Study of Neuropeptide Substance P as A Marker of Pain in Newborn Infant

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Abstract

BACKGROUND: Prolonged and repeated untreated pain in newborn infant may produce a relatively permanent adverse long-term sequelae.

AIM: The aim of this study was to evaluate the potential role for neuropeptides substance P (SP) as neurochemical pain marker in newborn infants in order to decrease unnecessary use of analgesics and protect the developing brain.

METHODS: This case-control study was conducted on 60 newborn infants. They were assigned to four groups, control preterm, sick preterm, control full-term, and sick full-term. All neonates were subjected to estimation of pain through neonatal infants pain score (NIPS) as well as Neuropeptide SP on the 1st and 5th day of life. The NIPS addresses five behavioral parameters (facial expression, crying, arm movement, leg movement, and state arousal) and one physiological parameter (breathing pattern). Results were further evaluated according to nature of the procedures; invasive and non-invasive procedures.

RESULTS: There was a significant increase in the severity of pain score among the sick preterm and full-term infants after invasive procedures. There was a significant increase in SP in the sick preterm group than the control preterm on the 1st and 5th day of life; p were =0.003 and = 0.037, while full-term infants showed significant increase on the 5th day; p = 0.005. Furthermore, there was no significant difference in SP values between the preterm and full-term infants on the 1st and 5th day of life. SP increased significantly after invasive procedures than non-invasive procedures in the sick full-term and sick preterm infants weather in the 1st or 5th day of life. There was a significant correlation between the pain score NIPS and SP level on the 1st day of life.

CONCLUSION: SP can be used as pain marker in sick preterm and full-term newborn infants. It showed increase with invasive procedures, acute and chronic pain.

Introduction

Majority of newborn infants in neonatal intensive care unit (NICU) are subjected to some grade of pain and stress. They expose to repetitive handling, painful procedures, and separation from mothers during a period of rapid brain growth [1]. Even procedures as bathing, weighing, noxious sounds, lights, and diaper changes are perceived as stress to the infant [2].

Newborn infants respond to harmful stimuli by a succession of multiple biochemical, physiologic, and behavioral changes. The untreated pain has been reported to cause negative effects on both short-term and long-term consequences in the infant's brain development and quality of life.

Prolonged and repeated untreated pain in the newborn period, may produce a relatively permanent shift in basal autonomic arousal, which may have long-term sequelae. In the long run, the most significant clinical effects of early pain exposure may be on neurodevelopment, contributing to later attention, learning, and behavior problems in these vulnerable children. There is functional change in cortical pain sensitivity processing in the brain in older ex-preterm children that may allow the development of a more rational approach to pain management in NICU [3]. One of the seven neuroprotective core measures for family-centered developmental care of the premature neonate is minimizing stress and pain [4].

The neonatal infant pain scale (NIPS) is a behavioral scale and can be utilized with both full-term and pre-term infants. It is composed of six indicators that allow for the estimation of pain grade in newborn infants [5].

Substance P (SP) is a highly conserved member of the tachykinin neuropeptide family. SP mRNAs are present in striatal neuronal populations from week 12 of fetal life. SP is involved in a multitude of neuronal signaling pathways, mediating sensations and emotional responses and transmission and modulation of pain signals by activating the neurokinin-1 (NK-1) receptor [6]. It transmits nociceptive signals via primary afferent fibers to spinal and brainstem second-order neurons.
Pain control is important for many reasons but overuse of morphine or benzodiazepines may have undesirable long-term effects [7]. Animal evidence suggests that the neonatal brain is affected differently when exposed to morphine administered in the absence of pain than in the presence of pain. Safety and efficacy are the major concerns in the selection of an appropriate pain-relieving treatment in infants [8], [9].

Although the effect of pain and stress exposure on neurodevelopment in preterm infants is well-documented, the potential role for SP as neurochemical markers of pain in neonates has not been fully investigated. SP in plasma of adult has been associated with pain.

**Research questions**

Can SP be used as potential neurochemical marker for diagnosis and management of pain in sick full term and preterm infants?

**Objectives**

Evaluate the potential role for neuropeptides SP as neurochemical pain marker in newborn infants in order to manage pain adequately, decrease unnecessary use of sedatives and analgesics, protect developing brain and promote better long-term outcome.

**Materials and Methods**

**Subjects**

The present study included 60 neonates, who were admitted to NICU. They were allocated to two groups according to gestational (GA) age; full-term and preterm groups. The full term group was subdivided into control group (10 normal full term) and 20 sick full-term neonates. The preterm group included control group (10) and sick preterm term infants (20) cases.

The sick preterm aged from 32 to 36 weeks of gestations while full-term GA ranged from 37 to 42 weeks.

**Study design**

This was case-control study to evaluate the potential role of SP as pain markers. All neonates were subjected to full complete history taking and thorough clinical examination, estimation of NIPS before and after procedures of checkup, routine care, or painful procedures. The NIPS addresses 5 behavioral parameters (facial expression, crying, arm movement, leg movement, and state arousal) and 1 physiological parameter (breathing pattern). Each behavioral indicator is scored with 0 or 1 except “cry,” which has three possible descriptors, therefore, being scored with a 0, 1, or 2 [10]. Infants were observed for 1 min in order to fully assess each indicator. Total pain scores range from 0 to 7. We considered mild or no pain if 0–2, mild-to-moderate if 3–4, moderate-to-severe 4–5 severe if >5. It was done at the 1st day, before and after the invasive procedures as well as on the 5th day.

Serum level of SP was estimated in the 1st and 5th days using Assay Designs’ Correlate EIA™ SP kit. The samples were taken in relation to the routine investigations in the morning for the newborn infants whether control or sick groups.

Invasive procedure includes skin-breaking procedures, mechanical ventilation, arterial puncture, bronchoscopy, endoscopy, heel lancing, lumbar puncture, suprapubic bladder tap, venipuncture, bladder catheterization, central line insertion/removal, chest tube insertion/removal, intramuscular injection laser therapy for retinopathy, peripheral venous catheterization.

Noninvasive procedures include removal of adhesive tape, wound treatment, apply eye shield during phototherapy, apply monitor electrode, change diaper, eye examination, chest physiotherapy, gavage tube insertion, postural drainage, suture removal, and retinopathy of prematurity examination.

Informed consent was taken from the parents of the involved neonates and the study was approved by the ethical committee of the Faculty of Medicine for Girls, AL-Azhar University, number 202103751. The registration number is IRB00012239.

**Statistical analysis of data**

The collected data were validated and verified, organized, tabulated, and analyzed using statistical package for social software statistical computer package version 12. For qualitative data number and percent were calculated and for comparison between the variables Chi-square ($\chi^2$) test was used. For quantitative data mean and standard deviations were calculated and independent samples students (t) test was used to compare between two means. For comparison between more than two means, one-way analysis of variance was used. Paired, samples (t) test was used to compare two means at different times (1st and 5th day). Pearson's correlation coefficient was used for correlation between NIPS and SP. p value considered to be significant when <0.05.

**Results**

The results are shown from Tables 1-6.
The study was performed on 60 newborn infants with gestation age ranged from 32 to 42 weeks. There was significant difference in GA, birth weight (BW), and length in the preterm groups than the full-term group and non-significant difference as regard to gender.

Table 2: Pain score response to invasive and non-invasive procedures among the studied groups

Table 3: Substance P at 1st and 5th day in the studied groups

Table 4: Comparison of substance P levels at the 1st and 5th day after invasive procedures among the studied groups

Concerning BW, post hoc test, showed that BW was not quite significantly different between control preterm and sick preterm as p = 0.0552, but there was significant decrease of BW in sick preterm than sick full term infants, p < 0.0001. Post hoc test showed also, significant decrease in BW of sick preterm than control full term and in control preterm infants than control full-term infants, p < 0.001.

Further study of significance between the studied variables by post hoc test, showed that GA age was significantly decreased in control preterm and sick preterm than control full term, p = 0.029, and <0.001. Furthermore, there was a significant decrease in the GA of sick preterm than sick full term, p = 0 <0.001. There were no significant differences between control and sick preterm groups, p = 0.3427 as well as control and sick full-term groups as p = 0.1392, also no significant difference between the sick and control preterm infant and control full term hence p = 0.0123. No significant differences in length between the control and sick full-term infants as p = 0.1392, also no significant difference between the sick and control preterm infants as p = 0.6937.

Table 5: Comparison between cases need invasive procedures and non-invasive procedure

Further study of differences in Apgar score at 1 min by post hoc test, showed that there were no differences between all groups, except between sick preterm infant and control full term hence p = 0.0123.

Considering Apgar score at 5 min, post hoc test showed that, there was significant increase in the value of Apgar score at 5 min in the control preterm than sick preterm as p = 0.0037, also there was increase in the control full-term infants than sick full infants because p = 0.0198 and increase in the control full term than sick preterm infants as p = 0.0019.

There was significant increase in grade of severe pain among sick preterm infants due to invasive procedures, while response to noninvasive procedures was mild or moderate response p < 0.001. The sick full-term neonates response to invasive procedure was moderate to severe in 50% of cases and severe in 40% of cases, while the response to noninvasive procedures was mild or no in 40% of cases and moderate to non-invasive in 60%, p = 0.011 (Table 2).

The study was performed on 60 newborn infants with gestation age ranged from 32 to 42 weeks. There was significant difference in GA, birth weight (BW), and length in the preterm groups than the full-term group and non-significant difference as regard to gender.
There was a significant increase in SP mean values in the 5th day of postnatal age than the 1st day in control full term, preterm and sick preterm groups, \( p = 0.046, < 0.001, \) and \( 0.025 \) respectively (Table 3).

Comparing SP in the control groups to the sick newborn groups showed that there was a significant difference in the sick preterm group than the control preterm on the 1st and 5th day of life; \( p = 0.003 \) and 0.037. There was a significant increase between control and sick full term on the 5th day; \( P \) value = 0.005. Furthermore, there was no significant increase in SP between the preterm and full-term infants on the 1st and 5th day of life (Table 4).

SP was increased significantly after invasive procedures than non-invasive procedure in the sick full-term and sick preterm infants wether in the 1st or 5th day of life (Table 5).

There was significant correlation between NIPS and SP on the 1st day of life. Furthermore, there was positive, moderate, significant correlation between NIPS and SP after invasive procedures in preterm and full-term groups. It means that there was increase in SP with increase severity of pain score (Table 6).

| Substance | Basic At 1st day | After invasive procedure in preterm neonate | After invasive procedure in Full term neonate |
|-----------|-----------------|------------------------------------------|------------------------------------------|
| r         | 0.36            | 0.33                                     | 0.39                                     |
| p         | 0.009           | 0.002                                    | 0.005                                    |

| Pain Scale | After invasive procedure in preterm neonate | After invasive procedure in Full term neonate |
|------------|------------------------------------------|------------------------------------------|
| r          | 0.075                                    | 0.12                                     |
| p          | 0.61                                     | 0.59                                     | 0.024                                    |

Table 6: Correlation between pain score NIPS at the 1st and 5th day and substance P levels

Discussion

Pain assessment tools have inadequate indicative significances in the sickest and preterm infants. These scores were endorsed for acute pain, while still no useful tool for chronic or persistent pain. Newborn infants respond and process pain in distinct way as they have different neurobiology than older infants, children, and adults. The exposure to recurrent agonizing stimuli, strong painful procedures, or repeated mild procedures may permanently modify individual pain processing. The current study looked at the level of SP in normal and sick newborn infants and compared the findings between preterm and full-term infants as well as studied the relation to type of pain and pain score (NIPS) during the first 5 days of life. Furthermore, the study evaluates the difference between the sick newborn infants and the control groups.

There was a significant increase in SP levels in sick preterm infants in comparison to the control preterm group on the 1st and 5th day of life.

The sick full-term showed non-significant elevation on the 1st day and significant elevation of SP on the 5th day of life in comparison to the control full-term infants. The increase level of SP among sick groups may be due to the frequent repeated exposure to invasive and noninvasive procedures than the control groups. SP is synthesized in small- and medium-sized neurons of dorsal root ganglia and stored in dense core vesicles and transported by fast axonal transport to both spinal and peripheral nerve terminals [11].

Chronic and persistent pain have an effect on the level of SP. As far as we know there was no reported data compare normal to sick newborn before. There are studies compared SP in infants with sudden infant death syndrome and in normal infants [12], [13], [14].

The study showed significant increase in SP on the 5th day of life whether in control or sick preterm and full-term infants. This is in agreement with a previous study that showed a gradual rise in SP during the first 3 days, which decreased again by day 14 among normal neonates [13].

These data may indicate possibility of exposure of sick infants whether preterm or full term to chronic pain; chronic pain is not simply a temporal continuum of acute pain. In the setting of persistent injury, functional and structural reorganization of neuronal circuits in the CNS leads to long-term changes in perception and behavior [14].

Furthermore, the repeated painful procedures are associated with long-term adverse events as perceptual sensitization blunting of the hypothalamic-pituitary-adrenal axis response with subsequent lower thresholds for withdrawal responses that persist for at least the 1st year of life, lower cognitive and motor development at 8 and 18 months, increased sensitivity to childhood injuries, and higher incidence of somatic complaints [16], [17], [18].

There was non-significant increase in the levels of SP among sick preterm infants than full-term infants. This may be due to more exposure to painful procedures among preterm group. In preterm infants there is imbalance between the plentiful afferent excitatory pain neurotransmitters at birth with the descending inhibitory neurotransmitters that subjected the preterm infants to limited ability to modulate pain [19], it leads to increased nociceptive signaling in the central nervous system in preterm infants. Specific cell populations in the central nervous system of preterm neonates are particularly vulnerable to excitotoxicity, oxidative stress, and inflammation [20]. On coronary Wang et al. reported that gestational age has no significant correlation with SP concentrations among normal infants [13].
The current study showed a significant increase in the level of SP after invasive procedures in preterm and full-term infants on the 1st and 5th day of life, these results were not reported before as far as we know. This means that aversive and stressful stimuli cause increase in the SP. This finding confirms the rule of SP in acute pain and its association to severity of pain too. SP and its preferred NK-1 receptor has been found within brain areas known to be involved in the regulation of stress and anxiety responses. Stress increases SP efflux in specific limbic structures such as amygdala and septum and that the magnitude of this effect depends on the severity of the stressor. There are changes in SP brain tissue content and subsequent intracerebral SP concentration related to the stressful and aversive stimuli [21].

Tissue damage occurs at birth or shortly after, lead to intense and continuing dendritic sprouting in the local sensory nerve terminals with lowered mechanical threshold and hyperinnervation that persist into adult [19], [22]. Painful stimuli activate more than 1000 genes within the dorsal root ganglion [23]. This trigger unpredicted long-term epigenetic changes when happened during vulnerable period of neuronal plasticity due to early life stress [24].

The study revealed a significant positive correlation between pain score (NIPS) and SP on the 1st day following invasive procedures in sick preterm as $P = 0.002$ and in sick full-term $P = 0.005$, consequently SP increased with increase severity of pain. It could be as result to modulation effect of SP during early postnatal life on medullary cardiorespiratory and autonomic control [25], [26], whereas, changing breathing pattern is one of the items of NIPS.

It is crucial for neonatologist to diagnose, estimate the grade of pain as early as possible and to minimize the invasive procedures in order to treat neonatal pain effectively. Animal studies have shown that persistent or repeated pain increases apoptosis of neurons, and lead to anxiety-like behaviors during adulthood. In humans, greater exposure to neonatal pain-related stress has been associated with altered brain microstructure and stress hormone levels, that instigate poorer cognitive, motor, and behavioral neurodevelopment in infants and children born very preterm. On the other side, there is evidence that suggests that routine morphine administration in ventilated neonates has no beneficial effects on pain expression and may result in adverse neurological outcomes, and contributes to negative effects on cognitive functioning at 5 years of age [18].

Therefore, it is important that pain-related stress in preterm neonates is accurately identified, appropriately managed, and that pain management strategies are evaluated for protective or adverse effects in the long term [20].

Limitation of the study: There was small number of cases in control groups (10 cases in full term and 10 cases in preterm). We could not include control preterm <32 weeks of gestation as they need NICU and were subjected to painful procedures.

**Conclusion**

SP might have important role as pain marker in sick preterm and full-term infants, it increases significantly with invasive procedures. Adequate management of pain before the invasive procedure is mandatory. In addition, decrease the frequency of invasive and noninvasive procedures to ensure safe care and improve the quality of life of newborn infants.

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**Ethical approval**

The approval for this study was obtained from the Ethics Committee of Faculty of Medicine for Girls, AL-Azhar University (approval ID: number 202103751. The registration number is IRB00012239).

The study was run according to the Declaration of Helsinki adopted in 1975 and revised in 2008, and the ethical principles were completely respected.

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