Salivary Cortisol, Alpha-Amylase and Heart Rate Variation in Response to Dental Treatment in Children

Furlan NF * / Gavião MBD ** / Barbosa TS *** / Nicolau J **** / Castelo PM *****

Objective. *Anxiety and stress are usually related to the dental treatment situation. The objective was to investigate salivary cortisol and alpha-amylase levels (salivary biomarkers) and heart rate in children undergoing a minor dental procedure (dental prophylaxis). Study design. In total, 31 children (range 84–95 months) of both genders without caries or history of dental treatment/pain/trauma were selected. Three saliva samples were gathered: one prior to dental prophylaxis, one immediately after, and one ten minutes later. Weight and height were assessed, and heart rate was evaluated prior to and during the procedure. Data were analyzed by correlation tests and t-test/Wilcoxon (α = 0.05). Results. Higher cortisol and amylase levels were observed before prophylaxis compared to afterward. Cortisol and amylase levels did not show a significant correlation, nor did salivary biomarkers and body mass index. However, heart rate and amylase levels showed a significant positive correlation. Conclusions. In the studied sample, certain anticipation of the dental treatment was observed because higher cortisol and amylase levels were observed before, rather than after, the event; moreover, a significant correlation between amylase levels and heart rate was observed. Thus, salivary biomarkers may be a valuable tool for evaluating anxiety-producing events, such as dental treatment, in children.*

Keywords: *Alpha-amylases, Children, Dental Prophylaxis, Heart rate, Cortisol, Saliva.*
J Clin Pediatr Dent 37(1): 83–88, 2012

INTRODUCTION

Stress is commonly defined as physiological and psychological reactions that mobilize an organism's defense against external or internal threats (stressors) and has at least two main components.[1] One component involves the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis and the secretion of glucocorticoids (e.g., cortisol) into the circulating blood stream. The second involves activation of the autonomic nervous system (ANS) and the release of catecholamines (e.g., norepinephrine) into the blood stream.[2] The sympathetic component of the ANS is responsible for a host of effects, including elevated cardiovascular tone, heart and respiratory rate, blood flow to muscles and blood glucose. Individual differences in the activity of the HPA axis and sympathetic nervous system (SNS) can be measured non-invasively in saliva. Over the past decade, there has been a dramatic increase in studies evaluating salivary biomarkers of stress, as this process minimizes some of the potential confounding variables associated with blood sampling.[3] Recently, the strategy of measuring SNS activity via salivary alpha-amylase (sAA) has become possible.[4]

Stress and the diurnal rhythm are modulating factors that can upregulate the production of cortisol. Its secretory activity is characterized by peak levels 20-30 minutes after awakening and a declining pattern thereafter. Salivary cortisol is known to be an indicator of the concentration of unbound cortisol in the serum.[1] Moreover, researchers have consistently reported increases in sAA in response to laboratory stress procedures. Thus, sAA may serve as a non-invasive measure of adrenergic activity in humans.[5]

Dental treatment is often considered anxiety-producing and stressful, and the anticipation of an event,[6] particularly where "horror stories" abound, is likely a reliable cause of stress.[7,8] However, there is no consensus on the amount of stress generated by different kinds of dental procedures.[6,9,10] To date, few studies have investigated the physiological response to dental procedures, particularly in children.[1]

* Natália Fontanello Furlan, DDS, Piracicaba Dental School, State University of Campinas, Piracicaba.
** Maria Beatriz Duarte Gavião, DDS, PhD, Professor, Department of Pediatric Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba.
*** Taís de Souza Barbosa DDS, PhD, Post Doctoral Student, Researcher of the Department of Pediatric Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba.
**** José Nicolau, DDS, PhD, Professor, Oral Biology Research Center, Dental School, University of São Paulo.
***** Paula Midori Castelo DDS, PhD, Professor, Department of Biological Sciences, Federal University of São Paulo, Diadema.

Send all correspondence to: Prof. Paula Midori Castelo Depto. Ciências Biológicas, Universidade Federal de São Paulo (UNIFESP - Diadema) R. São Nicolau, 210 – 1. andar, Diadema – SP – Brasil /09913-030

E-mail: pcastelo@yahoo.com

Body composition, age, pubertal development, and time of awakening have potential confounding effects on the cortisol-psychopathology relationship and, therefore, should be investigated, or at least controlled.[11-13] One recent study in adults has shown that body mass index (BMI) may be a strong predictor of sAA increase.[14]

There is a lack of information concerning the evaluation of salivary biomarkers of stress in dental treatment, especially in pediatric dentistry, where behavior management is often necessary and may be monitored by physiological markers. Thus, the aims of this study were to evaluate salivary cortisol and sAA responses to dental prophylaxis in young children and to examine the correlation between salivary biomarkers, heart rate and BMI in a specific dental procedure that may represent a stressor event in children without previous experience.

MATERIAL AND METHOD

A cross-sectional study design was used with subjects recruited as a convenience sample from public schools of Piracicaba, São Paulo (SP), Brazil. Children with ages ranging between 84 and 95 months (approximately seven years) of both genders were evaluated, and 31 were selected after the conduction of complete anamnesis and clinical examination to verify their medical and dental history as well as body variables (weight and height). The inclusion criteria were the presence of mixed dentition, no history of dental pain or trauma and no dental treatment experience. The exclusion criteria were: dental caries, early tooth loss, parafunctional habits (pacifier/digit sucking, nutritive sucking habit, bruxism and/or nail biting), systemic and/or mental developmental disorders (including diseases of the endocrine and metabolic systems) and medications that would interfere with the central nervous system.

At the first appointment, the study design was explained to the children and their parents, and their informed consent was obtained (Ethical Committee of the Piracicaba Dental School, protocol no. 021/2007).

In the study done by Kivlighan and Granger (2006),[23] the correlation coefficient obtained between salivary alpha-amylase and cortisol 20 minutes after a competition was -.46. Considering an alpha level of 0.05 and power of the test=80%, 36 subjects would be enough to conduct a similar evaluation. In the present study, 40 children were recruited after screening, but only 31 fulfilled the inclusion criteria. However, this study included children with a very restricted age range (between 84 and 95 months) to minimize potential confounding factors.

Experimental protocol

All dental procedures involved a standardized, routine dental prophylaxis, performed by a pediatric dentist at the second appointment, which consisted of polishing of teeth using a slow speed hand piece, soft rubber cup and paste without fluoride for 15 minutes. Patients were in the supine position, and prophylaxis was performed without local anesthesia. Those responsible for each child were instructed to wake the child between 6:30 and 7:00 AM and not to give him or her food or drink for at least 1 h before the session. Children did not take any medication for at least 15 days before the dental procedure nor drink beverages with caffeine for one day preceding the session. To minimize the effects of circadian rhythms, all procedures were initiated between 9:00 AM and 9:30 AM on a weekday.[7] Three saliva samples were taken: (00:00) 10 minutes before the start of dental prophylaxis, for cortisol and alpha-amylase analysis; (00:25) at the end of the procedure, for amylase; and (00:35) 10 minutes after the end of the procedure, for cortisol measurement, as proposed by Blomqvist *et al* [1] The first saliva sample (00:00) was taken in a calm room, in the presence of child's parents/guardians and with the patient in the sitting position. For standardization of the method, the presence of parents/guardians during dental attendance was not permitted and uncooperative children were excluded.

Stimulated saliva samples at both the dental clinic and at home were collected by chewing cotton rolls for one to two minutes, until they were soaked with saliva (Salivettes, Sarstedt, Numbrecht, Germany). For home saliva samples collection (one week later), the subjects woke up as usual on a weekday. The first sample was taken while lying in bed and the second sample was taken 30 minutes after awakening (fasting). After chewing, the cotton rolls were placed in the plastic tube of a *salivette*. Home saliva samples were considered to evaluate the normal function of HPA axis of the selected subjects, since free cortisol concentrations in saliva increase by between 50% and 160% in the first 30 minutes immediately post-awakening.[14] The *salivettes* were transported on ice to the Pediatric Dentistry Laboratory on the same day and were centrifuged (at 3500 rpm for 5 min) and stored at -80°C until analysis.[15]

The average and maximum heart rates were evaluated using a cardiac monitor (S625x, Polar, Finland), which consisted of a wrist receptor and a chest belt sensor. Heart rates were assessed before (sitting position, 10 minutes) and during the whole dental treatment (supine position), and the data obtained were uploaded to a personal computer for further analysis.[16]

Analysis of saliva

Cortisol: To minimize variation, all samples from the same subject were assayed in the same batch (in duplicate). Salivary cortisol was assayed using a commercial, highly sensitive enzyme immunoassay kit (Salimetrics, State College, PA, USA) according to the manufacturer's directions. Twenty five µL of whole saliva was added to each well of the microtiter plate, which was read at 450 nm in a microplate reader (Stat Fax 2100, Awareness Tech. Inc., Palm City, FL, USA). Standard curves were fitted by a weighted regression analysis, and the lower limit of sensitivity was 0.003 µg/dL.

Alpha-amylase: sAA activity was assayed using a kit ($CNPG_3$, Synermed, Westfield, IN, USA). The assay employs a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of alpha-amylase on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm using a spectrophotometer (DU800 UV/Vis, Beckman Coulter, Fullerton, CA, USA). The amount of amylase activity present in the sample is directly proportional to the increase (over a 3-min period at 37˚C) in absorbance at 405 nm, expressed as international units per milliliter of saliva (U/ml). Protein concentration was determined by the method of Lowry *et al.*[17] using bovine serum albumin as standard.[18] sAA levels were expressed as amylase corrected by protein concentration (U/mg).

Table 1. Demographic data and body variables of the studied sample.

n = 31	Age (months)	Weight (kg)	Height (m)	BMI(Kg/m²)
♀ = 17	89.77	26.35	1.26	16.57
♂ = 14	(±3.37)	(±5.24)	(±0.04)	(±2.76)

BMI, body mass index.

Table 2. Average (±SD) salivary concentrations of total protein, alpha-amylase, and cortisol before and after dental treatment and awakening levels one week later.

	Total protein (mg/ml)	sAA		Cortisol (μg/dl)
		sAA (U/ml)	sAA/total protein (U/mg)	
Before (00:00)	0.57** (0.42)	77.07*** (43.59)	347.72 (419.95)	0.17* (0.11)
sAA collection (00:25)	0.44** (0.33)	55.56*** (38.15)	347.15 (496.23)	-
Cortisol collection (00:35)	-	-	-	0.12* (0.08)
Awakening (one week later)	1.34 (1.91)	51.79 (43.00)	215.49 (495.93)	0.17 (0.10)
30 minutes after awakening (one week later)	0.74 (0.65)	53.32 (37.39)	283.43 (486.94)	0.30 (0.17)

sAA, alpha-amylase
* p=0.037 (paired *t*-test; t = 2.182 with 30 degrees of freedom; CI (95%) = 0.00321 to 0.0968)
** p=0.005 (Wilcoxon test)
*** p=0.001 (paired *t*-test; t = 3.7022 with 30 degrees of freedom; CI (95%) = 9.6486 to 33.3837)

Statistics

Statistical analysis was performed using Sigma Stat (3.1 Sigma Stat Software Inc., Richmond, CA, USA) with a 5% significance level, and normality was assessed using the Shapiro-Wilk *W*-test.

A paired *t*-test or Wilcoxon test was used to analyze the difference in salivary concentrations of cortisol and amylase before and after dental treatment, as well as differences in heart rate at rest and during dental attendance. Correlations between salivary cortisol and alpha-amylase concentrations, heart rate and BMI were analyzed by means of Pearson's or Spearman's correlation test, where appropriate.

RESULTS

The demographic characteristics and body variables of the studied sample are shown in Table 1. Table 2 shows the average ± standard deviation (SD) of the salivary concentrations of cortisol, sAA and total protein during dental treatment and awakening levels one week later. Cortisol, total protein and sAA levels were significantly higher before dental prophylaxis than afterward. Cortisol levels prior to dental prophylaxis were comparable to those observed upon awakening, but sAA concentration per total protein did not differ significantly before and after dental treatment.

The average heart rate was higher prior to dental treatment (sitting position) than during the session (supine posi-

Table 3. Average (±SD) heart rate before and during dental treatment.

HR before† (bpm)		HR during dental treatment‡ (bpm)	
Average	Maximum	Average	Maximum
92.74*	102.97	87.74*	105.35
(9.56)	(9.54)	(11.99)	(14.24)

HR, heart rate; bpm, beats per minute.
† sitting position
‡ supine position
* p < 0.0001 (paired *t*-test; t = -3.9265 with degrees of freedom = 30; CI (95%) = -2.3998 to -7.6002)

Table 4. Correlation coefficients among salivary concentrations of cortisol, alpha-amylase and heart rate in response to dental treatment.

	sAA before	sAA after dental treatment	HR before (average)	HR before (maximum)	HR during dental treatment (average)	HR during dental treatment (maximum)
Cortisol before	0.21	-	-0.01	-0.03	-	-
sAA before	-	-	0.37*	0.34	-	-
Cortisol after dental treatment	-	0.30	-	-	0.24	0.25
sAA after dental treatment	-	-	-	-	0.36*	0.41*

HR, heart rate; sAA, alpha-amylase.
* p ≤ 0.05 (Pearson's correlation test)

tion), and this difference was statistically significant. However, the maximum heart rates observed prior to and during dental treatment did not differ significantly (Table 3).

The studied salivary biomarkers did not show any significant correlation with BMI. Moreover, no significant correlation between salivary cortisol and sAA levels before and after dental treatment was observed. Evaluation of the correlation between heart rate and sAA levels showed significant positive correlation coefficients in both observation periods (Table 4), while cortisol did not correlate significantly with heart rate.

DISCUSSION

Anxiety is a special variety of fear, experienced in anticipation of threatening stimuli, which is associated with a change in blood pressure and heart rate. Corroborating the results found, a previous study showed a significant change in the systolic pressure and heart rate in all the situations in the dental operatory area, such waiting in the reception area, examination, oral prophylaxis, cavity preparation, and extraction.[8] Moreover, the salivary levels of the evaluated biomarkers were comparable to previous studies done in children in terms of total protein,[18] alpha-amylase[19] and cortisol.[13] The study design took into account recent studies that have shown that sAA reactivity peaks in response to mild-to-moderate level challenge within 10 minutes of the event,[2] more quickly than the cortisol response. Dental prophylaxis was chosen due to its replicable and easy pattern and because it is a procedure that does not interfere with saliva collection and assaying.[20]

Higher levels of salivary cortisol, total protein and alpha-amylase were observed before dental prophylaxis, compared to after the procedure, even in samples from patients without any history of dental treatment. Thus, a certain amount of anticipation of the event was observed, corroborating the study done by Rank *et al* [21] Those authors observed that in children aged 4-6 years with no previous dental treatment experience, the percentage displaying negative self analysis of emotional reactions (such as fear, rage and panic) decreased after dental prophylaxis at the first appointment, and more children expressed “happiness” or “no anxiety” after the procedure. Moreover, it was observed that cortisol levels prior to dental prophylaxis were similar to the level upon awakening one week later. According to Nater *et al*[12] and Strahler *et al*[14] morning cortisol samples may represent the highest levels of cortisol in the diurnal rhythm. After dental prophylaxis (lasting 35 minutes), the cortisol levels decreased significantly. The fact that the dental procedure was designed not to involve pain or discomfort might serve to minimize the increase in cortisol and amylase levels after the end of the procedure. A previous study in older children with low dental anxiety as controls showed cortisol levels prior to and after dental examination reaching about half of those observed in saliva collection upon awakening and 30 minutes after awakening.[1]

The study done by Miller *et al* [7] did not observe significant differences in cortisol levels in adults undergoing routine dental procedures. However, previous studies involving dental procedures with associated distress and pain, such as third molar extraction, showed slight alterations in HPA biomarkers like cortisol,[10] but significant alterations in SNS biomarkers like catecholamines.[9] Other studies involving the evaluation of sAA and dental anxiety were not found for comparison. In adults, cortisol and amylase levels have shown to increase after stressor events, such a stressful video viewing[22] and sports competitions.[23] The diurnal profile of sAA shows a pronounced decrease in the first 30 minutes after awakening and steadily rising levels about one hour after awakening continuing into the afternoon and evening.[12] Prior to dental treatment, higher levels of total protein and amylase were observed, compared to after the event, and this result differs from the pattern of the diurnal rhythm of amylase.[12,14] In terms of amylase content per total protein, no significant difference between the two time points was observed.

Average heart rate was higher prior to dental treatment than during the session, although this comparison must be drawn with caution because measures were done in different positions (sitting and supine, respectively). Past studies have shown that in the supine position, the parasympathetic activation in the sino-atrial node is higher than the sympathetic tonus, as compared to the sitting position[24] and standing position.[16] In a study done by Goldstein *et al* [9] an increase in heart rate was observed during third molar extraction; nevertheless, the use of sedation did not decrease heart rate or systolic pressure, and the authors suggested that other systems besides the SNS may influence the circulatory response to stress. The present study reports a positive correlation between average and maximum heart rates and sAA levels, prior to and during dental treatment, confirming the assumption that amylase release into the saliva is primarily controlled by sympathetic arousal and, in particular, by beta-adrenergic activity.[25]

The results found did not show any significant correlation between levels of salivary stress biomarkers and BMI, corroborating the study done by Rosmalen *et al* [11] in children aged 10-12 years, although past studies have found positive correlation between cortisol levels and BMI in adults.[26] No significant correlation between cortisol levels and sAA was noted, showing a clear distinction between these salivary biomarkers representing different stress-sensitive system (SNS and HPA axis, respectively).[27] This study included children with a very restricted age range to minimize potential confounding factors related to age. However, limitations of this study may include the lack of evaluation of gender differences. The few data available seem to point towards similar stress-related cortisol responses in younger and older children, with no evident gender differences.[28] Other limitation may include the lack of use of a scale to identify self-perception of dental anxiety for the event.

The extent of anxiety a child experiences does not relate directly to dental knowledge, but may be related to personal experiences, family concerns, disease levels and general personality traits,[29] which means that it is no easy task to measure dental anxiety. In children, controlled stress studies are rare, and the results found suggest several prospect directions. Future studies should examine the response in sAA and cortisol to various kinds of dental procedures. Salivary biomarkers may be of importance in evaluation of behavioral strategies to manage uncooperative children during dental treatment.

CONCLUSIONS

This study showed that certain anticipation of the dental treatment was observed because higher cortisol and amylase salivary levels were found before, rather than after, the dental prophylaxis; moreover, a significant correlation between amylase levels and heart rate was observed. Thus, salivary biomarkers may be a valuable tool for evaluating anxiety-producing events, such as dental treatment, in children. Salivary stress biomarkers may be of importance in evaluation of behavioral strategies to manage uncooperative children during dental treatment.

ACKNOWLEDGMENTS

The first author would like to thank Dr. TSB for direct supervision. This research was carried out under grants from the State of São Paulo Research Foundation (FAPESP, SP, Brazil, n. 2006/06338-0, 2007/05760-2 and 2008/05103-4).

REFERENCES

1. Blomqvist M, Holmberg K, Lindblad F, Fernell E, Ek U, Dahllöf G. Salivary cortisol levels and dental anxiety in children with attention deficit hyperactivity disorder. Eur J Oral Sci, 115: 1–6, 2007.
2. Gordis EB, Granger DA, Susman EJ, Trickett PK. Salivary alpha amylase–cortisol asymmetry in maltreated youth. Horm Behav, 53: 96–103, 2008
3. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. Clin Endocrinol, 63: 336–41, 2005.
4. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology, 34: 486–96, 2009.
5. Rohleder N, Nater UM, Wolf JM, Ehlert U, Kirschbaum C. Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity? Ann NY Acad Sci, 1032: 258–63, 2004.
6. Krueger TH, Heller HW, Hauffa BP, Haake P, Exton MS, Schedlowski M. The dental anxiety scale and effects of dental fear on salivary cortisol. Percept Mot Skills, 100: 109–17, 2005.
7. Miller CS, Dembo JB, Falace DA, Kaplan AL. Salivary cortisol response to dental treatment of varying stress. Oral Surg Oral Med Oral Pathol, 79: 436–41, 1995.
8. Rayen R, Muthu MS, Chandrasekhar Rao R, Sivakumar N. Evaluation of physiological and behavioral measures in relation to dental anxiety during sequential dental visits in children. Indian J Dent Res, 17: 27–34, 2006.
9. Goldstein DS, Dionne R, Sweet J, Gracely R, Brewer Jr HB, Gregg R, Keiser HR. Circulatory, plasma catecholamine, cortisol, lipid, and psychological responses to a real-life stress (third molar extractions): effects of diazepam sedation and of inclusion of epinephrine with the local anesthetic. Psychosom Med, 44: 259–72, 1982.
10. Hill CM, Walker RV. Salivary cortisol determinations and self-rating scales in the assessment of stress in patients undergoing the extraction of wisdom teeth. Br Dent J, 191: 513–15, 2001.
11. Rosmalen JG, Oldehinkel AJ, Ormel J, de Winter AF, Buitelaar JK, Verhulst FC. Determinants of salivary cortisol levels in 10-12 year old children; a population-based study of individual differences. Psychoneuroendocrinology, 30: 483–95, 2005.
12. Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology, 32: 392–401, 2007.
13. Räikkönen K, Matthews KA, Pesonen AK, Pyhälä R, Paavonen EJ, Feldt K, Jones A, Phillips DI, Seckl JR, Heinonen K, Lahti J, Komsi N, Järvenpää AL, Eriksson JG, Strandberg TE, Kajantie E. Poor sleep and altered hypothalamic-pituitary-adrenocortical and sympatho-adrenal-medullary system activity in children. J Clin Endocrinol Metab, 95: 2254–61, 2010.
14. Strahler J, Berndt C, Kirschbaum C, Rohleder N. Aging diurnal rhythms and chronic stress: distinct alteration of diurnal rhythmicity of salivary alpha-amylase and cortisol. Biol Psychol, 84: 248–56, 2010.
15. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology, 19: 313–3, 1994.
16. Paschoal MA, Volanti VM, Pires CS, Fernandes FC. Variabilidade da frequência cardíaca em diferentes faixas etárias. Rev Bras Fisioter, 10: 413–19, 2006.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem, 193: 265–75, 1951.
18. Dezan CC, Nicolau J, Souza DN, Walter LRF. Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30 and 422 months attending a baby clinic. Arch Oral Biol, 47: 423–7, 2002.
19. de Farias DG, Bezerra AC. Salivary antibodies, amylase and protein from children with early childhood caries. Clin Oral Investig, 7: 154–7, 2003.
20. Gröschl M, Wagner R, Rauh M, Dörr HG. Stability of salivary steroids: the influences of storage, food and dental care. Steroids, 66: 737–41, 2001.
21. Rank RCC, Carvalho AS, Raggio DP, Cecanho R, Imparato JCP. Children emotional reactions after dental treatment. RGO Rev Gaúcha Odontol, 53: 176–80, 2005.
22. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K, Nishikawa Y. Gender-specific differences in salivary biomarker responses to acute psychological stress. Ann NY Acad Sci, 1098: 510–5, 2007.
23. Kivlighan KT, Granger DA. Salivary a-amylase response to competition: relation to gender, previous experience, and attitudes. Psychoneuroendocrinology, 31: 703–14, 2006.
24. Zuttin RS, Moreno MA, César MC, Martins LEB, Catai AM, Silva E. Evaluation of autonomic heart rate modulation among sedentary young men, in sitting and supine postures. Rev Bras Fisioter, 12: 7–12, 2008.
25. Bosch JA, De Geus EJ, Veerman EC, Hoogstraten J. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. Psychosom Med, 65: 245–58, 2003.
26. Tornhage CJ, Alfven G. Diurnal salivary cortisol concentration in school-aged children: increase morning cortisol concentration and total cortisol concentration negatively correlated to body mass index in children with recurrent abdominal pain of psychosomatic origin. J Pediatr Endocrinol Metab, 19: 843–54, 2006.
27. Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, Ehlert U. Stress-induced changes in human salivary alpha-amylase activity – associations with adrenergic activity. Psychoneuroendocrinology, 31: 49–58, 2006.
28. Buske-Kirschbaum A, von Auer K, Krieger S, Weis S, Rauh W, Hellhammer D. Blunted cortisol responses to psychosocial stress in asthmatic children: a general feature of atopic disease? Psychosom Med, 65: 806–10, 2003.
29. Nicolas E, Bessadet M, Collado V, Carrasco P, Rogerleroi V, Hennequin M. Factors affecting dental fear in French children aged 5-12 years. Int J Paediatr Dent, 20: 366–73, 2010.