

Use of Salivary Alpha Amylase to Evaluate Dental Stress in Children during their First and Subsequent Visits: A Pilot Study

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ABSTRACT

Introduction: Dental anxiety is very frequent among population of many countries. It is assessed with a noninvasive biomarker, Salivary alpha amylase. Assessment of dental anxiety or stress can be a vital part of children management during treatment as it affects the children behaviour.

Aim: To evaluate acute psychological stress undergone by children during their first visit and subsequent dental visit using salivary biomarker of stress namely salivary alpha amylase.

Materials and Methods: This cross-sectional study was conducted in Department of Paediatric and Preventive Dentistry, Saveetha Dental College, Chennai, Tamil Nadu, India. Twenty children were selected for the study. To measure anxiety, the child's parent completed Modified Dental Anxiety Scale (MDAS).

Unstimulated whole salivary samples were collected from 20 children in their first dental visit and in their second visit. Salivary alpha amylase concentrations were obtained using an automatic analyser and assay kits from Roche. Paired t-test was used to compare the two study groups (new and returning patients).

Results: Moderate and severe anxiety was present in seven and twelve children out of twenty new patients. Six children had moderate anxiety while no one reported severe anxiety among returning children. Salivary alpha amylase levels were higher in children in their first dental visit (61.29 ± 4.51) compared to their second visit (45.56 ± 3.58).

Conclusion: Children during their first dental visit experienced higher levels of stress compared to subsequent visit.

Keywords: Anxiety, Biomarker, Dental treatment

INTRODUCTION

Stress is often defined as the physiological and psychological reaction that prepare an organism's defence against external or internal threats which is also known as stressors [1]. There are two groups of stressors: physical, either systemic or relative and psychological, either emotional or processing [2]. Various biomarkers are used in quantification of stress including cortisol levels, immunoglobulins, chromogranin-A, cardiovascular parameters, etc. Of the total salivary proteins, 40% to 50% is alpha amylase enzyme. They are secreted the most from the parotid gland [3,4]. Stressors can bring about the activation of the Autonomic Nervous System (ANS), which prepares the body for the fight-or-flight reaction, and the Hypothalamic-Pituitary-Adrenal (HPA) axis. By triggering ANS, it begins the release of epinephrine and norepinephrine from the adrenal medulla [5]. The secretion of salivary alpha amylase from the acinar cells of the parotid and submandibular salivary gland is being elevated by the release of norepinephrine [6]. The level of alpha amylase in saliva is regarded to be the major biomarker of stress. Some temporal, situational issues and diurnal variations are evident due to hormonal changes after using salivary biomarkers [7].

Dental anxiety is very frequent among population of many countries. The origin of dental anxiety in children is multifactorial and complex. Dental caries, dental pain, previous dental treatment, family experiences and environmental characteristics are the main cause for dental anxiety. Dental treatment is one of the most stressful environments inducing anxiety and fear, especially in children [8]. Hence, the level of dental anxiety among children must be monitored prior to the treatment for proper management of the children.

To access the dental anxiety of patients, many questionnaires based scales were developed such as Modified Dental Anxiety Scale (MDAS) [9], Corah's Dental Anxiety Scale [10], Dental Fear Survey [11], General Geer Fear Scale [12]. Among these scales, MDAS is the most commonly used questionnaire which is a modified version of Corah's Dental Anxiety Scale. Currently, research on the use of

salivary biomarker, namely alpha amylase, is considered to be a useful tool for assessing the Sympathetic Adrenal Medullary System (SAMS) [13,14] and to predict dental anxiety in children during their first dental visit and subsequent visit.

Other than traditional blood and urine sampling methods, saliva is increasingly used in the qualitative and quantitative analysis of biologically active components. Saliva is considered as easier, safer, intrusive, iterate, stress free which does not require any particular training and thus becoming even more agreeable [15]. Moreover, for medical practitioners, salivary samples are considered to cause less harm than serum. The latter is more likely to reveal operators with blood-borne diseases [16].

Anxiety before dental treatment is very common, especially in children which makes it difficult for the dental practitioners. In the present study, by measuring stress levels using salivary alpha-amylase, the treatment can be planned according to the child's comfort which will reduce stress in the initial stages by performing preventive procedures followed by complex procedures. This will not only benefit the child and the dentist but will also change the approach of the child.

This study is an attempt to monitor changes in Salivary Alpha-Amylase (SAA) levels related to dental fear in children having their first dental visit and those returning for dental treatment in subsequent visit.

MATERIALS AND METHODS

This cross-sectional study was carried out in the Department of Paediatric and Preventive Dentistry, Saveetha Dental College, Chennai after obtaining ethical clearance from the Institutional Ethical committee (SRB/MDS/PEDO/18-19/0006) from September 2018-January 2019. The study involved measurement of salivary alpha-amylase levels in children during their first and their subsequent visit.

This study recruited a total of 20 healthy children aged between 6-12 years. Stone and Church have classified 6-12 year-old as middle year children [17]. Hence, we decided to take children from 6-12 years. Sample size was determined using the non-inferiority

design, based on our pilot study among five samples. Power was set at 80% with 95% confidence interval, and an error rate of 5% yielded a sample size of 20 inclusive of 15% loss to follow-up.

Hence, total of 20 children were recruited for the study. Children in the study were either new patients having their first dental visit or were returning to continue previous dental treatment. Only non-invasive treatment was done for children who received the dental treatment. Only simple procedures like scaling and restorations were done in both the visits to standardise the study.

Children with diagnosed medical problems were excluded from the study. The study was explained in detail to the child's parents/guardians who consented to join the study. Instructions to take part in the research were explained to the child's parents initially and then to the child. Parents were allowed to accompany their children during the collection of salivary samples. The questionnaire was designed to be answered by parents because it has a complex language difficult to understand for child. The parent who answered the questionnaire witnessed and accompanied the child throughout the procedure.

After signing a consent agreement, the child's parent completed the MDAS questionnaire [10]. Tell-Show-Do was done for all the patients in both the visits to demonstrate what procedure would be performed. All procedure was performed in the same setting by the same operator.

The scale includes 5 questions, which assesses the dental anxiety levels in various dental situations. The 5 responses in scale include "not anxious" to "extremely anxious". The scoring of each response is from 1 to 5. So, a child who is "not anxious" is scored 1 and an "extremely anxious" child is scored 5. Children with scores of 6 to 11, 12 to 18, and 19 to 25 were considered as patients with low, moderate, and severe anxiety [18]. MDAS is not only simple to access but also less time consuming.

After the questionnaires were completed, unstimulated salivary samples were taken from all recruited children. Each child was instructed to rinse his/her mouth with water to get rid of debris, sit in an up-right position and then spit 2 mL saliva in the sampling container once a minute for five minutes. Each container was then labelled with the child's file number, time and date of sample collection. One sample was taken in first dental visit and the second sample was taken after the completion of the second visit from the same patient.

To exempt any bias occurring due to diurnal variation, all the samples were collected at the same time, i.e., between 9 am to 12 pm. The containers of saliva samples were stored at -80°C until biochemical analysis took place. After thawing, samples were centrifuged at 3000 rpm for 5 minutes and then used for the analysis [Table/Fig-1]. Alpha amylase activity was determined by using an automatic analyser and assay kits from Roche (Cobas-Bio; Roche Diagnostic Systems, Nutley).



[Table/Fig-1]: Centrifuging the saliva samples.

STATISTICAL ANALYSIS

Data was analysed using Statistical Package for the Social Sciences software (SPSS) version 16.0 (SPSS, Chicago, Illinois, USA). A p-value of <0.05 was taken as a cut-off for statistical significance. The proportions, arithmetic means, standard deviations and medians were used as summary statistics. Paired t-test was used to compare the two study groups (new and returning patients) regarding categorical variables.

RESULTS

A total of 11 girls and 9 boys with a mean age of 8.425 ± 1.89 years participated in the study. Out of recruited children, 20 were new dental patients and the same 20 children were returning for completion of treatment. Based on the total MDAS score, it was noted that 60% of children had severe anxiety scoring more than 19-25 on MDAS scale during their first dental visit whereas, 35% and 5% were moderately and mildly anxious about dental treatments. However, 70% of children had mild anxiety and 30% had moderate anxiety during their second visit [Table/Fig-2]. Comparing new and returning patients the results show that new patients had significantly higher levels of salivary alpha-amylase levels as depicted in [Table/Fig-3].

MDAS	New patients	Returning patients
Low anxiety (6-11)	1 (5%)	14 (70%)
Moderate anxiety (12-18)	7 (35%)	6 (30%)
Severe anxiety (19-25)	12 (60%)	0

[Table/Fig-2]: MDAS depicting anxiety level in new patients and returning patients.

Group (u/mL)	N	Mean	Standard deviation	p-value
SAA -First visit	20	61.2935	4.50713	<0.001*
SAA-Second visit	20	45.5560	3.57638	

[Table/Fig-3]: Comparison of salivary amylase level between new and returning patients.

Paired t-test results; p-value <0.05 was taken as statistical significance; SAA: Salivary alpha-amylase
* Significant

DISCUSSION

Dental treatment is the main cause of triggering stress/anxiety in children. The environment of the dental operator instills fear in the child and the sight of other children being treated, the noise of their cries, the sight of needles and other equipment used in operatory increases their level of anxiety.

Alpha amylase is an important stress marker of the human body. To help in digestion of starch [19], SAA are produced by salivary glands [20]. These glands have an enormous amount of norepinephrine-stimulated beta-adrenergic receptors [20,21]. Psychological stress leads to increase in plasma vasoconstrictor which in turn increases the production and release of SAA from acinar cells of parotid and submandibular salivary glands. This is caused by SAMS activation that is a part of autonomic nervous system [22,23]. Pain, a stressful agent [24], increases production and secretion of alpha amylase, and therefore, can be used as a biomarker of pain sensation [25,26].

This study showed that s- α -amylase level was significantly higher in new patients compared to returning patients. It seems that children became more stressed by seeing dental instruments and dental equipment for the first time at the dental operator. The results are in accordance with those of Takai N et al., and Noto Y et al., who looked at the correlation between dental anxiety and SAA level showing a significant correlation between them [22,27]. Results given by Alaki SM et al., showed a significant increase in s- α -amylase in returning patients, which may be a reflection of the present cumulative experience [28]. Certain dental procedures cause discomfort and loud noises which could elevate patient's fear and anxiety level [29]. In a study done by Sadi H et al., analysed the levels of Salivary cortisol and Salivary alpha-amylase by salimetrics and observed that a significant correlation was not established between dental anxiety score, salivary cortisol and salivary alpha-amylase [30].

According to the results of MDAS questionnaire in the present study, children with higher anxiety level showed more increase in the alpha amylase level in first dental visit compared to their subsequent visit. Study done by Sadi H et al., did not find any correlation between scores of Corah's Dental Anxiety scale and SAA level [30]. In a study done by Koh D et al., which was similar to this study, it was noted that a clear correlation was evident between State-Trait Anxiety Inventor (STAI) questionnaire scores and the SAA level before displaying corneal surgery film [31]. Takai N et al., used a mental arithmetic task process as stressful stimuli, a significant relationship was found between STAI and SAA level before a stressful process [22].

A significant increase in the post dental treatment SAA levels when compared to the pretreatment levels at the first appointment was observed. These results suggest that the stressors selected in present study were indeed appropriate. Such a trend of increase in the postdental treatment SAA over pretreatment levels at the first appointment was not evident in the second appointment, which could be due to adaptation to the stressors by the second appointment [32]. According to Phukela SS et al., the stress level was minimum in healthy patient and highest when patient underwent implant placement [33]. Even simple procedures like dental prophylaxis can cause stress in young children [34].

Hence, stress should be monitored in children during dental treatment. By providing a friendly environment and enjoyable distractions, the child's behaviour towards dental treatment can be modified.

Limitation(s)

Comparative analysis with the normal subjects was not done. Further research is required in this area, e.g., studies with larger sample size.

CONCLUSION(S)

The results of this study indicate that cumulative salivary biomarkers respond markedly to the stress of dental environment. The dental stress experienced by children in their first visit was higher compared to their subsequent visit with a significant increase in salivary alpha amylase level. Monitoring salivary alpha amylase is essential to measure anxiety in children. As the biomarker used in the present study is non-invasive, it helps measure anxiety with accuracy. It can be concluded that the alpha amylase is promising and can be used to measure anxiety in future studies also.

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