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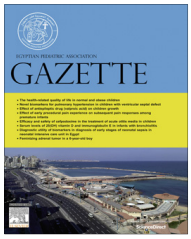

Effect of early procedural pain experience on subsequent pain responses among premature infants

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Abstract *Background and objectives:* Preterm newborns are exposed to repeated procedural pain during their NICU stay. Acute pain has negative short-term effects and may have adverse neurodevelopmental sequelae. Disagreement among researchers exists in the direction of pain responses. We aimed at evaluating the short-term effects of early procedural pain exposure on subsequent behavioral and physiological responses among preterm infants; and to define possible contributing factors.

Patients and methods: A prospective study included 203 preterm newborns recruited from 2 community centers, excluding cases having conditions that may affect pain responses. They were categorized into: group I including cases who were exposed to painful procedures; and group II were not exposed. Pain response to *heel-stick procedure* was assessed by *Neonatal Infant Pain Scale* to measure behavioral response, and changes in heart rate and oxygen saturation to evaluate physiological responses.

Results: History of pain exposure and number of procedures were the only independent variables that predicted subsequent pain responses while other contextual factors had no significant impact. The behavioral pain responses were blunted in group I with lower pain scores during and after the heel-stick test, while physiological responses were exaggerated with a higher heart rate and oxygen saturation variability.

Conclusion: On studying the physiological and behavioral responses to pain in the premature infants, it was found that prior pain exposure and the number of procedures predict dampened behavioral and exaggerated physiologic subsequent pain responses. Protocols for minimization of pain exposure and pain control need to be implemented to avoid infant distress and long-term neurodevelopmental sequelae.

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Introduction

Pain associated with investigations, treatment or procedures is defined as procedural pain. It may be for an isolated intervention, but not uncommonly a period of treatment or investigation requires a repeated number of such episodes.[1]

Preterm infants are exposed to repeated procedural pain-related stress, as part of their care in the neonatal intensive care unit (NICU). Acute pain can have negative short-term and long-term effects on the premature neonate including deleterious acute physiologic responses and possible neuromodulation leading to altered responses to noxious stimuli.[2–4] It may adversely affect their neurodevelopment during a period of physiological vulnerability and rapid brain development.[5,6] Immaturity, coupled with reduced abilities of these infants in regulating their autonomic, motor, and state organization heightens their vulnerability to noxious stimulation.[7]

Despite growing scientific evidence, several gaps in the research remain. Most researchers agree that multiple exposures to pain in the NICU alter the responses of premature infants.[8,9] However, disagreement remains about the direction of these changes and behavioral versus physiological responses.[10,11] It is not yet clear whether repeated exposure to pain in preterm infants intensifies or diminishes their behavioral and physiologic responses, nor is it ascertained which factors heighten or dampen their responses.[10]

Accurate pain assessment in preterm infants is complex, it should be comprehensive and multidimensional including contextual, behavioral and physiological tools.[12]

Current protocols for pain management include pain treatment and prophylaxis in infants subjected to continuous or persistent pain, however no universally-acceptable regimens exist for those exposed to few procedures.[6,13] An analysis of the short- and long-term sequelae of pain and its contributing factors will help identify the subjects in need for pain management.

The aim of the present study was to evaluate the short-term effects of early procedural pain exposure among preterm infants on the subsequent behavioral and physiological pain responses; and to define the possible contributing factors.

Patients and methods

Study design and settings

This prospective study was conducted at Bab-El-Shaeria University Hospital and El-Hussein University Hospital from July 2012 to December 2013. These two hospitals in Cairo, Egypt, were selected since they have comparable NICU capacities with no protocols for procedural pain control in either hospital. The study was approved by both local ethics committees. Informed parental consent was obtained prior to enrollment in the study.

Study population

All preterm newborns admitted to the NICU or nursery, who had a normal course of pregnancy and labor, stable postnatal condition, and breathing spontaneously without oxygen supplementation were eligible for the study. A total of 203 newborns (32–36 completed weeks of gestation) were included. Exclusion criteria included conditions that might potentially alter the response to painful procedures; namely: 5-min Apgar score <7, apparent genetic or congenital anomalies, seizures or any central nervous system disease, any cardiac disease that may interfere with vital indices, any condition requiring ventilatory support, analgesics or sedatives given to the mother or infants, and clinical or laboratory evidence of sepsis.

According to exposure to painful procedures infants were categorized into two groups: preterm infants exposed to painful procedures (group I), and those who had not been exposed to any painful procedure (group II).

Study methods

Data collection

Data included detailed perinatal history (maternal illness; mode of delivery; Apgar scores at 1 and 5 min; gestational age; gender; clinical course; medications including analgesics or sedatives; and type of feeding), and history of exposure to any painful procedure. Comprehensive clinical and laboratory examination executing the eligibility criteria were prospectively collected.

Painful procedures

A painful procedure is defined as a medical, diagnostic, or therapeutic activity performed in the NICU. The number and type of performed procedures were identified and documented. Neonatal pain exposure has been quantified as the number of painful procedures performed during hospitalization in the NICU either one, two or $\geqslant 3$ procedures.

Assessment of subsequent pain responses

All studied candidates were subject to the *Heel stick* (*lance*) procedure to assess their subsequent behavioral and physiological pain responses during their routine follow up after one month of postnatal age.

Pain responses were assessed and recorded by a single trained examiner at three different time points: before the procedure (baseline), during the procedure (lance/squeeze phase, when maximal response was observed), and 3 min after the procedure (recovery phase, when cotton wool was applied).

Heel stick (lance) procedure was selected being the most frequently reported procedure in the NICU and very easy to perform.[10] Before starting the procedure, every effort was made to ensure that the baby was resting quietly, awake and not crying. The procedure was done 30 min after feeding to avoid hunger pain. The baby was contained in warm sheets and was not removed from the incubator or crib during the entire procedure. The heel was wiped with alcohol, pricked with a lancet and squeezed to collect 0.25 ml volume of blood. A cotton wool ball was applied to prevent bleeding.

The Neonatal Infant Pain Scale (NIPS),[14] is a multidimensional scale that was used to measure the behavioral pain response to the heel-stick in preterm infants. NIPS evaluates six parameters to assess procedural pain: five behavioral parameters (facial expression, cry, state of arousal, position of arms and legs) and one physiological parameter (breathing pattern). Each indicator is scored 0 or 1 with the exception of cry, which is scored 0–2, resulting in a total score between 0 and 7 for minimum and maximum rates, respectively.[15,16] It

Table 1 Characteristics of the whole study population (n = 203).

Characteristic	Value
Gestational age	34.4 ± 1.3
Gender (male/female)	122/81
MOD (NVD/CS)	85/118
History of prior pain exposure	130 (64.0)
Postnatal age at time of pain assessment (days)	31.6 ± 1.7
NIPS pain score	
During procedure	5 (2–7)
After procedure	3 (1–6)
Heart rate (b/min)	
Before procedure	126 ± 6
During procedure	143 ± 9
After procedure	134 ± 8
% change during procedure	12.9 (2.2–36.1)
% change after procedure	5.6 (−7.0 to 27.7)
Oxygen saturation (%)	
Before procedure	99 ± 1 (98–100)
During procedure	95 ± 2 (91–98)
After procedure	97 ± 2 (94–99)
% change during procedure	−5.0 (−9.0 to 0.0)
% change after procedure	−2.0 (−5.0 to 1.0)

Data are expressed as ratio, frequency (percentage), mean ± SD (range), and median (range).
NIPS, *Neonatal Infant Pain Scale*.

Table 2 Correlation between pain scores, gestational and postnatal ages of the study population (n = 203).

Variable	NIPS during the procedure		NIPS after the procedure	
	r	p	r	p
Gestational age	−0.071	0.314	−0.058	0.411
Postnatal age	−0.144	0.065	−0.135	0.054

Correlation coefficient (r) by Pearson product moment method.

is easy to use, practical in application and does not require additional assessment skills or equipment.[17] Its validity and reliability has been supported in studies of preterm neonates.[18]

Physiological responses to pain were evaluated by changes in heart rate and oxygen saturation. Heart rate and oxygen saturation were measured using a pulse oximeter with the sensor placed on the foot 10 min before starting the procedure.

Statistical analysis

Data were analyzed using IBM SPSS Advanced Statistics version 20.0 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean, standard deviation, median and range. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between the qualitative variables. For quantitative data, comparison between the two groups was done using independent sample t-test or Mann–Whitney test as appropriate. Comparison of consecutive measures was done using an ANOVA or Wilcoxon Signed Ranks test for repeated measures. Pearson product moment was used to estimate correlation between variables. Multiple linear regression models were used to evaluate the predictor variables of pain responses. p-value <0.05 was considered statistically significant.

Results

The characteristics of the study population are demonstrated in Table 1. The mean gestational age was 34.4 ± 1.3 weeks (range: 32–36 completed weeks of gestation), and the mean postnatal age at time of the pain assessment was 31.6 ± 1.7 days (range: 31–36 days).

On analyzing the data of the whole group, it was found that the behavioral and physiological changes in response to the painful heel stick procedure according to the NIPS pain score showed a significant decrease after the procedure than during the procedure ($p < 0.001$). The mean HR values during the procedure significantly increased with a significant decrease in the mean O_2 saturation in relation to baseline values. After the procedure, the mean HR significantly decreased and the mean O_2 saturation significantly increased in relation to values during the procedure. However, both values were still significantly different after the procedure compared to baseline readings (p values < 0.001).

NIPS pain scores during and after the procedure had non-significant correlation with the gestational and postnatal ages (p values > 0.05) (Table 2).

Also, the NIPS pain scores of the whole study population during and after the procedure were not affected by either gender or mode of delivery (p values > 0.05) (Table 3).

Out of the total 203 preterm infants enrolled in the study, 130 (64%) were exposed to painful procedures (group I) and 73 (36%) were not exposed to pain (group II). Both groups were comparable regarding gestational age (p = 0.228), gender (p = 0.248), mode of delivery (p = 0.236), and postnatal age at time of the heel stick procedure (p = 0.212) (Table 4).

Out of the 130 infants in group I: 26 (20%) experienced one procedure (venipuncture); 32 (24.6%) experienced two procedures (venipuncture and nasopharyngeal suctioning); and 72

Table 3 NIPS pain scores in relation to gender and mode of delivery of the study population (n = 203).

NIPS pain score	Gender		p value	Mode of delivery		p value
	Male	Female		NVD	CS	
During procedure	4 (2–7)	5 (2–7)	0.098	5 (3–7)	4 (2–7)	0.352
After procedure	3 (1–6)	3 (1–6)	0.105	3 (1–6)	3 (1–6)	0.193

Data are expressed as median (range).
Analysis by: Mann–Whitney test.

Table 4 Characteristics of the studied groups.

Variable	Group I (n = 130)	Group II (n = 73)	p value
Gestational age	34.5 ± 1.3	34.3 ± 1.2	0.228
Gender (male/female)	82/48	40/33	0.248*
Mode of delivery (NVD/CS)	50/80	35/38	0.236*
Postnatal age at time of assessment (days)	31.9 ± 1.6	31.6 ± 1.7	0.212
NIPS pain score			
During procedure	4 (2–6)	7 (6–7)	< 0.001†
After procedure	2 (1–4)	5 (4–6)	< 0.001†
Heart rate (b/m)			
Before procedure	127 ± 6	124 ± 6	0.008
During procedure	148 ± 7	135 ± 5	< 0.001
After procedure	138 ± 7	127 ± 4	< 0.001
% change during procedure	16.8 (2.9–36.1)	8.2 (2.2–17.1)	< 0.001†
% change after procedure	8.9 (−3.7 to 27.7)	2.3 (−7.0 to 8.5)	< 0.001†
Oxygen saturation (%)			
Before procedure	99 ± 1	99 ± 1	0.061
During procedure	93 ± 2	97 ± 1	< 0.001
After procedure	96 ± 1	98 ± 1	< 0.001
% change during procedure	−6.1(−9.0 to −1.0)	−2.0 (−4.0 to 0.0)	< 0.001†
% change after procedure	−3.1 (−5.1 to 1.0)	−1.0 (−3.0 to 1.0)	< 0.001†

Data are expressed as ratio, mean ± SD, and median (range).
Analysis by: t-test for independent samples.
* Chi-square test.
† Mann–Whitney test.

Table 5 Correlation between pain responses during and after procedure with number of procedures and duration of exposure among group I preterm infants (n = 130).

Pain response	Number of procedures		Duration of exposure	
	r	p	r	p
NIPS during procedure	−0.834	< 0.001	−0.764	< 0.001
NIPS after procedure	−0.887	< 0.001	−0.830	< 0.001
% change HR during procedure	0.397	< 0.001	0.398	< 0.001
% change HR after procedure	0.369	< 0.001	0.363	< 0.001
% change O_2 saturation during procedure	−0.568	< 0.001	−0.490	< 0.001
% change O_2 saturation after procedure	−0.436	< 0.001	−0.382	< 0.001

Correlation coefficient (r) by Pearson product moment method.

(55.4%) experienced three to six procedures (either venipuncture, nasopharyngeal suctioning, heel stick, gastric tube insertion, intravenous cannulation, or removal of adhesive tape). The mean number of painful procedures was 2.6 ± 1.1 (range: 1–6), performed within a mean duration of 6.9 ± 3.5 days (range: 1–13 days).

In group I, the median NIPS pain scores during and after procedure were significantly lower ($p < 0.001$); and the mean HR scores before, during and after procedure were significantly higher ($p = 0.008$ and $p < 0.001$) than group II. The mean O_2 saturation was significantly lower in group I during and after the procedure ($p < 0.001$), although baseline values before the procedure had shown a non-significant difference between the two groups ($p = 0.061$). On considering the percent change in HR and O_2 saturation, the median values during and after the procedure relative to baseline were significantly higher in group I ($p < 0.001$).

The NIPS pain scores of group I during and after the procedure had strong inverse correlation with the number of painful procedures ($r = -0.834$, -0.887; p values < 0.001), and with the duration of exposure to pain ($r = -0.764$, -0.830; p values < 0.001).

The percent change of HR during and after procedure showed moderate positive correlation with the number of painful procedures ($r = 0.397$, 0.398; p values < 0.001), and the duration of exposure to pain ($r = 0.369$, 0.363; p values < 0.001). Meanwhile, the percent change of O_2 saturation during and after the procedure had moderate inverse correlation with the number of painful procedures ($r = -0.568$, -0.490; p values < 0.001), and with the duration of exposure to pain ($r = -0.436$, -0.382; p values < 0.001) (Table 5).

The multiple linear regression analysis in group I indicated that the number of procedures significantly predicted NIPS pain scores; percent change O_2 saturation during and after the procedure; and percent change HR after the procedure. Nevertheless, the duration of pain exposure significantly predicted the percent change HR during the procedure (Table 6).

Multiple linear regression analysis was performed on all 203 cases, revealing that the number of procedures and history of previous exposure to pain significantly predicted the NIPS pain scores during and after the procedure (Table 7).

Discussion

Pain in preterm infants has an important hemodynamic impact and possibly affects the behavioral neurodevelopmental outcomes. Premature infants respond somewhat differently to

Table 6 Multiple linear regression models for the predictors of behavioral and physiological pain responses of group I (n = 130).

Variable	B	SE	p value	95.0% CI for B	
				Lower bound	Upper bound
NIPS during (R^2 = 0.696)					
Number of procedures	−0.856	0.050	<0.001	−0.956	−0.757
(Constant)	5.749				
NIPS after (R^2 = 0.789)					
Number of procedures	−1.011	0.047	<0.001	−1.103	−0.919
(Constant)	4.256				
% change HR during (R^2 = 0.158)					
Duration of pain exposure	0.779	0.159	<0.001	0.465	1.092
(Constant)	11.666				
% change HR after (R^2 = 0.136)					
Number of procedures	2.961	0.659	<0.001	1.657	4.265
(Constant)	2.439				
% change O_2 saturation during (R^2 = 0.32)					
Number of procedures	−1.234	0.158	<0.001	−1.547	−0.922
(Constant)	−3.085				
% change O_2 saturation after (R^2 = 0.190)					
Number of procedures	−0.706	0.129	<0.001	−0.961	−0.451
(Constant)	−1.429				

B: Regression Coefficient; SE: Standard Error; CI: Confidence Interval.

Table 7 Multiple linear regression models for the predictors of NIPS pain scores of the study population (n = 203).

Variable	B	SE	p value	95.0% CI for B	
				Lower bound	Upper bound
NIPS during (R^2 = 0.902)					
Number of procedures	−0.856	0.052	<0.001	−0.959	−0.754
History of exposure to pain	−0.826	0.134	<0.001	−1.091	−0.561
(Constant)	6.575				
NIPS after (R^2 = 0.924)					
Number of procedures	−1.011	0.048	<0.001	−1.106	−0.916
History of exposure to pain	−0.594	0.125	<0.001	−0.840	−0.347
(Constant)	4.849				

B: Regression Coefficient; SE: Standard Error; CI: Confidence Interval.

pain than term infants and children.[4,19] This work aimed at studying the response of premature infants to pain and the factors that affect subsequent pain responses, which will aid clinicians to minimize such disagreeable consequences and guide the use of pain-control methods when indicated.

The studied population was a homogenous group recruited from two similar centers dealing with premature infants, however with no protocol for pain control. The group that was exposed to painful procedures (group I) were identified according to the number of procedures, the types of procedures being unified in each subset. These routinely-performed procedures at the NICU ranged from painful to very painful.[13,20]

On analyzing the whole study population, it was found that the mere history of pain exposure and the number of procedures were the only independent variables that predicted the subsequent behavioral pain responses while the other contextual factors including gestational age, gender, mode of delivery, postnatal age at time of study and duration of pain exposure had no significant impact. Other researchers have debated the effect of gestational age whether their pain responses are exaggerated due to low pain threshold[11,21,22] or the lack the ability to respond appropriately to pain.[23,24] Regarding gender, the majority of studies found no significant sex differences[25–27]; while few studies has reported more pronounced responses in males.[22,28] Also, postnatal age was found to have no significant impact on behavioral pain responses.[10]

However, there is an agreement that prior pain experience alters the subsequent responses to pain.[8,9,29] Nonetheless, disagreement remains about the direction of these changes and behavioral versus physiologic responses, where some described an inverse correlation of pain scores with the number of painful procedures,[24,30] while others report a more vigorous response with the number of procedures or time spent at the NICU.[29,31] The state of arousal has a role in pain perception

and thus affects the pain responses,[32,33] however this effect was nullified in the current study as the pain assessment was performed on awake infants.

On comparing both groups, we found that the behavioral pain responses were blunted in group I exposed to painful procedures, where they had lower scores during and after the heel stick procedure. This habituation reflects the ability of the central nervous system of the preterm to regulate pain pathways to adapt to procedural pain[19] especially if repeated. Grunau et al.[34] demonstrated that cumulative procedural pain was associated with diminished behavioral response to pain, due to an early dampening of the hypothalamic–pituitary–adrenal axis. Such mechanisms are associated with functional and structural alterations in pain pathways,[34,35] indicating that prolonged or even life-long impairment in pain responses and also altered neurodevelopmental outcomes might be implicated.[19,35]

Additionally, in infants previously exposed to painful procedures, the number of these procedures was the only independent factor predicting pain response. This is in agreement with the findings of Brummelte et al.[5] who documented that the number of tissue-breaking procedures was associated with a reduction in cerebral white matter and subcortical gray matter. Furthermore, long-term sequelae of repeated painful procedures include a delay in corticospinal development[36] as well as lower postnatal growth.[3] This might result in a variety of neurodevelopmental, behavioral, and cognitive deficits that manifest in later childhood.[8,37] Also, structural and functional reorganization of the nervous system as a result of repeated painful stimuli might alter future pain responses.[30,38] On the contrary, the earlier report of Johnston and Stevens[29] stated that the number of invasive procedures had no effect on subsequent pain responses. An exception to this was the percent change in heart rate during the procedure that was predicted by the duration of pain exposure, this could be explained by autonomic immaturity,[23,24] and the fact that heart rate is affected by multiple co-existing variables other than pain.[10]

The physiological responses to pain were exaggerated with a higher alteration of heart rate and oxygen saturation in infants previously exposed to painful procedures. This is supported by the earlier results of Stevens et al.,[33] Grunau et al.[34] and McIntosh et al.[39] This might reflect the immature nature of these autonomic responses, however, an agreement exists upon O_2 saturation that is reliably decreased with pain.[10] These physiological responses, however, are non-specific to pain and could be a manifestation of anger or distress.[27,40]

Lastly, pain significantly affects the premature infant acutely and over the long-term. This implies that painful procedures should be minimized, and adequate pain control measures should be taken with any painful maneuver to avoid or ameliorate such undesirable sequelae. Several protocols exist, including non-pharmacologic interventions[16] such as swaddling and nonnutritive sucking, and pharmacological agents whether local or systemic analgesia.[41] Each unit should adopt the suitable regimen for its practice, with regular audit and feedback loops to improve neonatal outcomes especially those requiring intensive care and thus inherently exposed to repeated pain.[42]

Conclusion

On studying the physiological and behavioral responses to pain in the premature infants exposed to procedural pain and compared them to the non-exposed, it was found that the fact of prior pain exposure and the number of procedures predicts dampened behavioral and exaggerated physiologic subsequent pain responses. Protocols for minimization of pain exposure and pain control need to be implemented to avoid infant distress and long-term neurodevelopmental sequelae.

Conflict of interest

The authors declare no conflict of interest.

Specific contribution of each author to the study

1. *Atef El-Sayed Donia:* Contributed to the conception and design of the study; acquisition of data; reviewed and approved the final version of manuscript to be published.
2. *Omar Atef Tolba:* Contributed to the conception and design of the study; analysis and interpretation of data; performed the statistical analysis and wrote the manuscript; revised it critically for important intellectual content; critically reviewed the manuscript; and approved the final version of manuscript to be published.

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