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The Effect of Psychological Stress on Salivary Alpha-Amylase in Pediatric Patients

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Abstract

Aim of the study: This study aimed to investigate the relationship between psychological stress and salivary alpha-amylase levels in pediatric dental patients visiting specialist versus non-specialist dental clinics.

Material and method: Eighty male children aged 4–6 years with their first dental visit were recruited from specialist and non-specialist dental clinics in Iraq. Salivary samples were collected at three time points, and alpha amylase levels were analyzed using ELISA assays.

Results: In the non-specialized clinic, mean values rose from 2.66 ± 0.68 prior to therapy to 4.06 ± 0.70 during diagnosis, ultimately reaching 5.37 ± 1.14 post-treatment ($F = 243.383$, $p < 0.001$). At the specialized clinic, levels increased from 2.27 ± 0.75 to 2.00 ± 0.74, and subsequently to 3.34 ± 1.02 following therapy ($F = 37.958$, $p < 0.001$). Comparisons between groups revealed statistically significant variations in α-amylase levels at all time intervals ($p < 0.05$), with continuously elevated values noted in the non-specialist clinic.

Conclusion: Children attending pediatric dental clinics are less prone to stress than those treated at regular dental clinics. Salivary alpha-amylase is a reliable physiological biomarker of dental stress. Environmental and emotional stresses exert a greater influence on SAA secretion than self-perceived stress.

Keywords: alpha-amylase, dental anxiety, salivary biomarker, stress, sympathetic nervous system, pediatric dentistry

Introduction

The majority of children at dental practices are often obstinate during dental procedures. Overseeing a child in a dental office can be extremely challenging at times. These management difficulties pertain to the child's diverse emotional distress and the technical aspects of treatment. Stress, anxiety, and apprehension are the predominant emotional disturbances manifested during dental procedures (Mittal and Sharma 2012).

Anxiety is characterized as a condition of discomfort associated with the apprehension of internal or external dangers. The outcome is contingent upon the capacity for imagination (Riskind and Calvete 2020).

Anxiety is a prevalent issue in the dental operatory and is a significant difficulty for Pediatric Dentists, since many children with intense anxiety may completely evade dental examinations and decline treatment. Dental anxiety is characterized as a form of state anxiety arising from dental operations, along with negative anticipations, frequently rooted in prior traumatic experiences and adverse familial attitudes (Hamzah, Gao *et al*. 2014).

Stress is a physiological and psychological response exhibited by individuals when confronted with unfamiliar or challenging situations.

There are many ways that stress can show itself. When a person's body and mind respond to things in their environment,

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homeostasis changes. This answer leads to the change. The broad and vague idea of stress is connected to many different things and ideas. Stress's many sides can be both a good and a bad thing. The depth and thorough examination of diverse everyday events, alongside its distinctive characteristics. Stress can also be defined as a mismatch between a person and their environment, which leads to resource depletion and trouble adapting. If people believe something When people think a situation is too much for them to handle and beyond their means, they feel stressed, which can make them sick. In today's world, it is more important than ever to measure stress levels because too much stress can cause long-term health problems like high blood pressure, heart disease, cancer, and even death in the worst cases (Al-awadei and Qasim 2024.(

Stressful stimuli provoke physiological responses in specific regions of the central and peripheral nervous systems. When the body is under stress, the hypothalamus and brainstem are activated, the hypothalamic–pituitary–adrenal (HPA) axis is stimulated, and the autonomic nervous system (ANS), which includes the efferent sympathetic–adrenal–medullary (SAM) system, is involved.

In the past 15 years, salivary alpha-amylase (SAA) has become a reliable and accurate measure of autonomic nervous system (ANS) activity in studies of stress.

Alpha-amylase is an enzyme in saliva that helps break down starches and carbohydrates. It can be quickly and easily tested by collecting saliva, and it is often used in research on acute stress as a sign of sympathetic arousal, especially in studies that want to make things easier for participants or where samples need to be collected at home.

It is essential to acknowledge the ongoing discourse in the literature regarding whether SAA levels assessed during stress exclusively indicate sympathetic or parasympathetic activity, or a synthesis of both. Research suggests that SAA may signify central noradrenergic activity, as demonstrated by Nater and Rohleder, and more recently by Warren et al.

Accordingly, depending on the research context—whether acute stress is created or a pharmacological challenge is utilized—SAA may be considered an indicator of autonomic nervous system (ANS) activity and could function as a biomarker for assessing ANS performance in behavioral medicine. The next sections will focus on studies in behavioral medicine that have employed SAA as a stress indicator (Ali and Nater 2020).

Analyzing saliva can help get around these problems. Saliva has been an important diagnostic tool in the last 10 years because it can be used for many different tests and is easy to collect without hurting anyone (Wang, Schipper *et al*. 2015). The absence of dentists' specialization in pediatric dentistry, the lack of a kid-friendly atmosphere, and inadequately trained support staff in child behavior management may hinder the efficacy of pediatric dental therapy. The disparity between professional specialty and environmental support may adversely affect the overall dental experience for children. The objective of this study is to compare the psychological stress in pediatric patients aged 4 to 6 years with their first dental visit attending specialist versus non-specialist dental clinics using salivary biomarker. The alternative hypothesis stated there is a difference in psychological stress levels among pediatric patients attending specialist versus a non-specialist dental clinic. Null hypothesis states there is no difference in psychological stress levels among pediatric

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patients attending specialist private versus non-specialist clinics.

material and method:

Ethical consent

The Research Ethics Committee at the educational institution of Dentistry, University of Mustansiriyah, has evaluated the research project for ethical approval.

The decision was sanctioned based on the items presented, which have been received and evaluated by the committee. Reference number: REC145, Date: 01 December 2023, Project No. MUPRV010, the trial was registered in a clinical Trail. gov under registration number (NCT069179776).

study design

A comparative cross-sectional study was conducted with a sample of 80 male patients aged between 4 and 6 years who attended specialized pediatric dental clinic and non-specialized pediatric clinic, and had their first dental visit, with the age selected based on the period when the child commenced attending the clinic.

The sample size was 80, 40 patients from a specialized pediatric dentistry clinic and 40 from a nonspecialized dental clinic.

sample size calculation

Making use of G power 3.1.9.7 (an application created at the University of Kiel in Germany by Franz-Faul). The sample size is about 80 people, with 40 in each group. The power of the study is 85%, the alpha error is 0.05 on both sides, the effect size of Cohen D is 0.6, which is a middle effect size, and there are two groups.

Small is 0.3, medium is 0.5, and big is 0.8 for effect size D.

Inclusion and exclusion criteria

Inclusion criteria included:

male children within 4 to 6 age groups, patients with their first dental visit, healthy children without any systemic disease, and children with initial caries who need treatment with SDF material only.

Exclusion criteria included:

any child not within the specified age range, Subjects with physically and medically challenged states, particularly those on corticosteroid medicines, any individual with a systemic illness, any youngster or parent who is not willing to partake in the research.

Criteria in a specialized dental clinic: A specialist pediatric dental clinic features a welcome and waiting space exclusively built for children. The environment is lively and magical, with creative decorations, wall murals, and a lot of toys, especially those that have to do with painting and drawing. This child-friendly environment is meant to lower anxiety and make dentist visits better for kids.

The clinic has pediatric dentists on staff, who are dentists who have been trained to work with kids. The whole staff, including receptionists and dental assistants, is well-trained in how to handle children's behavior so that they may interact with them in a kind and effective way. In this situation, it's very vital to provide a safe, friendly, and developmentally appropriate space that fits the needs of young dental patients. On the other hand, many dental clinics offer a broad ambiance that isn't simply for kids. Kids don't like the waiting and welcome areas in these establishments because they don't have any design elements that would appeal to them, such bright colors, toys that are appropriate for their age, or items that catch their eye. The environment is usually more neutral or geared toward adults, which could make it harder for young patients to feel less anxious or more at ease.

Materials and instruments

Materials and instruments instrument used in oral examination and treatment.

Cotton roll, Disposable probe, Dental mirrors, Dental tweezers, Disposable

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bonding brush, Eye glass, Gloves (Silk Touch), and masks (U CARE), Kidney dishes, silver diamine fluoride material (silver sense SDF, centrix).

Materials and instruments used in saliva collection, storage, and analysis:
High-speed Centrifuge (Rotina 35, Hettich Zentrifuge/ Hettich), Cooling box and ice, Disposable collection cup, Double distilled water or deionized water, Eliso Uno-Human Device, human amya (amylase alpha) ELISA kit (ELK Biotechnology), Graduated disposable test tubes, Marker pen and pencil, PH meter (Checker by HANNA) with buffer solution pH 7, Refrigerator (ANGELATONI/ALS).

Method

A cross-sectional study was conducted with a sample of 80 randomly selected males. 40 participants were recruited from a pediatric specialized dental clinic and 40 from a general dentistry clinic. patients aged 4 to 6 years old with their first dental visit who presented to the Department of Pedodontics and Preventive Dentistry.

Between 10 and 12 AM, unstimulated saliva samples were collected via passive drooling into a graduated sterile cup for five minutes, in accordance with the protocols established by the University of Southern California School of Dentistry for saliva collection. The child was positioned in the Coach-man's posture, with the head slightly tilted downward, and advised to avoid swallowing or moving the tongue or lips during saliva collection.

The patient was directed to gather saliva in the mouth cavity for 5 minutes and then permit it to flow passively into the collection vessel (Ali and Nater 2020).

Three samples of unstimulated saliva were collected from each participant using the following protocol: T0, obtained while the patient was in the reception area prior to entering the diagnostic room; T1, collected 30 minutes after the initial screening without any intervention; and T2, collected 30 minutes after the commencement of treatment.

All patients underwent the identical treatment protocol, incorporating the application of silver diamine fluoride (SDF) as a standard intervention.

Subjective stress assessments were evaluated by using the well-regarded CFSS-DS scale (Children Fear Survey Schedule-Dental Subscale), which was established by Cuthbert and Melamed (Mobin, Khan et al. 2023)

To eradicate any bias from diurnal variation, all samples were collected concurrently, especially between 10 AM and 12 PM; 2 mL of saliva from each sample was extracted and stored at –20° according to the manufacturer instructions (ELK Biotechnology LTD)until analysis.

The serum alpha-amylase concentration was evaluated utilizing "The New Life alpha-amylase ELISA Kit", which implements a solid-phase competitive enzyme-linked immunosorbent test (ELISA).

Statistical Analysis

SPSS version 22, Chicago in press, Illionis, USA is a statistical package for the social sciences. served to describe, assess, and show the result.

The following statistical analyses were used:
Descriptive statistics
These include Minimum, maximum, mean, and standard deviation (SD) for a quantitative variable
Inferential statistics
These include Shapiro-Wilk test used to see if the distribution of a quantitative measure across groups is normal .Repeated Measure ANOVA: check to find if there are significant differences in means across multiple.

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time points within the same subjects. post hoc multiple pairwise comparison using sidak: to determine which specific group differences are significant.
There are two significance levels: statistically significant (P<0.05) and not statistically significant (P>0.05).

Result

Marked elevations in SAA were noted from pre- to post-screening in both general and specialist clinics ($p < 0.001$). Nonetheless, no substantial relationships were seen between SAA and self-reported stress ($p > 0.05$).
Salivary α-amylase levels were assessed at three intervals: prior to the dental operation, during diagnosis, and post-treatment. In both general and specialized clinics, α-amylase levels exhibited a statistically significant elevation with time. In the general clinic, mean values rose from 2.66 ± 0.68 prior to therapy to 4.06 ± 0.70 during diagnosis, ultimately reaching 5.37 ± 1.14 post-treatment ($F = 243.383$, $p < 0.001$). At the specialized clinic, levels increased from 2.27 ± 0.75 to 2.00 ± 0.74, and subsequently to 3.34 ± 1.02 following therapy ($F = 37.958$, $p < 0.001$). Comparisons between groups revealed statistically significant variations in α-amylase levels at all time intervals ($p < 0.05$), with continuously elevated values noted in the general clinic.

Pearson correlation coefficients between α-amylase and stress levels varied from 0.104 to 0.173, with p-values significantly exceeding 0.05. In the specialty clinic, r values varied from 0.045 to 0.160, signifying weak and non-significant correlations.

Table 1. Normality test of studied variables among clinics and time.

	Clinic			
	General		Specialist	
	Statistic	P value	Statistic	P value
Amylase-before	0.940	0.058	0.959	0.158
Amylase-Diagnosis	0.947	0.062	0.957	0.155
Amylase-Treatment	0.959	0.153	0.952	0.086
Stress-scale	0.940	0.055	0.966	0.164

Table 2. Descriptive and statistical test of α-amylase among clinics and time.

Clinic		Before	After Diagnosis	After treatment
General	Minimum	1.06	2.43	3.450
	Maximum	3.65	5.23	7.660
	Mean	2.66	4.06	5.368
	±SD	0.68	0.70	1.144

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Specialist	Minimum	1.08	1.01	1.360
	Maximum	3.98	3.95	4.920
	Mean	2.27	2.00	3.340
	±SD	0.75	0.74	1.024

SD: standard deviation

Table 3. Test of between subjects' effect

Source	Type III Sum of Squares	df	Mean Square	F	P value	Partial Eta Squared
Time	150.090	2	75.045	213.598	**0.000**	0.733
Clinics	133.880	1	133.880	88.951	**0.000**	0.533
Time*Clinics	36.257	2	18.128	51.598	**0.000**	0.398

Both the main effect of time, clinic, and the interaction between them have a significant effect on salivary amylase.

Table 4. Statistical test of α-amylase among clinics

	Mean difference	Sum of Squares	df	Mean Square	F test	P value
Before	0.395	3.113	1	3.113	6.127	**0.015**
		39.635	78	.508		
After Diagnosis	2.058	84.707	1	84.707	162.663	**0.000**
		40.619	78	.521		
After treatment	2.029	82.317	1	82.317	69.826	**0.000**
		91.953	78	1.179		

Df : degree of freedom

Table 5. repeated measure ANOVA of alpha amylase over time.

Clinic		F	df	P value	Partial Eta Squared
General	Wilks' lambda	243.383	2	**0.000**	0.863
Specialist	Wilks' lambda	37.958	2	**0.000**	0.496

Df: degree of freedom

Table 6. post hoc multiple pairwise comparison of salivary α amylase among times using sidak.

Clinic	Time		Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
General	Before	Diagnosis	-1.397	0.082	**0.000**	-1.598	-1.196
		Treatment	-2.704	0.150	**0.000**	-3.071	-2.338
	Diagnosis	Treatment	-1.308	0.153	**0.000**	-1.680	-0.935
Specialist	Before	Diagnosis	.267	0.082	**0.005**	.066	0.468
		Treatment	-1.070	0.150	**0.000**	-1.437	-0.703

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	Diagnosis	Treatment	-1.337	0.153	**0.000**	-1.710	-0.964

Std: standard deviation

Discussion

Children's dental anxiety and stress may adversely affect their oral health(Luoto, Lahti *et al*. 2009). This anxiety may persist into adolescence, leading to disruptive and irresponsible behavior during dental treatment or total avoidance of such therapy (Paryab and Hosseinbor 2013)

The research cohorts. Only male youngsters were selected as target participants in this investigation to eliminate any influence of early puberty or hormonal changes, and female heightened anxiety on the stress response. The findings corresponded with a study indicating (Mensah, Bayer *et al*. 2013, Shim, Kim *et al.* 2015)

A study at the University of Toronto revealed that although females may demonstrate heightened anxiety before dental procedures compared to males. Numerous studies suggest that the child's age during their first session is not of substantial significance; rather, the dentist's attitude and the comfort they afford the patient are of utmost importance (Shindova and Belcheva 2021)

Aside from the patient-doctor connection, environmental factors significantly influence the exacerbation or alleviation of dental anxiety. This study mostly addressed environmental issues. Bares and Dundes' research showed that the look of a dental clinic is very important. They found that clinics with bright wall colors, a pleasant smell, and well-stocked waiting rooms with magazines and books can greatly lower dental fear and anxiety (Bare and Dundes 2004).

This research resulted in the creation of a specialized dental clinic with a reception and waiting area specifically for children. There are a lot of toys in the room, especially ones for drawing and painting. The decorations and art on the walls make the place lively and interesting. This kid-friendly setting is meant to make going to the dentist less scary and more fun for kids.

There are dentists at the clinic who only work on kids' teeth. Everyone who works here, from the dental assistants to the receptionists, knows how to handle kids' behavior so that every interaction with them is friendly and helpful. This incident shows how important it is to make sure that young dental patients are in a safe, friendly, and age-appropriate environment that meets their needs. In contrast, the atmosphere of many dental offices is the same for everyone and doesn't change for kids. In these places, the reception and waiting areas may not have any design features that are good for kids, like bright colors, appropriate toys, or interesting visual stimuli. The setting is mostly neutral or geared toward adults, which may make it harder for young patients to feel better or more comfortable. A sensory-adapted dental environment has been effective in reducing dental anxiety (Shapiro, Melmed et al. 2007). In the context of patient care, this idea is meant to improve the main senses of sight, touch, and smell. Shapiro et al. conducted a study employing this strategy to alleviate dental fear and anxiety, utilizing dimmed lights, calming music, and a specialized butterfly vest that provides a comforting pressure sensation to the child. The exceedingly low ratio of patients to dentists in a specialist dental clinic significantly diminished the time a dentist allocated to controlling a kid's behavior prior to the procedure. A child's initial dental appointment is essential for shaping their attitude towards dental care, as evidenced by research (Xia, Wang *et al*. 2011). Studies show that dentists who are rude, dental staff

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who aren't welcoming, and short treatment times can all make people anxious about going to the dentist. A study was conducted on patients aged 4 to 6 years, which is the period when individuals typically attend a dental clinic for the first time .

This study's strengths include being the first of its kind in Iraq's pediatric population to compare dental stress and anxiety among children across various dental settings, utilizing a rigorously validated tool (CFSS-DS) and salivary biomarker (alpha amylase) to assess dental stress. Cuthbert and Melamed came up with the CFSS-DS in 1982. It is used to find out if someone is stressed or anxious about going to the dentist. Numerous studies have utilized this measure, and their results indicate its substantial reliability and validity(Nakai, Hirakawa et al. 2005; Arapostathis, Coolidge *et al.* 2008).

Salivary alpha-amylase (SAA) has been a dependable and valid measure of autonomic nervous system (ANS) activity in stress studies during the past 15 years, with indications that SAA may also represent activity in the central noradrenergic system. This evidence originates from Nater and Rohleder, as well as Warren et al., who are more recent sources. Consequently, SAA is frequently considered an indicator of autonomic nervous system function due to its presence. Recent findings indicate a correlation between SAA secretion and the emergence of stress-inducing circumstances. The concentration of enzymes increases in response to physical stressors, such as treadmill exercise, running, cycling, and cold exposure, as well as psychological stressors, including exposure to distressing emotional imagery of mutilation or accidents, participation in collegiate-level individual athletic competitions, undertaking written examinations, and engaging in the Trier Social Stress Test (TSST)(Arhakis, Karagiannis *et al*. 2013).

Distinct diurnal pattern of salivary alpha-amylase activity, characterized by a significant decline in the initial 30 minutes post-awakening, followed by a gradual increase during the afternoon and evening. For this reason, in order to avoid any circadian rhythm variations or differences in the enzymatic regulatory system, we used samples collected between 10-12 AM (Nater, Rohleder *et al*. 2007).

The results of this study show that alpha-amylase levels change a lot at different times in both expert and non-specialist clinics. In the non-specialist clinics, alpha-amylase levels increased significantly from pre-diagnosis to post-diagnosis, followed by a further elevation following therapy. This implies that persons in non-specialist clinics demonstrate more significant physiological responses, potentially attributable to the stress inherent in the clinical environment or the therapy process.

In contrast, the alterations in alpha-amylase levels at the specialist clinics were less pronounced. There were huge differences from the period before the diagnosis and the time after the treatment. But the difference in levels between diagnosis and therapy was not as great as it was in a clinical setting that didn't specialize in the area. It seems that the numbers go down from 2.27 ± 0.75 to 2.00 ± 0.74 and then back up to 3.34 ± 1.02. Putting a youngster in a safe setting lowers their stress, which lowers the level of salivary alpha amylase. So, alpha-amylase levels go down for a brief time and may go back to normal about 20 minutes after the body is stressed. The clinical aspects of the dental process and the dentist's behavior can induce anxiety and tension, affecting salivary alpha-amylase levels (Reis, Sanchoten et al. 2016).

This means that patients at specialty clinics get more tailored care. This could help them respond better, either by helping them deal with stress better or by making the therapy more organized.

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These findings indicate that alpha-amylase levels ought to be monitored as a potential stress biomarker in clinical environments. The significant rise in non-specialist clinics may indicate elevated stress levels, whereas the minimal fluctuations in expert clinics suggest that specialized therapy may alleviate stress responses.

Limitations

The present study, despite its noteworthy findings, possesses intrinsic limitations that necessitate acknowledgment. The sample size, although adequate for statistical analysis, may limit the generalizability of the findings to wider populations and diverse dental settings. Second, the study did not take into account possible confounding factors, such as nutrition and sleep quality. Third, the groups are all the same age. One big worry was that only workers from two clinics could be hired. This might have made the results less believable in other areas. Adding more clinics to the study could give a better picture and maybe even change the results. Ultimately, completing the surveys in the presence of parents may have made the replies less private, which could have caused bias in the responses.

Suggestions

Despite the limitations of our work, we identified a potential avenue for further research.

1 .Expanded Multicenter Research: Subsequent studies ought to incorporate a larger and more heterogeneous sample from various institutions to enhance the external validity of the results.

2 .Longitudinal Design: A longitudinal methodology would yield a thorough examination of the temporal fluctuations in stress biomarkers across consecutive dental consultations.

3 .Enhanced Psychological Evaluation: Utilizing diverse validated psychological instruments can yield a more thorough assessment of perceived stress, anxiety, and coping strategies.

4 .Management of Confounding Variables: Future research must consider variables affecting salivary biomarkers, including pharmacological treatments, dietary intake, and sleep patterns.

5 .Intervention Studies: Evaluating the effectiveness of anxiety-reducing therapies, such as music therapy, cognitive-behavioral techniques, or sedation methods, may aid in the creation of dental environments that alleviate stress.

6. Regular Stress Monitoring: Adding non-invasive salivary biomarker tests may help find patients who are more stressed and could benefit from personalized treatment plans.

Conclusion

Kids who go to pediatric dental clinics for treatment feel less stressed than kids who go to general dentistry clinics for treatment.

Salivary alpha-amylase rises during dental procedures and acts as a sensitive indicator of psychological stress and treatment assessment in various clinical environments. Environmental and emotional stressors have a more significant impact on SAA secretion than self-reported stress.

Supplementary Material

None.

Author Contributions

Summer Hilu Hammdallah: data curation, writing-original draft preparation. Mohammed Qays Mahmoud Fahmi:

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Conceptualization, methodology, writing-review and editing. Haider Al-Waeli: validation, formal analysis, investigation, supervision.

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Data Availability Statement

Data are available from the authors upon reasonable request.

Conflict of interest

The authors reported that they have no conflicts of interest.

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