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Salivary biomarkers are not suitable for pain assessment in newborns

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ABSTRACT

Background and aims: Newborns admitted to the neonatal intensive care unit are repeatedly subjected to painful or stressful procedures; therefore, objective assessment of their pain is essential. An increasing number of scales for neonatal pain assessment have been developed, many of which are based on physiological and behavioral factors. Recently, salivary biomarkers have been used to assess stress in adults and older infants. This study aimed to determine whether salivary biomarkers can be useful objective indices for assessing newborn pain.

Study design: A total of 47 healthy newborns were enrolled 3–4 days after birth. Heel lancing was performed to collect blood for a newborn screening test. Before and after heel lancing, saliva was collected to analyze hormone levels, a video was recorded for behavioral observations, and heart rate was recorded. Two investigators independently assessed newborn pain from the video observations using the Neonatal Infant Pain Scale (NIPS). Salivary chromogranin (sCgA) and salivary amylase (sAA) levels were measured using an enzyme-linked immunosorbent assay kit and a dry chemistry system, respectively.

Results: No definite changes in salivary biomarkers (sCgA or sAA) were detected before and after heel lancing. However, newborn sCgA levels were markedly higher than reported adult levels, with large inter- and intra-subject variability, whereas newborn sAA levels were lower than adult levels. NIPS score and heart rate were dramatically increased after heel lancing.

Conclusions: NIPS score (behavioral assessment) and heart rate are useful stress markers in newborns. However, neither sCgA nor sAA is suitable for assessing newborn pain.

1. Introduction

Current advances in medical care have facilitated the survival of newborn infants with life-threatening medical conditions such as extreme prematurity or congenital malformations [1,2]. However, such infants are also reported to be at increased risk of disordered psychological development [2]. Newborns admitted to the neonatal intensive care unit (NICU) are repeatedly subjected to painful or stressful diagnostic and interventional procedures during their hospital stay [3,4]. Perinatal brain vulnerability increases the risk of early painful or stressful events. There may be short- and long-term consequences to exposing hospitalized newborns to repeat painful procedures [5–7], and awareness of the need to minimize neonatal pain during such procedures is now growing. Guidelines for preventing or minimizing neonatal pain have thus been issued [8,9].

Pain is a subjective experience. Although verbal expression of pain is the gold standard for pain interpretation, newborns are incapable of self-reporting. Because newborns cannot verbalize their pain, they depend on others to recognize, assess, and manage it [9,10]. Pain assessment in newborns is challenging. Several scales have been developed, most of which are based on physiological and behavioral factors [7,11]. One of the most frequently used scales in neonatal pain research is the Neonatal Infant Pain Scale (NIPS) developed by Lawrence et al. [12]. It has high inter-rater reliability and internal consistency [11].

In addition to such physiological and behavioral measures, various hormones act as indices of pain or stress in adults and older children. Even in newborns, changes in the levels of various blood hormones in response to painful procedures have been reported [13,14]. The sympatho-adrenomedullary (SAM) system is one of the major stress response pathways. The activation of SAM induces the release of catecholamines such as epinephrine (adrenaline) and norepinephrine (noradrenaline) from the adrenal medulla into the bloodstream. Painful or stressful stimuli induce an increase in circulating catecholamines. sAA levels are significantly correlated with noradrenaline

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levels in the plasma [15], and some studies identified both sCgA and sAA as potential biomarkers for evaluating the activity of the SAM system following psychological and physical stress [16–18]. sAA secreted by the salivary glands is regulated by autonomic neuronal pathways, and studies in animals and humans have provided evidence that activation of the sympathetic nervous system leads to increased sAA secretion [19,20]. In addition, sCgA is a soluble protein that is co-stored with catecholamines and co-released from the adrenal medulla into an extracellular environment when the SAM system is stimulated [21–23]. Several studies have shown a correlation between sCgA levels and stress [24–26]. Therefore, sCgA or sAA can be useful markers of neonatal pain. However, the effectiveness of these biomarkers for newborn pain assessment is unknown. To determine whether these salivary biomarkers are useful objective indices for assessing newborn pain, we measured heart rates and NIPS scores as well as sCgA and sAA salivary biomarkers before and after a painful procedure.

2. Methods

2.1. Participants

The study was conducted at Kyoto University Hospital, Kyoto, Japan. A total of 47 healthy newborns (25 boys) from whom blood samples were required for clinical purposes were assessed to determine their responses to heel lancing performed in a standard manner. The mean postnatal age at the time of assessment was 4.0 days (3–4 days). All were term or near-term infants, and their mean gestational age at birth was 39.1 weeks (range, 36.7–41.3 weeks). None received any medications, including sedative and analgesic agents. The study protocol was approved by the ethics committee of Kyoto University Graduate School and Faculty of Medicine (No. E581), and informed written consent was obtained from the participants' parents.

2.2. Procedures

Heel lancing was performed when blood samples were required for a newborn screening test (Guthrie test). Heel lancing solely for the purpose of this study was not performed in any infant. Before and after the heel lancing procedure, saliva was collected to analyze hormone levels and video recordings and measurements of heart rate were obtained to calculate the pain score using NIPS.

2.3. Saliva analysis

Saliva samples were collected using a small super-absorbent sponge attached to a plastic shaft (Sorbette, Salimetrics, LLC., USA) that is commonly used to collect saliva from infants or toddlers. We collected saliva before and immediately after heel lancing. All saliva samples were centrifuged for 10 min at 3000 rpm and frozen at −30 °C until assay. sCgA and sAA levels were determined using an enzyme-linked immunosorbent assay kit (YK070 Human Chromogranin A EIA, Yanaihara Institute, Inc., Shizuoka, Japan) and a Cocoro meter® (Nipro Co, Osaka, Japan), respectively. Total protein level in the saliva was determined using a protein assay kit (Bio-Rad Protein Assay, Bio-Rad Laboratories, Inc., CA, USA), and sCgA and sAA levels were expressed as pmol/mg total protein and IU/ml, respectively.

2.4. Behavioral and physiological analysis

We used NIPS as a behavioral and physiological assessment tool for newborn pain. NIPS includes five behavioral components and one physiological component [12]. NIPS scores were independently assessed from videotape by two coders every 1 min. Krippendorff's α was 0.98, indicating high inter-rater reliability. Each component of the NIPS was scored as the highest level achieved by the infant every minute. NIPS score before and after heel lancing was determined as the 5-minute average of each one-minute total NIPS score before and after blood sampling. Heart rate was monitored every 2 s from 5 min before to 5 min after heel lancing. Heart rate was measured using a pulse oximeter (The OxiMax™ N-560™ pulse oximeter, Covidien-Nellcor Puritan Bennett LLC., USA) and analyzed with an in-house software program implemented in MatLab software (version 7.11; MathWorks, Inc., Natick, MA, USA). Heart rate before and after heel lancing was determined as the 5-minute average of each period.

2.5. Statistical analysis

Results were expressed as median ± MAD (median absolute deviation), or mean ± SD (standard deviation). The Wilcoxon signed rank test was used to verify differences in sCgA and sAA levels and NIPS scores before and after heel lancing. A paired *t*-test was used for the analysis of heart rate. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Infants' profiles

Forty-seven healthy newborn infants were recruited. Of these, 12 were excluded from our analysis because of saliva collection returning an insufficient volume for analysis of sCgA or sAA levels before and after the painful procedure. The remaining 35 newborns (18 males; mean birth weight, 2993 g; range, 2064–3800; Apgar score at 5 min, 8–10) were included in the study.

3.2. Salivary biomarkers

3.2.1. Salivary chromogranin A levels before and after pain induced by heel lancing

Among the 35 newborns, sCgA levels before and after the painful procedure could be determined in 33. The median sCgA level before and after heel lancing was 45.1 and 52.9 pmol/mg, respectively (Fig. 1A). A total of 20 of the 33 newborns (60.6%) had increased sCgA levels after heel lancing; however, this increase was not significant ($p = 0.53$, Wilcoxon signed rank test). The range of sCgA levels before the painful procedure (baseline value) was 2.16 to 614.46 pmol/mg, and the median absolute deviation was 34.7 pmol/mg, which showed the large variation of sCgA values.

3.2.2. Salivary amylase levels before and after pain induced by heel lancing

Among the 35 newborns assessed, sAA levels before and after the painful procedure could be measured in 24. The mean sAA level before and after heel lancing was 46.4 and 48.8 IU/ml, respectively (Fig. 1B), and no significant changes were detected in levels before and after the heel lancing procedure ($p = 0.36$, Wilcoxon signed rank test).

3.3. Behavioral and physiological analysis

3.3.1. NIPS scores

Of the 35 newborns, 30 (86%) cried immediately after the heel lancing procedure. The mean NIPS score before and after heel lancing was 2.46 and 5.64, respectively. This increase of 3.18 points was highly significant ($p = 0.00000076$, Wilcoxon signed rank test; Fig. 1D). Concerning the six subcomponents of NIPS, crying, breathing pattern, and state of arousal, were largely responsible for the observed increase in the total NIPS score (71.4% of the increase in total score; Fig. 1E). The score for crying increased from 0.38 to 1.51 (35.6% of the total increase), which reflected the large number of newborns