**ABSTRACT**

**Aim** Anxiety may influence dental treatment unfavourably, thus evaluation of the psychophysiological reactions of the child may reduce its negative effects. This study aimed to evaluate and correlate the levels of cortisol and α-amylase, vital signs and behaviour of children during their first dental visit.

**Methods** Study sample consisted of 32 children (11 male and 21 female patients) aged between 4 and 6 years, who would go to the dentist for the first time, with no pain or chronic illness, and without the use of medication. Three saliva samples were collected: before, immediately after and 20 minutes after the dental procedure (dental prophylaxis). Statistics: Data were analysed using Student’s t test and Pearson’s correlation test (p = 0.05).

**Results** In the comparison of the levels of α-amylase, the heart rate and blood pressure were higher after the dental visit and the levels of salivary cortisol were significantly higher before. There was no statistically significant difference in the correlation between the Frankl Behaviour Rating Scale and the levels of cortisol and α-amylase, but the results showed that the levels of these two salivary biomarkers increased gradually as the child had a less cooperative behaviour.

**Conclusion** The sample revealed that the first dental visit generated anxiety, which was manifested through physiological and behavioural alterations.

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**Keywords** Alpha-amylase; Dental anxiety; First dental visit; Salivary cortisol.

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**Introduction**

During childhood, the environment and dental procedures may be sources of anxiety and stress, interfering with the behaviour of children [Possobon et al., 2007; Folayan et al., 2004; Gustafsson et al., 2010; Lee et al., 2008] and representing an obstacle in dental care [Holmes and Girdler, 2005]. Hence, initial dental visits should not be motivated by emergencies such as pain or trauma, since these are directly associated with anxiety in children [Nicolas et al., 2010]. Anxiety may influence dental treatment unfavorably; thus, evaluating the psychophysiological reactions allows the professional to identify its occurrence and search for solutions to reduce its negative effects. Several methods are available and have been increasingly perfected to contribute more effectively to the physiological (objective measures) and the behavioural analysis, relating hormone, cognitive and emotional processes [Gunnar et al., 1997; Gil et al., 2002]. Many studies [Nicolas et al., 2010; Bakke et al., 2004; Furlan et al., 2012] have sought to understand and perfect the responses of patients, mainly of children, to dental treatment, evaluating both physiological and psychological aspects of anxiety.

Physiological responses to stress and anxiety result from the interaction between the environment and the information processed by the central nervous system [Mayer, 2000; Habib et al., 2001]. Psychic symptoms, such as tension, nervousness, uneasiness and insecurity, add to the somatic symptoms, such as increased autonomic tonus, breathing difficulties, tachycardia and sweating, resulting in a psychophysiological process triggered by the body [Wolf, 2002] through the activation of the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) [Blomqvist et al., 2007; Nater and Rohleder, 2009].

Based on the HPA axis and the SNS, the body produces mediators responsible for the response to stress, the corticotropin-releasing hormone, the adrenocorticotropic hormone, glucocorticoids and catecholamines [Tsigos and Chrousos, 2002; Holmes, 1997]. These mediators promote an increase in heart rate, blood pressure, cellular metabolism and mental activity [Almeida, 2010], aimed at a homeostatic adaptation of the body in face of a stressing agent, such as the dental visit. Hence, salivary components as diagnostic markers have great medical and scientific interest [Malamud, 2011], mainly because of the collection procedure, which is easy, non-invasive, and does not cause pain or discomfort like blood sample collections.

Assessment of cortisol and alpha-amylase levels in
the saliva aim to provide directions to the professionals in the evaluation of the alterations in behavioural and physiological functions for a broader and more accurate understanding of anxiety [Bakke et al., 2004]. Nevertheless, according to Furlan et al. [2012] there is a lack of information regarding the use of these salivary biomarkers in relation to anxiety, especially in paediatric dentistry, in which behaviour management is often necessary and which could be monitored by these physiological markers.

The aim of this study was to evaluate the levels of cortisol and alpha-amylase, the vital signs and the behavior of children without previous experience during their first dental visit, verifying whether anxiety is present and if there is a correlation between the physiological measures and the behaviour of the child.

Materials and methods

The study project was approved by the Human Research Ethics Committee of the Federal University of Uberlandia, as per resolution CNS 196/96 (Project record at CEP/UFU: 316/11) and with the Helsinki Declaration of 1975, as revised in 2000. Data were collected between March and August of 2012.

A cross-sectional study was developed with children recruited from a convenience sample of a waiting list of 150 children, from the Specialisation Program in Paediatric Dentistry of the Faculty of Dentistry at the Federal University of Uberlandia (FOUFU). The final sample consisted of 32 children, 11 male and 21 female patients, aged between 4 and 6 years (mean 5 years), who complied with the following inclusion criteria: first dental visit; absence of pain of dental origin; absence of chronic illness; and no use of medication.

The parents/guardians of the children were contacted by telephone, received a detailed explanation regarding the study purposes, and those who authorised the participation of their respective child in the study signed the Free and Informed Consent Form during the first visit. Date and time for the dental visit were scheduled at 9:00 am and 10:00 am.

The method of Granger [2007] was used to determine the salivary amylase activity. Ten microliters of saliva were added to a microplate followed by the addition of 320 µL of substrate (2-chlorine-4-nitrophenol-α-D-galactopiranosilmaltoside: GALG2-CNP) heated at 37°C. The enzyme activity of the human salivary amylase (U/mL) was determined with the formula: [(Difference of absorbency per minute × total volume total of the assay (328 ml) × dilution factor (200))/ [millimolar absorbency of 2-chlorine-4-nitrophenol (12.9) × sample volume (0.008 ml) × optical path (0.97)].

In order to analyse the salivary components, i.e. level of salivary amylase and salivary cortisol, of the HPA axis and the SNS (the objective measurements), samples of non-stimulated saliva were collected; moreover blood pressure (AP) and heart rate (HR) were also recorded.

In order to measure the BP (mmHg) and the HR (beats per minute [bpm]), the children were seated on the dental chair, and the measurements were taken on the right arm, which was always supported at the
heart level. An automatic wrist blood pressure monitor was used for the measurement (Techline® Z-43, Taipei, Taiwan, China).

**Behavioural measurement**

The Frankl Behavior Rating Scale [Frankl et al., 1962] was used to register the behaviour of the child during the visit, without the presence of the parents or other children so as not to influence him/her. In data analysis, the children were classified according to the Frankl rating scale, as follows: definitely positive behaviour (child is completely cooperative, has good communication with the dentist, is interested in the dental procedures, smiles and even enjoys the situation), positive behaviour (child shows acceptance towards the treatment, is cautious, willing to cooperate with the dentist, sometimes complains, but follows instructions and presents a reserved attitude), negative behaviour (child is reluctant to accept the treatment, uncooperative, shy and has evidenced negative attitude, yet not constant) and definitely negative behaviour (child refuses to be treated, cries, expresses fear or any other negative characteristic, being the worst possible behaviour).

**Results**

Before each analysis, the test of Kolmogorov-Smirnov was applied to evaluate data normality. Data were analysed by means of Student’s t test for the comparison before and after dental treatment and by means of Pearson’s or Spearman’s correlation coefficient to compare the variables. The level of significance was set at \( p = 0.05 \). The program GraphPad Prism Version 4.0 (GraphPad Software, Inc., San Diego, CA) was used for the analyses.

- Salivary amylase activity: levels were higher immediately after the dental procedure (Fig. 1). ANOVA showed that such differences were not statistically significant \( (p = 0.8840) \).
- Salivary cortisol: levels were higher before the dental procedure in comparison with 20 minutes afterwards (Fig. 2), constituting a significant difference \( (p = 0.0001) \).
- Heart rate: Figure 3 shows that there was no significant difference before and after the dental procedure \( (p = 0.3201) \).
- Blood pressure: systolic blood pressure (Fig. 4) was significantly different before and after dental procedure \( (p = 0.0480) \), whereas diastolic blood pressure (Fig. 5) presented no significant difference \( (p = 0.2137) \).
- Frankl Behaviour Rating Scale: according to the Frankl Behaviour Rating Scale (Table 1), 14 children presented definitely positive behaviour, 16 children presented positive behaviour, one child presented negative behaviour and one child presented definitely negative behaviour.

Correlation between objective and subjective measures: Table 1 shows that in the association between the level of anxiety of the objective measurements and that of the behavioural measurement, the children classified as having a definitely negative behaviour presented the highest level of cortisol and \( \alpha \)-amylase, but the results did not reach significance when compared. There was no statistically significant difference between the levels

**FIG. 1** Amylase activity during the different moments of the dental visit. CAA: Immediately before dental procedure, DAA: Immediately after dental procedure.

**FIG. 2** Levels of salivary cortisol during the different moments of the dental visit. CAA: Immediately before dental procedure and DAC: 20 minutes after the dental procedure.

**FIG. 3** Heart rate before and after dental procedure. CAA: Immediately before dental procedure and DAA: Immediately after dental procedure.
of salivary cortisol and α-amylase of the children classified as having a definitely negative, negative, positive and definitely positive behaviour, even though the levels decreased progressively (Table 1).

**Discussion**

According to the American Psychological Association, the tools for assessing fear and anxiety must refer to the phenomenon under evaluation, present reliability (given by means of the correlation in the test-retest or agreement inter-examiners), validation (expressed by the correlation with other instruments that evaluate the same phenomenon) and normative data [Schuurs and Hoogstraten, 1993].

For countless reasons, children may not be able to recognise and interpret the physiological and cognitive manifestations of anxiety, thus, evaluations often focus on the behavioural component of fear [Santos et al. 2012]. As a result, the use of more than one instrument to analyse the same variable, in this case, anxiety, is justified by the individual variations in its manifestation.

In order to identify anxiety during the first visit, different instruments were simultaneously used, such as records of the behaviour, vital signs and salivary biomarkers, aimed to overcome the inaccuracies and limitations of each method and allow a broader approach. The salivary enzyme α-amylase has been studied as a stress salivary marker since it represents the activity of the adrenal medullary system (AMS). Studies have demonstrated that its levels, in humans, increases under several conditions of physical and psychological stress [Nater and Rohleder, 2009]. In this study, the results showed that the levels of α-amylase after the dental procedure were higher than those registered before the procedure (Fig. 1). Divergent data were found in the study of Furlan [2012], who applied as inclusion criteria the absence of pain, trauma and dental treatment. Besides the criteria considered by Furlan, this study also applied the criterion that the child had never been to a dentist. In this context, it is understood that given the fact the child did not know what would happen in the dental office, this may have resulted in lower levels before the visit, and as the visit happened and the procedures were performed, anxiety increased. The analysis of the salivary cortisol was essential to determine the diagnosis of a stressful situation, regardless of whether it had psychological, physical or environmental origins [Luz, 2006]. The results showed that there was a difference between the levels of salivary cortisol before and after the dental visit (Fig. 2), and that these levels were significantly higher before the procedure (p=0.0001), which was also found in the study of Furlan (p=0.037) [Furlan et al., 2012]. Santos et al. [2012] also found higher levels before (0.74 µg/dl) compared to after (0.66 µg/dl) the procedure, but this difference was not statistically significant.

Regarding the heart rate, the values after the procedure were higher than those before, but when compared this difference was not statistically significant (Fig. 3). Previous studies which evaluated the heart rate demonstrated that it increased after stimuli such as the use of anaesthetic and absolute isolation, however this increase did not reach a significance level [Robin et al., 1998], and in others, the position (sitting or in supine position) of the child when the heart rate was measured resulted in different values before and after the procedure [Furlan et al., 2012].

In the blood pressure evaluation (Fig. 4), systolic blood pressure presented significantly higher results after the dental procedure (p=0.0480) when compared to those before, but in relation to the diastolic pressure (Fig. 5),
results showed no statistically significant difference.

In the analysis of the Frankl Behavior Rating Scale, most of the children exhibited positive behaviour (n=30), one presented negative behaviour and the one who presented a definitely negative behaviour also had higher levels of cortisol (0.216 µg/dl) and α-amylase (106.92 U/ml) (Table 1), showing that there was a positive relationship between salivary biomarkers and behaviour, similar to the study of Santos et al. [2012], in which the children with negative behaviour also presented higher levels of cortisol (0.78 µg/dl) and α-amylase (49.52 U/ml). Despite the fact that there was no statistically significant difference between the levels of cortisol and α-amylase among children classified with different types of behaviour, analysis of the association between the Frankl Behaviour Rating Scale and the levels of cortisol and α-amylase showed that the levels of these two salivary biomarkers increased gradually, as the child was classified as having a less cooperative type of behaviour, confirming the data found in a previous study [Santos et al., 2012]. In this study, it is possible to consider that the stressing agent, in this case the dental treatment, was minimum and asymptomatic, a dental prophylaxis. It is likely that, if the studied children had been submitted to anaesthesia or to a more radical treatment, such as oral surgery and pulp treatments, there would be a greater probability for the markers to present higher levels, reaching the significance found by authors like Miller et al. [1995] and Kanegane et al. [2009].

In this study, the use of physiological data (levels of salivary cortisol and α-amylase, blood pressure, heart rate) and their comparison with behaviour data (Frankl behaviour rating scale) allowed to verify a positive correlation (Table 1), showing the validity of objective and behavioural measurements in the verification of the child’s anxiety related to dental treatment.

It is important to acknowledge some limitations of the present study, such as its reduced and non-randomised sample. On the one hand, the inclusion criteria aimed to standardise the study subjects so that the comparisons were as accurate as possible. On the other hand, studies with a greater sampling number should be made in order to confirm the results found.

Based on the data found, it was assessed that the first dental visit of the child generated anxiety, which was demonstrated through physiological and behavioural alterations, with the existence of a correlation between them. The authors believe that, in the first contact, the professional must perceive the reaction of the child in face of the unknown, of something new, and that at this time conditioning strategies aimed to establish a professional-patient relationship of trust should be developed. It was possible to verify that studies have increasingly sought to use all the resources available for identification of anxiety related to dental treatment. Hence, contemporaneous paediatric dentistry has sought support in other areas, such as psychology, to perform an integrating approach, demanding from professionals skills that go beyond dentistry, such as the ability to recognize emotions and to evaluate behavioural alterations in face of different causes such as fear, pain and anxiety, in order to establish a professional-patient relationship of trust, which is fundamental for the success of dental care.

References

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