Comparing Dental Stress in New Child Patients and Returning Patients Using Salivary Cortisol, Immunoglobulin-A and Alpha- Amylase

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Objectives: *this study was aimed at comparing dental stress in children having their first dental visit to those returning for dental treatment using salivary biomarkers of stress including salivary cortisol (s-cortisol), Immunoglobulin-A (s-IgA) and alpha-amylase (s-α-amylase). Additionally, the study was aimed at monitoring the change in stress in new patients as they progressed from the waiting to the clinical areas.* ***Study design:*** *salivary samples were collected from 40 children who had not been to a dentist before and similar samples were collected from 40 children who were returning for completion of dental treatment. Salivary cortisol, s-IgA and s-α-amylase concentrations were obtained by Enzyme-linked Immunosorbent Assay (ELISA).* ***Results:*** *salivary cortisol levels were higher for new patients at the waiting area compared to that at the dental chair (p=0.05). Salivary alpha-amylase significantly increased in new patients while being seated in the dental chair. Returning patients had higher s-α-amylase (p=0.001) and s-IgA (p=0.016) compared to new patients. Returning patients had the lowest level of s-cortisol when providers were faculty pediatric dentists than with students and interns (p=0.035).* ***Conclusions:*** *children coming in for their first dental visit may experience dental stress at the waiting area before being seated for dental examination. Returning children may experience higher levels of stress compared to new child patients possibly due to previous dental exposure.*

Key words: *Stress, Salivary immunoglobulin-A, Salivary cortisol Salivary alpha-amylase, children.*

INTRODUCTION

Stress is commonly defined as the physiological and psychological reactions that mobilize an organism's defense against external or internal threats (stressors) [1]. Stress is probably the most prevalent psychosocial problem [2]. Dental treatment can be a major source of anxiety and fear among patients and is considered to be one of the most stressful environments to many people, especially children [3]. Dental stress can affect children's behavior during treatment and its assessment can be a vital part of children's management [2].

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Responses to stressful conditions can be observed on the physiological level in the form of increase in the activity of the hypothalamus-pituitary adrenal (HPA) axis with subsequent rise in salivary cortisol (s-cortisol) level [4]. Cortisol has a diurnal variation, and its secretory activity is characterized by peak levels 20-30 minutes after awakening and a declining pattern thereafter [5]. Salivary cortisol is known to be an indicator of the concentration of unbound cortisol in the serum [6]. However, in order to determine the serum cortisol concentration, a blood sample is needed. Venipuncture induces anxiety and thereby itself increases the serum cortisol level [7,8]. Therefore, a non-invasive method is preferable. Urine can be used to evaluate serum cortisol level but may not represent instantaneous changes because urine samples represent cumulative cortisol levels since last micturition [9]. Saliva can be used to evaluate serum cortisol levels because of its stress-free non-invasive manner [10].

Stress can also lead to variation in the IgA level, which is considered the most abundant antibody [11]. It became a focus of interest in psycho-immunological research since it has been shown to be sensitive to variations in subjective and objective stress levels and considered as one of stress biomarkers [12].

Salivary cortisol and salivary Immunoglobulin-A (s-IgA) are biomarkers, which can be used to study the effects of stress in an individual. Despite their proven role in determining the effects of stress, conflicting results have been found regarding the relationship between stress and s-IgA and cortisol. The causes of these

inconsistent results may be due to methodological reasons such as sampling time and collection techniques. Another cause may be the complex interplay of neurobiological events linking perceived stress to HPA-axis activation [13].

Salivary alpha-amylase (s-α-amylase) is one of the principal salivary proteins secreted by highly differentiated epithelial acinar cells of the exocrine salivary glands following activation of beta-adrenergic receptors. It gained interest as a non-invasive indicator of body changes associated with stress [14]. Salivary α-amylase activity is linked with the sympatho-adreno-medullary system and is significantly correlated with noradrenaline levels in saliva [15,16]. In humans, α-amylase levels have been reported to rise in response to physiological and psychological stress [17-20].

Studies show that children experience fear and anxiety in dental settings and that as much as 34% may have high to severe dental anxiety [21]. This anxiety can be a major cause of avoidance of regular dental care [22]. This study was aimed at comparing dental stress in children having their first dental visit and those returning for dental treatment using salivary biomarkers of stress including s-cortisol, s-immunoglobulin-A and s-α-amylase. Additionally, the study was aimed at monitoring the change in stress in new patients as they progressed from the waiting to the clinical areas.

MATERIALS AND METHOD

The present cross-sectional study was carried out at the Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia. The study recruited a total of 80 healthy children (Saudis and non-Saudis), ages 6-12 years. Recruited children were those treated by students, interns or pediatric dentist staff providers. Children with diagnosed medical problems were excluded from the study. Children in the study were either new patients having their first dental visit or were returning to continue previous dental treatment. Ethical approval to conduct the study was obtained prior to the commencement of the study. The study was explained in details to all recruited individuals who consented to joining the study.

The study instructions were explained to the child's parents first and then to the child. Parents were allowed to accompany their children during the collection of salivary samples. To overcome the language barrier in non-Arabic speaking children, the examiner demonstrated the sampling procedure to the parents who in turn showed their children what needed to be done using their native language. 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 saliva in the sampling container once a minute for five minutes. Each container was then labeled with the child's file number, and time and date of sample collection. New patients donated saliva at the waiting area then immediately after being seated in the dental chair. Returning patients donated saliva after being seated at the dental chair.

The containers of saliva samples were refrigerated at 4°C and analyzed within 14 days. Concentrations of s-cortisol (0-30 ng/ml), s-IgA (0-1200 µg/ml), and s-α-amylase (0-500 U/ml) were measured by Enzyme-linked Immunosorbent Assay (ELISA) from Euroimmune Germany, according to the procedures described by the manufacturer [23].

Statistical analysis

Data was analyzed 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 cutoff for statistical significance and all tests were two-sided. The proportions, arithmetic means, standard deviations and medians were used as summary statistics. Chi-square tests were used to compare the two study groups (new and returning patients) regarding categorical variables. The Mann-Whitney test was used to compare the distribution of salivary markers between the two study groups and Wilcoxon signed-ranks test was used for comparison of salivary biomarkers in new patients. To compare among groups in the mean age, the t-test was used and Kruskal-Wallis test was used to compare salivary markers by provider position categories.

RESULTS

This study recruited a total of 80 children of which 35 were males (43.75%) and 45 females (56.25%). Of the recruited children 40 were new dental patients (mean age 8.8 years) and 40 were returning for completion of treatment (mean age 9.1 years). (Table 1) shows the demographics of the sample. It can be seen that the demographics of the two groups of children were not significantly different. On the other hand, there were significantly more females and undergraduate dental students providing treatment in the study (p=0.003 and p=0.000, respectively).

Table 1–Sample demographics.

Variable	New patients No. (%)	Returning patients No. (%)	X^2	p value
Gender				
male	18 (45)	17 (42.5)	0.05	0.82
female	22 (55)	23 (57.5)		
Age (years)				
<9	19 (47.5)	14 (35)	1.29	0.26
9-12	21 (52.5)	26 (65)	t=0.47	0.57
mean age (years)	8.8	9.1		
Nationality				
Saudi	19 (47.5)	23 (57.5)	0.80	0.37
non-Saudi	21 (52.5)	17 (42.5)		
Provider gender				
male	5 (12.5)	17 (42.5)	9.028	0.003**
female	35 (87.5)	23 (57.5)		
Type of provider				
undergrad	0 (0)	31 (77.5)	52.18	0.000**
intern	13 (32.5)	5 (12.5)		
grad	2 (5)	1 (2.5)		
faculty	25 (62.5)	3 (7.5)		

Salivary biomarkers

The results show that for new patients, s-α-amylase significantly increased as the child was seated in the dental chair compared to that at the waiting area (p=0.019). The opposite was found with s-cortisol which appeared to be higher at the waiting area (p=0.05) (Table 2). Salivary flow and s-IgA did not show any significant change. Comparing new and returning patients the results show that returning patients had significantly higher levels of s-α-amylase and s-IgA (p=0.001 and p=0.016, respectively) as depicted in (Table 3).

Table 2–The difference in salivary biomarkers of stress for new patients during their dental visit.

Salivary biomarkers	at waiting area	in the dental chair	Wicoxon Signed Ranks Z	p value
volume (per 5 min)	1.3	1.2	0.47	0.64
s-cortisol	3	2.4	1.96	0.05**
s-α-amylase	429.2	473.5	2.34	0.019**
s-IgA	1114.8	1114.1	0.93	0.35

Table 3–The difference in salivary biomarkers of stress between new and returning patients.

Salivary biomarkers	New patients	Returning patients	Mann-Whitney Z	p value
volume (per 5 min)	1.2	1.4	0.64	0.53
s-cortisol	2.4	2.9	0.73	0.46
s-α-amylase	473.5	553.8	3.47	0.001**
s-IgA	1114.1	1200.6	2.42	0.016**

The study looked at the effect of demographical variables on the levels of salivary stress biomarkers in new and returning patients (Table 4). The results showed that child and provider gender as well as type of provider were associated with these levels. New male patients generally had higher levels of s-cortisol compared to new female patients (p=0.05). Salivary cortisol also showed higher levels in returning patients when the dental provider was a male (p=0.02). Returning patients had significantly higher levels of s-cortisol with interns and graduate student providers and lowest with pediatric dentists faculty providers (p=0.035). Salivary amylase on the other hand, was highest for returning patients with faculty providers (p=0.049). The table shows that child age and nationality had no effect on salivary stress biomarkers.

The effect of the type of dental provider on changes in the levels of salivary stress biomarkers is further depicted in the box plot in (Figure 1). It can be seen that with interns and graduate student providers the median s-cortisol level for new patients was the same at the waiting area and with the child seated in the dental chair and was lower than that of returning patients. On the other hand, the median s-cortisol level for new patients decreased after seating the child in the dental chair with faculty dental providers and became similar to that of returning patients. The opposite was observed with s-α-amylase levels in new patients as the median increased after seating the child in the dental chair but still remained lower than that for returning patients whether the provider was an intern, graduate student or pediatric dentist faculty staff member. The median level of s-IgA, showed a slight drop for new patients after being seated in the dental chair when providers were Interns or graduate students but remained generally lower than that for returning patients. When the provider of treatment was a faculty member, the median levels of s-IgA remained the same for both groups of patients.

DISCUSSION

Findings of the present study illustrate the capacity of the stressor "dental examination" to activate the HPA axis related stress reactions. The presence of higher cortisol level in the waiting area before entering to the dental clinic suggests that the major stressor for the child is anticipation of what will happen rather than entering the examination room and being seated in the dental chair. This is in agreement with a study by Benjamins *et al* [9] who found that in adult patients with severe dental anxiety, anticipation of a dental visit without any treatment resulted in increased cortisol levels. On the other hand, our data does not show a difference in the mean s-cortisol levels between new and returning patients, which may be explained by the ability of children to adapt to the stressors during the second appointment [24].

This study showed that s-α-amylase significantly increased in new patients while being seated in the dental chair compared to that at the waiting area. It seems that children became more stressed by seeing dental instruments and dental equipment at the dental operatory. Dental examination acts as a stressor, which activates the ANS leading to the release of epinephrine and nor-epinephrine. The latter is known to increase the secretion of s-α-amylase from acinar cells of the parotid and submandibular salivary glands [20]. Our results are in accordance with those of Noto *et al* [25] and Takai *et al* [26] who looked at the correlation between dental anxiety and s-α-amylase level showing a significant correlation between them. On the other hand, recent research on s-α-amylase showed that it is a reliable parameter for stress, although its exact connections to cortisol remains largely unclear. Our results also show a significant increase in s-α-amylase in returning patients, which may be a reflection of previous cumulative experience. The sound and possible discomfort associated with some dental procedures can increase patients' fear and anxiety [21].

The higher levels of s-IgA found in returning children me be reflecting the adaptive physiological responses to previous cumulative experience. High concentration of s-IgA in children with severe caries may be due to increased antigenic load, leading to high production of antibodies [27].

This study points out the importance of monitoring stress in children during the provision of dental care. Dental management should not only be at the dental operatory but should start at the waiting area, possibly by providing a child-friendly environment and enjoyable distractions for children. The manner in which the child is welcomed into dental practice may influence future patient behavior.

Table 4–The effect of demographical variables on the levels of salivary biomarkers of stress in new and returning patients.

Group Variable	Salivary stress biomarkers		
	Cortisol	Amylase	IGA
New patients **Gender**			
Males (n=18)	2.7	467.8	1091.2
Females (n=22)	2.1	478.1	1132.7
Mann-Whitney Z	1.29	0.00	-0.61
P value	0.05**	1.00	0.54
Returning patients	3.4	581.2	1197.7
Males (n=17)	2.5	533.4	1202.7
Females (n=23)	0.16	0.84	0.89
Mann-Whitney Z	0.87	0.4	0.38
p value			
New patients **Age**			
<9 y (n=19)	2.4	450.7	1149.5
9-12 y (n=21)	2.4	494.1	1082.0
Mann-Whitney Z	0.47	1.77	0.07
p value	0.64	0.08	0.94
Returning patients	3.8	596.1	1197.5
<9 y (n=14)	2.4	531	1202.3
9-12 y (n=26)	-0.44	-1.09	-0.11
Mann-Whitney Z	0.66	0.28	0.91
p value			
New patient **Nationality**			
Saudi (n=19)	2.1	447.3	1196.8
Non-Saudi (n=21)	2.7	497.2	1039.1
Mann-Whitney Z	0.94	0.06	1.36
p value	0.35	0.96	0.18
Returning patient	3.5	544.5	1151.5
Saudi (n=23)	2.1	566.2	1267.0
Non-Saudi (n=17)	-0.94	-0.06	-1.36
Mann-Whitney Z	0.35	0.95	0.18
p value			
New patient **Gender of provider**			
Male (n=5)	2.0	338.6	1030.4
Female (n=35)	2.5	492.7	1126.0
Mann-Whitney Z	0.97	1.93	0.72
p value	0.34	0.054	0.47
Returning patient	3.3	585.0	1234.0
Male (n=5)	2.6	530.7	1175.9
Female (n=35)	-2.32	-1.7	-0.31
Mann-Whitney Z	0.02**	0.09	0.76
p value			
New patient **Type of provider**			
Undergrad (n=0)	—-	—-	—-
Intern/grad (n=15)	2.6	438.6	1082.2
Faculty (n=25)	2.3	494.4	1133.2
Kruskal Wallis X^2	1.55	0.2	2.93
p value	0.21	0.65	0.09
Returning patient	2.4	554.9	1209.3
Undergrad (n=31)	6.2	491.5	1131.0
Intern/grad (n=6)	0.9	666.7	1250.0
Faculty (n=3)	6.72	6.03	0.25
Kruskal Wallis X2	0.035**	0.049**	0.88
p value			

CONCLUSIONS

Our results indicate that cumulative salivary biomarkers respond markedly to the stress of dental environment. Children coming in for their first dental visit may experience dental stress at the waiting area even before being seated on the dental chair. Our findings also shows that returning children may experience higher levels of stress compared to new ones possibly due to previous dental exposure.

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