk NM. Fetal plasma cortisol and beta – endorphin response to intrauterine needling. Lancet. 1994:344:77-81.
13. Antonini SR, Jorge SM, Moreira AC. The emergence of salivary cortisol circadian rhythm and its relationships to sleep activity in preterm infants. Clin Endocrinol. 2000;52:423-6.