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Caporal Reis, Alessandra; Corcini Sanchoten, Manoella; Rausch Pereira, Keila; Martini
Dalpian, Débora; Pasquali Dotto, Patricia; Dornelles Carpes, Adriana; Zimmermann
Santos, Bianca

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Original Article

Levels of Anxiety and Salivary Alpha-Amylase in Children During Restorative Dental Treatment

Alessandra Caporal Reis¹, Manoella Corcini Sanchotene¹, Keila Rausch Pereira², Débora Martini Dalpian¹, Patricia Pasquali Dotto¹, Adriana Dornelles Carpes¹, Bianca Zimmermann Santos¹

¹Department of Dentistry, Franciscan University Center, Santa Maria, RS, Brazil.

²Department of Dentistry, University of Southern Santa Catarina, Florianopolis, SC, Brazil.

Author to whom correspondence should be addressed: Bianca Zimmermann Santos, Rua Guilherme Cassel Sobrinho, 275 apto 902, Nossa Senhora de Lourdes, Santa Maria, RS, Brasil. 97050-270. Phone: +55 97050-270. E-mail: biancazsantos@hotmail.com.

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Abstract

Objective: To assess levels of anxiety and salivary alpha-amylase in children and its association with the use of informative or aversive behavioral management techniques during restorative dental procedures. **Material and Methods:** A cross-sectional study was performed with 69 children aged 4-12 years submitted to restorative dental treatment. The level of anxiety of patients was verified by means of the Modified Venham Picture Test and levels of salivary alpha-amylase were measured before and after the restorative procedure. The behavioral management techniques used during the dental procedure were registered. Associations between variables were analyzed using the chi-square test, considering statistically significant associations with $p \leq 0.05$. **Results:** Salivary alpha-amylase showed high and moderate levels prior to dental procedure in 47 children (68.1%). Variables gender and behavioral management techniques were not associated with anxiety, as measured by VPT and salivary alpha-amylase activity. However, younger children showed higher levels of alpha-amylase ($p = 0.001$). **Conclusion:** The sample revealed that the visit to the dentist has generated anxiety, manifested by behavioral and physiological changes, especially in children under 72 months; thus, no association between anxiety and the different management techniques was observed.

Keywords: Anxiety; Alpha-Amylases; Child; Pediatric Dentistry.

Introduction

Anxiety related to dental treatment can be defined as fear or dread related to dental appointments. It is common in patients of all age groups, but it is mainly developed during childhood and adolescence [1].

Childhood anxiety related to dental treatment has been widely studied [2] because it is recognized that the patient's emotions could help the dentist to identify the best behavior management technique to be used. In fact, by identifying factors associated with anxiety, it is possible to use appropriate strategies to control the child's behavior during dental treatment [1,3]. Among these factors, indirect fear - the fear that the child perceives in those surrounding him or being passed through reports - accounts for most of this anxiety [3,4].

The techniques that help control the child's behavior can be pharmacological and non-pharmacological. These techniques aim to assist the dentist in effectively and safely carry out dental care from the observation and recognition of the child's behaviors and signals [5]. In Brazil, non-pharmacological management techniques are the most commonly used, and pharmacological techniques are indicated only in extreme cases, when non-pharmacological techniques are not effective [6]. Thus, proper management of the child behavior is critical in building a relationship of trust between dentist and patient, able to alleviate fear, anxiety and stress [7,8].

Given the above, there are different ways to assess these feelings, some are subjective and use fear and anxiety scales such as the Frankl Behavior Scale [9], the Anxiety Rating Scale [10], the Uncooperative Behavior Rating Scale [10] and the Venham Picture Test [11]. There are also objective measures such as the measurement of biomarkers present in urine, blood and saliva [12-14].

When submitted to any kind of stress, including those generated by the dental appointment, the organism generates hormonal responses through activation of the hypothalamic-pituitary-adrenal axis and these responses can be measured by the levels of salivary alpha-amylase and cortisol [5,15]. Recent studies have suggested that any psychological or physiological change is followed by an increase of these stress biomarkers [13,14]. In addition, salivary biomarkers have gained popularity because they are reliable, easy to use, non-invasive and of rapid collection, compared to samples such as blood and urine [12].

In this context, the aim of this study was to assess anxiety through the Venham Picture Test and the level of alpha-amylase in the saliva of patients aged 4-12 years treated at the Children's Dental Clinic and its association with the use of informative or aversive behavior management techniques during restorative dental procedures in order to check if the patient's behavior during dental treatment could be predicted by any of these variables.

Material and Methods

Subjects and Study Design

This is a quantitative, exploratory and cross-sectional study conducted with patients aged 4-12 years at the Children's Dental Clinic of the *Centro Universitário Franciscano* in Santa Maria / RS / Brazil, from May to November 2014.

To be included in the study, the patient should be in need of at least one restorative dental procedure, with local anesthesia. This procedure used 3% mepivacaine without vasoconstrictor (New DFL), This procedure used 3% mepivacaine without vasoconstrictor (New DFL) Repetido, because the anesthetic most commonly used for the performance of restorative procedures, with vasoconstrictor, could influence the values of salivary alpha-amylase. Only primary teeth that received restorations (class I and class II) were considered, executed with absolute isolation. Teeth requiring pulpotomy were not included, and only tooth restored in the session were included.

Exclusion criteria were patients with delayed cognitive development, birth defects, neurological and / or psychological problems and children who do not live with biological family.

Sample size calculation was based on the following formula proposed for finite populations:

$$n = (N \cdot z^2 \cdot p \cdot (1 - p)) / e^2 \cdot (n - 1 + z^2 \cdot p \cdot (1 - p)),$$

where N = estimated sample size of children annually attended in the dental clinic (80), Z = confidence level (95%) expressed in deviation (1.96); e = prevalence of anxiety due to dental appointment among children aged 6-12 years (55.33% or 16) and margin of error of 5%. This calculation determined a sample size of 66 children. To compensate for possible losses, 5% was increased, resulting in a final sample of 69 children.

Data Collection

All children were accompanied by parents / guardians during dental appointments, and treatments of all children participating in the research were performed in the afternoon. The level of children's anxiety was assessed before and after treatment by means of a projective test with self-analysis based on drawings of human pictures, namely, the modified Venham Picture Test (VPT) [11]. The modified VPT consists of eight pairs of pictures of children displaying various emotional states, which were presented to patients before and after completion of the dental procedure, in a half A4 sized paper with drawings of females for girls and males for boys, also considering their ethnicity. Each set contained a drawing of an anxious child and another with a drawing of a non-anxious child and patients were encouraged to choose the figures that most reflected their emotions at the time. If they chose the anxious figure, value 1 was scored and if they chose the non-anxious child, value 0 was scored. For analysis of the scale [2], children were grouped according to Table 1.

Table 1. Child anxiety rated by the modified VPT scale.

Anxiety rating	Sum of scores in the VPT scale
No anxiety	0
Low level of anxiety	1 I—I 2
Moderate level of anxiety	3 I—I 5
High level of anxiety	6 I—I 8

In addition, before and after the completion of the dental procedure, saliva samples were collected. Parents / guardians were instructed to carefully brush their children's teeth after lunch to avoid the risk of gingival bleeding. In addition, children should avoid physical activity at least one hour before collection of saliva samples. When collecting samples, children were kept in a separate room in order to ensure a comfortable and cozy environment to avoid previous stress of participants. Immediately prior to saliva collection, patients were instructed to perform mouth rinsing with 20 ml of distilled water and remained seated with their mouth open until some amount of saliva was formed in the oral cavity. Then, saliva was collected using a sterile, disposable 3 ml Pasteur pipette and transferred to a 2 ml eppendorf.

After collection, samples were kept in a refrigerated container and taken to the laboratory of Clinical Biochemistry - *Centro Universitário Franciscano* - to determine the α -amylase enzyme activity. In the laboratory, samples were centrifuged at 3,000 rpm for 15 minutes. The supernatant obtained after centrifugation was diluted 500 times in NaCl solution at 150 mmol / L (0.85%) and a 0.01 ml aliquot of this diluted sample was used for laboratory analysis, which was performed in triplicate for each collected sample. For determination of the salivary alpha amylase enzyme activity, Labtest® commercial kit was used, which is based on a colorimetric method for fixed time kinetics reaction with reading at final point. This kit follows the modified methodology of Caraway, where the sample is incubated with a starch substrate and the decrease in blue color, after the addition of iodine, is compared with a control, being proportional to the amylase activity in the sample. The test protocol followed procedure described in the Kit, in which each tube was initially added of 0.5 ml of reagent 1 (starch), maintained for 2 minutes in water bath at 37°C and added of 10 μ L of diluted saliva, homogenized and kept for exactly 7.5 minutes in water bath at 37°C. Then, 0.5 ml of reagent containing iodine and 4 ml of distilled and deionized water were added. The same procedure was carried out with control tube, but it was not added of diluted saliva. At the end of the technical procedure, test and control tubes were taken to the spectrophotometer where absorbance reading was conducted using wavelength of 660 nm. The device was reset with distilled and deionized water. Thus, the absorbance values (Abs) obtained were converted into concentration using the following equation: Amylase (U/dL) = Absorbance of control - Absorbance of sample x calibration factor (800) x sample dilution (500). The enzyme level classification was based on tertiles of the first collection (1st quartile (1Q) up to 33.03 dL, 2nd quartile (2Q) up to 75.14 dL and 3rd quartile (3Q) values above 75.14 dL and from them, the following categories were created: low (1st Q), moderate (2nd Q) and high (3rd Q) enzyme level. To maintain the established pattern, the second collection was categorized according to criteria of the first collection.

To carry out the restorative dental procedure, 3% mepivacaine without vasoconstrictor was used (New DFL). During dental procedure, the non-pharmacological behavioral management technique used for each patient was also recorded. These were classified as informative (tell-show-do, distraction, positive reinforcement and modeling) or aversive (voice control and physical restraint).

Data were collected by three blind examiners. One examiner performed the restorative dental procedures, another applied the Venham Picture Test (VPT) and collected saliva samples and the third examiner identified the behavioral management techniques used during treatment. Prior to data collection, the examiner responsible for identifying behavior management techniques was calibrated in order to study the diagnostic criteria and enable the reproducibility assessment. In order to perform the calibration, 10 videos of patients treated in the children's dental clinic of the institution were recorded, which were attended on two occasions, the second one week after the first. It has been found that the intra-examiner agreement for the assessment of behavioral management techniques used during treatments was 100% (Kappa Test = 1).

A pilot study was conducted with 10% of the total sample including children not participating in the sampling plan to assess the feasibility of the proposed methodology. It was observed that such proposal was feasible without need for adjustments.

Study Variables

Anxiety (measured by the Venham Picture Test) and levels of alpha-amylase of patients before and after the restorative dental procedure were considered dependent variables in this study. Age, sex and behavioral management techniques used to perform these procedures were considered independent variables.

Statistical Analysis

The chi-square test was used to show associations among variables, and analyses were performed with the SPSS20.0 software, considering statistically significant associations with $p \leq 0.05$.

Ethical Aspects

The study project was submitted to the Ethics Research Committee on Human Beings of the *Centro Universitário Franciscano* and approved under number 206 296.

Results

The mean age of children in the study was 7.45 years (SD = 2.12). In relation to sex, 35 boys (mean age of 7.76 years (SD = 1.74)) and 34 girls (mean age of 7.16 years (SD = 2.42)) were included.

Table 2 shows the distribution of the 69 children included in the survey, showing anxiety before and after the completion of the dental procedure and behavioral management techniques used.

Anxiety decreased in 16 (23.2%) patients after dental treatment, remained unchanged in 38 (55.1%), and increased in 15 (21.7%). Levels of alpha-amylase decreased in 1 (1.4%) patient after dental treatment, remained unchanged in 43 (62.3%) and increased in 25 children (36.2%).

In relation to informative behavioral management techniques, tell-show-do was carried out in 66 (95.7%) children, positive reinforcement in 48 (69.6%), distraction in 48 (69.6%) and modeling

in 7 (10.1%) patients. In relation to aversive techniques, voice control technique was used in 17 (24.6%) children, physical containment in 7 (10.1 threat for responsible leave 5 (7.2%), request for responsible leave in 3 (4.3%) patients. The hand-over-mouth technique was not used in any child.

Table 2. Distribution of children in relation to anxiety, levels of alpha-amylase before and after the completion of the dental procedure and behavioral management techniques used (n = 69).

Variables	N	%
Anxiety prior to the dental procedure		
No anxiety	49	71.0
Low level of anxiety	18	26.1
Moderate level of anxiety	2	2.9
High level of anxiety	0	0.0
Anxiety after the completion of the dental procedure		
No anxiety	51	73.9
Low level of anxiety	10	14.5
Moderate level of anxiety	6	8.7
High level of anxiety	2	2.9
Levels of alpha-amylase before dental procedure		
Low level	22	31.9
Moderate level	13	18.8
High level	34	49.3
Levels of alpha-amylase after dental procedure		
Low level	12	17.4
Moderate level	20	29.0
High level	37	53.6
Behavioral management techniques		
Informative	52	75.4
Aversive	17	24.6

Table 3 shows possible associations between anxiety before completion of the dental procedure and age, sex, and behavioral management techniques.

Table 3. Association between anxiety before dental procedure and independent variables (n = 69).

Baseline Anxiety					
Variables	Absent		Present		p-value*
	N	%	N	%	
Age					0.086
Less than 72 months	10	55.6	8	44.4	
Greater than 72 months	39	76.5	12	23.5	
Gender					0.255
Male	27	55.1	8	40.0	
Female	22	44.9	12	60.0	
Behavioral management techniques					0.964
Informative	37	75.5	12	24.5	
Aversive	15	75.0	5	25.0	

*Chi-square test.

Table 4 shows the association between levels of alpha-amylase before dental treatment and age, sex, and behavioral management techniques. There was association between younger children and higher levels of alpha-amylase ($p = 0.001$).

Table 4. Association between levels of salivary alpha-amylase before dental procedure and independent variables studied (n = 69).

Variables studied (n = 66).							
Variables	Level of alpha-amylase						p-value*
	Low		Moderate		High		
	N	%	N	%	N	%	
Age							0.001**
Less than 72 months	0	0.0	3	16.7	15	83.3	
Greater than 72 months	22	43.1	10	19.6	19	37.3	
Gender							0.834
Male	12	34.3	7	20.0	16	45.7	
Female	10	29.4	6	17.6	18	52.9	
Behavioral management techniques							0.848
Informative	17	32.7	9	17.3	26	50.0	
Aversive	5	29.4	4	23.5	8	47.1	

*Chi-square test, **statistically significant.

Table 5 shows the association between variation in the levels of anxiety and alpha-amylase before and after dental procedure and behavioral management techniques used during dental treatment.

Table 5. Association between variation in the levels of anxiety and alpha-amylase before and after the completion of the dental procedure and behavioral management techniques used during dental care (n = 69).

65%

Variables	Behavioral management techniques				p-value*
	Informative		Aversive		
	N	%	N	%	
Anxiety variation					0.974
Decreased	12	23.1	4	23.5	
Remained unchanged	29	55.8	9	52.9	
Increased	11	21.2	4	23.5	
Alpha-amylase variation					0.234
Decreased	35	67.3	8	47.1	
Remained unchanged	1	1.9	0	0.0	
Increased	16	30.8	9	52.9	

*Chi-square test.

Discussion

The clinical aspects of the dental procedure and the dentist's behavior can lead to anxiety, fear and stress, and therefore, avoidance responses in the patient [2,5,7,17,18], compromising the quality of dental care. Thus, dental anxiety has been studied because it can lead patients to avoid seeking dental care and, thus having a negative impact on the oral health of individuals [19,20].

There has been a great progress in relation to the technical training of dentists in recent years; however, with regard to the management of patients with dental anxiety, there are still gaps in literature [21]. Specifically in Pediatric Dentistry, the situation becomes worse as the use of aversive techniques for the management of infant patients during dental procedures has always been much criticized [5,21], which justifies the need for research aimed at analyzing the impact of the use of these techniques on pediatric patients.

In this research, most children included showed no anxiety, as measured by VPT during dental treatment, unlike results obtained in previous studies [2,16].

Before dental appointment, VPT showed that 71% of children were not anxious and 29% had some degree of anxiety. After completion of the dental procedure, 73.9% of patients were not anxious and 26.1% had some degree of anxiety. It was also observed that more than half of patients had no change in the level of anxiety after dental procedure. According to another study [18], the period immediately before dental appointment is the main cause of high levels of anxiety compared to the dental treatment itself, a fact that may explain the results found in this study.

The levels of alpha-amylase were not the same as those verified with the subjective instrument (VPT), in which before dental care, 68.1% of children in the sample had moderate or high salivary alpha-amylase enzyme activity, and after treatment, this prevalence increased to 82.6%. Previous studies [13,22] comparing the levels of salivary alpha-amylase before and after dental procedure in children with and without delay in overall development showed that all had high levels of salivary alpha-amylase both before and after dental procedure. The determination of the levels of salivary alpha-amylase for most studies found [23-25] made use of quantitative kinetic enzymatic method in microplates, as described by Strahler [25]. Possibly, the method is a limiting factor in this study, since the Caraway method was used to determine the alpha-amylase activity in various biological samples, but not in saliva. It is known that the isoform of the salivary enzyme (S-form) differs from the predominant isoform in other biological samples (P-form), which may explain the need for a specific method when analyzing saliva. The use of categories in the expression of values obtained for amylase allowed the use of results and their correlation with other data evaluated in the survey.

Several hypotheses were considered to explain the high results found in the determination of the alpha-amylase activity for the tested samples. Initially, the form of sample collection was evaluated, researching the interference of unstimulated collection instead of using salivette®. However, there is evidence in literature [23] that unstimulated collection (passive) does not influence the levels of alpha-amylase activity, and Decaro [24] in a specific study for this purpose showed that unstimulated collection instead of using devices containing cotton roll is recommended, since the incomplete saturation of cotton with volumes less than 1 ml of saliva tends to result in variations in the activity of this enzyme.

Recognizing and managing the patient's anxiety is critical to the dentist [26], as often fear and anxiety regarding dental procedures can cause patients to avoid numerous times a dental appointment [6].

As for the use of behavior management techniques, informative techniques were used more frequently since educational practices and cognitive strategies allow managing behavior without the need for more aversive contingencies [7].

There was no association between anxiety, as measured by VPT, and levels of alpha-amylase enzyme prior to completion of the dental procedure with patient gender. Other studies have also shown no relationship between patient gender and dental anxiety [16,27].

However, since age was not associated with anxiety, measured by VPT, it was associated with levels of alpha-amylase enzyme prior to completion of the dental procedure, as the group corresponding to the age group > 72 months had higher levels of enzyme activity. Previous studies have shown that younger children are more prone to anxiety due to their emotional immaturity, as the pre-school age is a period that includes great emotional and physical development, with increased fear, and, in a dental office, there are noises, unexpected movements, pain, new people and strange images and smells. It was also observed that children under 4 years of age are unable to communicate precisely and are physically and psychologically dependent on the presence of parents, which can be related to higher fear and anxiety in relation to the unknown [17,28]. Although painless, sample collection represented something new to participants, and it is possible that this new feeling caused by this unknown procedure has aroused anxiety in children, resulting in sympathetic activation with greater release of alpha-amylase enzyme in saliva before dental procedure.

In this study, no association was found between anxiety, measured by VPT, and levels of alpha salivary amylase measured before dental procedure, with different behavioral management techniques used. This result indicates that the use of aversive techniques do not make children more anxious or stressed. No other studies checking these associations were found in literature.

In pediatric dentistry, behavioral management suggests a continuous professional interaction with the patient in order to decrease fear and anxiety in children [5,21]. Thus, considering the patient's behavior, the professional should choose the most appropriate strategy. In addition, when choosing a behavior management technique, the dentist should consider the perception and opinion of parents and should use aversive techniques only upon their authorization [5,21]. Parents whose children have difficult behavior usually tend to accept more easily the use of aversive techniques [6,8,21].

Corroborating previous findings, no association between behavioral management techniques used during dental care and variations in the levels of anxiety measured by VPT were found, as well as variations in the levels of alpha-amylase enzyme before and after dental procedure. It is possible that anxiety and stress in the dental context are associated with aspects of children's behavior and personality, negative previous experiences and seizure of behavioral models through observation [17,20,29], and not due to the management techniques used.

Non-pharmacological techniques to control children's behavior are used in order to decrease anxiety, stress and fear, providing safety for children and for parents during treatment 5, allowing the child to understand that there is no reason for these feelings.

The assessment of anxiety and stress through questionnaires is a highly subjective method, since methods that use biological tests are more reliable and objective. Questionnaires or self-reports, in most cases, do not generate a very precise response because many individuals who suffer from

stress tend to deny or exaggerate the real condition, which could cause bias in results. The level of salivary alpha-amylase increases in patients with chronic psychosocial stress, but may not be an indicator to suggest the development of stress related to changes in oral mucosa. The levels of salivary alpha-amylase reflect the activity of the sympathetic nervous system [12] and can be used as a stress biomarker since physiological and psychological changes cause an increase in their levels. Thus, this may be an important objective marker of child stress [13].

The fact that the “hand on the mouth” technique was not used may be a study limitation, not allowing the results of this study to extend to this type of management. Therefore, the level of patient anxiety through this technique was not assessed. In addition, due to the sample size, it was not possible to assess the association of each management technique and the levels of anxiety reported by patients. Patients in this study were not submitted to dental procedure for the first time, which may have interfered in their levels of anxiety, since they already had a previous dental experience.

Properly managing the behavior of children during dental care is a major challenge for both the general dentist and the pediatric dentist [19]. Thus, the early identification of anxious behavior before the dental care can significantly contribute to treatment, since the professional will suit to the specific needs of each patient, so their level of cooperation will improve and anxiety will be reduced [2].

Conclusion

The sample revealed that the visit to the dentist has generated anxiety, manifested by behavioral and physiological changes, especially in children under 72 months; thus, no association between anxiety and the different management techniques was observed.

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