Effectiveness of salivary chromogranin A as a stress index in young children during dental treatment

Chieko Mitsuhata¹*, Yukari Ohara¹, Meiko Tachikake², Yuko Iwamoto¹ and Katsuyuki Kozai¹

¹ Department of Pediatric Dentistry, Integrated Health Sciences, Institute of Biomedical & Health Sciences, Hiroshima University
1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, JAPAN
² Department of Pediatric Dentistry, Hiroshima University Hospital
1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, JAPAN

Abstract It is important to understand the level of stress experienced by children during dental treatment, which can be stressful and even lead to permanent dental phobia. We investigated whether chromogranin A (CgA), a stress marker in adults, is useful for determining stress levels in children. Saliva samples were collected before and after treatment from 5 children (3–5 years) who required treatment more than 5 times in a relatively short period. Their parents completed a questionnaire about their child liking/disliking the clinic visit, how they explained to their children the purpose of the dental visit and the type of treatment sought, and to explain descriptively the difference they noticed in their child’s behavior. The CgA levels were significantly higher before than after the treatment in all cases. Pre-treatment CgA values were not always related to dislike for the clinic visit, type of treatment sought, and behavioral reaction to the treatment. The CgA values may have been influenced by the children’s previous experience of the treatment. The results suggest that CgA might be appropriate for verifying children’s stress levels during dental treatment and stress tendency towards dental treatment.

Introduction It is well established that dental treatment involves stress. In severe cases, this stress can lead to dental fear anxiety (DFA) or dental behavior management problems (DBMP) for future dental care. Dental phobia can remain in adulthood. DFA and DBMP have multifaceted origins, and several potential etiological factors have been proposed, including general fear¹,²), temperament²,³), general behavior, and attention⁴). Pain and negative experiences from dental treatment are also considered as major causes of DFA and DBMP⁵). The precise level of stress experienced by children during dental procedures is currently unknown. The prevalence of dental anxiety in children has been reported to be between 5.7% and 6.7%⁶–⁸), with anxiety decreasing with increasing age⁹).

Many measurement techniques have been used to examine the condition of children during dental procedures, including behavioral ratings, psychometric scales, and physiological measures. The Dental Subscale of Children’s Fear Survey (CFSS-DS)⁹), Dental Fear Scale (DFS)¹⁰), and Dental Anxiety Scale (DAS)¹¹) are relatively commonly used as assessments of DFA. The other hand, Frankl scale¹²) and the Behavior Profile Scale (BPRS)¹³) are used as scales of DBMP.

Stress is usually defined as the combination of physiological and psychological reactions that mobilize an organism’s defense against external and internal threats (stressors). Stress reaction includes activation of the hypothalamus-pituitary-adrenal...
The classical stress hormones, catecholamine (CA) and cortisol, have been used in previous research as physiological and/or psychological markers of various stresses. Recently, other biological materials have been used as stress markers, in an effort to simplify detection and improve sensitivity. The measurement of salivary chromogranin A (CgA) concentration has been developed as a method to evaluate psychological stress.

CgA is a 457 amino-acid acidic glycoprotein in adrenal chromaffin cells and adrenergic neurons. CgA is secreted with CA into human blood, and is considered a valuable indicator of sympatho-adrenal activity. CgA is present in the submandibular glands ducts, and is secreted into saliva during stress. Salivary CgA has been proposed as a marker of sympathetic nervous activity involving the sympathetic-adrenomedullary system. It has been reported that salivary CgA might be promising as a sensitive index for psychosomatic stress in adults. Salivary CgA changes more rapidly and is more sensitive to psychological stressors than salivary cortisol. This is because the cortisol levels tend to remain high for quite some time even when the person is free from stress. This has been well proved by Nakane et al. who states in his study that CgA levels respond rapidly. After relief of stress these values go down immediately and attains a stress free level.

Biochemical markers in saliva are useful for objectively assessing physiological and/or psychological stress because the collection of saliva is convenient, noninvasive and relatively non-stressful. As such, it is a useful measure in studies where children are experimental subjects.

We verified whether saliva CgA might also be used as an index of stress in children. Forty-two children patients ranging from 5 to 9 years old (mean 7.5 years old) in our clinic were investigated. CgA values of 83.8% of children decreased after the dental treatment. There was a tendency that pre-treatment CgA values of kindergarten children were higher than school children. These results suggested that CgA might be used to verify child’s stress during dental treatment. Meanwhile, it was reported that an experience of past dental treatment influenced the formation of present dental fear in adults. It’s said that 4 years old child is brought about much fear by visual and audio stimulus.

The purpose of the present study was to investigate whether salivary CgA may also be used as an index of stress in younger children during dental treatment. Our results suggest that CgA might be an appropriate measure for verifying young children’s stress levels during dental treatment.

**Patients and Methods**

**Subjects**

Our subjects were 5 boys aged between 3 years 3 months and 4 years 8 months who visited Hiroshima University Hospital Pediatric Dental Clinic for treatment of caries in 4 months. Of the 5 children, 3 were undergoing dental treatment for the first time while the other 2 children had dental treatment before. All patients were brought to the clinic complaining of spontaneous toothache. All the patients had between 4 and 16 teeth that required treatment, including pulpectomy or root canal treatment.

We started observation and saliva sampling after the second visit, and repeated the sampling 6 or 7 times from the same participant in a random manner. All the children had cavities and required more than 5 visits to the dental office. None of the participants had a systemic illness, prescribed drugs and none had growth or mental development problems. Ethical approval for the study was granted by an independent ethical committee of Hiroshima University (D73-1). The study was performed in accordance with the committee’s ethical guidelines. Before the experiment, we explained the purpose of this study to the guardians and children, and obtained written consent from all the guardians.

**Experimental protocol**

We randomly chose children who needed more than 5 treatments. For each treatment, patients’ saliva was collected before and after dental treatment. The parents of the children answered the questionnaire for each trip, and we produced a report on each day of treatment and the child’s behavior during dental treatment. Each child was treated by a dentist in attendance at all visits.

**Saliva sampling and CgA analyses**

Saliva samples were collected just prior to and after each dental treatment in the waiting room using a cotton swab, which was placed inside the mouth for 2 min, and then deposited in a sample tube (Salivette, Sarstedt, Germany). Each child visited our clinic at about the same time period and we asked guardians to stop eating and drinking in 1–2
hours before treatments. The tubes were stored on ice until centrifugation for 10 min at 3,000 rpm. The collected saliva was stored at −30°C until analysis. The concentration of saliva CgA was measured by an enzyme-linked immunosorbent assay (ELISA) using the YK070 Human CgA EIA Kit (Yanaihara Institute Inc., Japan). Salivary protein concentration was determined using a protein assay (BioRad, USA). The CgA concentration was corrected using protein concentration, and data are presented as pmol/mg protein.

Saliva samplings and CgA analyses were performed in faithful accordance with the manufacture protocol. Pre- and post-treatment saliva CgA values were compared using a two-sided Wilcoxon’s signed rank test. A Kruskal-Wallis rank test was used within each group.

**Questionnaire and treatment record**

A questionnaire was administered to the parents, asking them to report whether their child particularly liked/disliked the clinic visit, how they explained to their children about the purpose of the visit to the dentist and the type of treatment to be sought, and to write freely on what they noticed about their child’s behavior during the visit to the dental clinic and dental treatment.

The treatment record included treatment content, time spent on the chair, use of local anesthesia, and child’s cooperation. We used the Frankl scale to classify the degree of cooperation. Each child’s behavior was classified into one of the following categories: (1) definitively negative, (2) negative, (3) positive, and (4) definitively positive.

**Results**

Figure 1 shows salivary CgA values for all patients at every clinic visit. The results of a two-sided Wilcoxon’s signed rank test showed that post-treatment CgA values were significantly lower than the pre-treatment values ($P<0.0001$). The median of pre-treatment and post-treatment CgA values was 5.94 pmol/mg protein and 1.58 pmol/mg protein, respectively. This indicates that the patients were
Fig. 2 Dental trip details for each child


E—_: upper right second deciduous molar, E—_—_: upper left second deciduous molar, E—__: lower right second deciduous molar, E—_—__: lower left second deciduous molar, D—__: upper right second deciduous molar, D—_—__: upper left second deciduous molar, D—__: lower right second deciduous molar, D—_—__: lower left second deciduous molar, C—__: upper right deciduous canine, C—_—__: lower right deciduous canine, B—__: lower right deciduous lateral incisor.
under stress before start of the treatment, and were relieved from stress soon after the treatment ended.

Each child’s CgA values are shown in Table 1 and Fig. 2. Pre-treatment CgA values varied widely and exceeded the detection limits in 2 of the 5 cases. The level of stress that the children experienced differed on each visit. Sometimes the same treatment given to the same child on subsequent visits resulted in different pre-treatment CgA values. Similar pre-treatment CgA values were observed even in patients who had similar rating for whether they disliked or liked the clinic visit. The distribution of pre-treatment CgA values were significantly higher than the post-treatment distribution for every child except one (Patient No.4), who showed relatively low pre-treatment CgA values higher values after treatment compared to other participants. Patients’ CgA values were analyzed separately before and after the treatment using a Kruskal-Wallis rank test. Post-treatment CgA values were significantly different (P = 0.0077) among all children, while pre-treatment values were not.

The Frankl scale score was found to be between 1 and 3 for all children. There was no correlation between children’s cooperation and pre-CgA values. We found that there were some children who had low pre-CgA value showed less co-operation (Frankl score 1) while some children having high pre-CgA value were more cooperative (Frankl score 3). Each child’s condition is shown in Fig. 2.

**Discussion**

Children often do not sufficiently understand the need for dental treatment, and some children might express fear that appears “excessive” or “unreasonable”, similar to “dental phobia”. In contrast, some children may endure the pain and continue to exhibit good behavior despite their fear. It is widely believed that dental treatment can induce stress in children, but the precise degree of stress experienced remained unclear. In addition, dental stress might contribute to dental phobia, similar to the contribution of stress to Post-traumatic stress disorder: PTSD. The vast majority of dental-care anxiety cases have been reported to stem from aversive dental experiences.

And some studies have investigated the molecular mechanism involved in cognitive vulnerability stress in vivo and in vitro. It is possible that patients might have cognitive vulnerability to stress. Some transgenic mice were indicative of high response against stress due to stress vulnerability. Thus, it is important to determine the level of stress experienced by children during dental treatments.

It was hypothesized that pre-treatment CgA values indicate the stress associated with dental treatment, and post-treatment CgA values indicate relief from stress after the treatment. The results revealed no significant correlations, but showed that children with a high CFSS-DS score tended to have a higher CgA value. We also reported that 83.8% of the children who were targeted between 5 years old and 9 years old on our pediatric dental clinic had lower CgA after treatment and the pre- and post-treatment CgA values of kindergarten boys (5–6 years old) receiving caries treatment differed significantly. In this research, we targeted younger children. The distribution of CgA values as in previous studies was found to decrease significantly after dental treatment (Fig. 1, Table 1). It was a tendency that younger children had higher values and wide variation in pre-treatment CgA and had similar values in post-treatment CgA compared to prior investigation. This result means that CgA values can be used as a stress marker in younger children.

Some recent reports have investigated psychological stress caused through multiple stressors, using salivary CgA as an indicator. The stressors examined include psychosomatic stress during computer operation, stress related to academic assessment, and stress related to psychological tension before surgery or anesthesia. Other studies have reported that there were no significant differences in CgA levels between the pre- and post-test saliva samples, because the stressors used were not strong enough to change CgA levels.

In this study, we found that CgA values across all children were significantly higher in the pre-treatment stage, compared with after treatment. This finding suggests that CgA is effective as a biological marker for stress in young children, although CgA values did not correlate with the content of the prearranged dental treatment, and did not always affect the child’s behavior during treatment. We predicted that the same dental treatment triggered similar level of stress in the same child if he/she understood the content of the prearranged dental treatment. So we planned to get samples more 5 times from the same child to prove our prediction. Young children tend to experience some degree of stress in any dental treatment. We found that
pre-treatment stress was influenced by experiences that they had on previous treatments. We did not find significant individual differences in pre-treatment CgA values. A possible explanation for the wide variation in CgA values is that in some cases, children who are usually well-behaved suddenly become uncooperative, even for relatively simple dental treatments, due to fear or anxiety. Many factors such as mood swings, differing feelings etc. are involved, and this behavior is not solely related to the dental treatment itself as the CgA readings vary even if the same stress was induced to the same child at a different time point. However, by checking CgA levels several times, it is possible to approximate the level of stress. From each child’s median pre-treatment CgA value, it is possible that patients 1, 3 and 4 were more stressed than patient 5. Patient 4 had the highest median post-treatment CgA value. This may be because this patient’s steady-state levels are higher than normal or that he needs more time to recover from stress. Each child’s median CgA value might indicate the tendency of his/her stress level.

It is important to know child’s stress levels when planning and conducting dental treatment, in order to prevent dental fear in the future. In particular, when we deal with patients with high pre-treatment CgA values and good cooperation, they need more detailed support best suited them by dentists and dental staff. Our results suggest that salivary CgA might be useful as a stress marker in young children. Dentists, dental staffs, and other practitioners should know patient’s stress level objectively using some stress indicators like CgA level estimations while they are visiting dental office or undergoing dental treatment and deal with it appropriately in order to prevent dental phobia in future.

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Declaration of interest

The authors report no conflict of interest.

References


