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Salivary Cortisol, Salivary Alpha Amylase, and the Dental Anxiety Scale

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Abstract

Aim: The aim of this study was to investigate the correlation between dental anxiety (measured using Corah's Dental Anxiety Scale), salivary cortisol, and salivary alpha amylase levels. Furthermore, the aim was to look into individual differences such as age, race, gender, any existing pain, or traumatic dental experience and their effect on dental anxiety.

Introduction: Dental anxiety has negative ramifications on people's quality of life and remains as a barrier for many people from seeking proper dental care. Dental anxiety is a form of psychological stress, and its effects are seen via activation of two pathways: First, the Hypothalamic-Pituitary-Adrenal axis (HPA), which causes the release of cortisol to all body fluids. Second, the autonomic nervous system (ANS); which causes the release of epinephrine and norepinephrine. Literature has shown that when the ANS gets activated, the level of salivary alpha amylase (sAA) increases in the saliva. The interest in sAA, as a biomarker of stress, has increased in bio-behavioral research, as it reflects the ANS activity and is non-invasive to obtain. Cortisol correlation with anxiety has been demonstrated in prior literature; however, to our knowledge its correlation with sAA has not been reported. Therefore, this study looked primarily at the effect of dental anxiety on salivary cortisol and sAA levels.

Methods: This study followed a cross-sectional design and included a convenience sample of 46. Every patient was asked to complete the Dental Anxiety Scale, and a basic demographic/dental history questionnaire. A saliva sample, utilizing the method of

passive drooling, was then collected in 2ml cryovials. Samples were analyzed for cortisol and sAA levels, which were the primary outcomes.

Results: Significant associations were found between DAS scores and presence of pain and traumatic experience in women. No significant correlations were found between DAS, cortisol, and sAA levels for both genders.

Conclusion: The level of dental anxiety is affected by presence of pain and a traumatic dental history, especially in females. Our study was the first to our knowledge to test the correlation between DAS and salivary alpha amylase, nevertheless, our results failed to show any correlation between DAS, cortisol, and sAA levels.

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I- Introduction

Dental anxiety is a very common phenomenon. Despite all the technological advances in dentistry, its prevalence remains relatively stable as time passes by. It is a global issue and has been extensively studied since the 1960s. Multiple studies have been conducted in an attempt to assess it, quantify it, and compare it to different groups of people where varying results have been achieved. The general consensus is, however, that it poses problems for both patients and dentists, and has an overall negative effect on oral health and oral health related quality of life.

i. Etiology of Dental Anxiety

The etiology of dental anxiety has been proposed to be variable and multifactorial. In a study conducted by Thomson et al.[1], it was suggested that dental anxiety might arise from either endogenous (vulnerability) factors or exogenous (conditioning) factors. Endogenous factors, such as personality traits, were considered important in the development of dental anxiety, hence the author's development of the Multidisciplinary Personality Questionnaire in an attempt to study the natural progression of dental anxiety in a birth cohort. Their results revealed that 90% of the cohort was found to develop dental anxiety due to exogenous factors. Nevertheless, personality traits did contribute to the severity level of dental anxiety[1].

Other proposed causes of dental anxiety are congenital determinants, and traumatic or negative dental experiences[2]. A study by Van Wijk et. al.[3], revealed that an early negative experience is probably the most stated single cause of dental anxiety. However, negative experiences don't always lead to this outcome. This is explained by Davey's

latent inhibition theory (1989) which states that positive or neutral experiences decrease the likelihood of anxiety while negative experiences may act as a one shot conditioner to leaving patients feeling anxious about treatment[3]. Previous literature showed that 18% of patients who are anxious are more likely to have had a previous traumatic exposure at the dentist [4]. Examples of previous traumatic events may involve pain, serious treatment errors, distressing events, invasive treatment, negative dentist remarks, and strong negative emotional responses [4].

Fear of pain was described as a state of distress in anticipation or in the presence of perceived danger, and can be a specific stimulus that causes anxiety[3]. Anxious patients tend to overestimate their pain. Therefore, people who respond fearfully to pain are at increased risk of ending up in a vicious circle of anxiety that ultimately leads to avoidance of treatment [3].

ii. Dental Anxiety and Dental fear/phobia

It is important to note that dental anxiety and dental fear/phobia are similar but not entirely defined the same way. Nevertheless, they are interrelated and their definitions are somewhat collapsed. Marks definition of a phobia (1969) states that: "when fears arise that are considered out of proportion to the demands of the situation, cannot be explained or reasoned away, are beyond voluntary control, and lead to avoidance of the fear situation, then a phobia is said to exist" [5]. The issue of dental phobia is significant and is classified under the subdivision of blood injury-injection (BII) phobia in the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV)[6]. It is estimated that 5-13% of people who have this BII phobia also develop fear of the dentist[6]. In a study comparing dental fear to other types of fears listed in the DSM-IV-TR criteria, it was

noted that it ranked fourth with a prevalence of less than 25% [6]. Dental fear was observed to be more severe and strongly associated with intrusive re-experiencing and avoidance of treatment [6].

Anxiety, on the other hand, is defined as a cognitive, emotional, or a physical reaction to a dangerous situation or anticipation of a threat [7]. Mark's anxiety theory states that: "It's the emotion that helps organisms defend against a variety of threats" [5]. Eitne et al [7] demonstrated that dental anxiety arises from a dysregulation of normal defensive responses, while a dental phobia is a complex construct involving anxiety, fear, and phobia. Stimuli like fear of pain, dental drills, and practice atmosphere can act as triggers [7]. It was suggested at the time that therapeutic methods involving continued exposure to the phobic situation might extinguish the anxiety and avoidance behaviors [7].

iii. Dental anxiety vs Trait and State Anxiety

It is important to distinguish between trait and state anxiety and their relationship to dental anxiety. An anxiety trait has been defined as a relatively stable tendency towards the kind of anxiety that anyone can suffer when facing situations that are perceived as threatening [8].

State anxiety is a transitory emotional condition of the human body, characterized by a subjective and consciously perceived strain, apprehension feelings, and hyperactivity of the autonomic nervous system. Dental anxiety has been included under state anxiety as it is more situational and transitory state [8].

iv. Consequences of Dental Anxiety

There are multiple consequences of dental anxiety on patients. First, it has been suggested that dental anxiety has an effect on appointment attendance, with a tendency for these patients to have frequent cancellations and missed appointments [8]. Second, a high anxious state may have an effect on pain threshold where patients experience more discomfort [8]. As a result, this affects their cooperation and compliance during their dental appointments[8]. Additionally, it has been observed that patients with dental anxiety tend to seek treatment only when in pain [9]. This poses potential complications in treatment, increases stress for the dentist, decreases patient comprehensive care, thus, compromising oral health and reducing the oral health related quality of life [9]. Furthermore, it has been observed that patients with dental anxiety have more missing, decayed, and filled teeth, more periradicular lesions, and more bone loss compared to non-anxious patients [9]. Finally, with respect to the dentist/patient relationship, anxious patients predictably have a jeopardized relationship with their dentists, where it acts as a predictor of cancelled appointments as well [9].

Studies by Milgrom et al, Locker et al and Liddel et al [10-12] revealed that patients with dental fear and dental anxiety have poor oral health perception, and are likely to perceive greater needs of dental care, rate oral health as poor, and report chewing problems [10-12]. As a result of dental care avoidance, anxious patients experience deterioration in their oral health, and in some cases an increase in embarrassment and guilt, decrease in self-esteem, inhibited smiling, and low quality of life [13, 14].

In most of the studies that were conducted, women were shown to be more involved than men. In addition, younger age groups had more anxiety compared to older age

groups[14]. As far as the age is concerned, Pohjola et al [14] showed that it was still unclear whether the age differences depended on the cohort or was due to the decline in fear with age. Generally speaking, nonetheless, there is a trend for a negative correlation between dental fear and age and its usually correlated as well with the number of missing and sound teeth [14].

v. Dental Anxiety and Pain Perception

Dental anxiety has a direct relationship with pain perception. According to the International Association for the Study of Pain, pain is defined as: " An unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described by patients in terms of such damage. Pain can occur if there is a persistent nociception, inflammation, functional, or structural alterations with the central and peripheral nervous systems"[15].

Rhudy and Meagher [16] suggested that emotional stress modulates the human pain reactivity. Pain was suggested to have emotional as well as physiological constructs and depends on the psychological factors, such as the emotional and motivational states [17]. Loggia et al [17] suggested that the patient's state of mind, like the attentional state, affects pain perception; a negative emotional state leads to more pain while distraction (using odors, music, and movies) from painful stimuli may reduce the pain perception. Social factors and social modeling were also suggested to be involved in pain perception in addition to administration of a placebo, where a belief that the treatment will be effective reduces the pain perception [17].

Anxiety and Pain are both negative feelings associated with dental treatment [17]. Anxiety was described as a multidimensional construct that consists of somatic,

cognitive, and emotional elements and describes a general state that is not stimulus specific [17]. Some anxiety and pain provoking stimuli in dentistry are the sight of the injection needle, dental surgery, root canal treatment, and insufficient anesthesia [17].

Pain and anxiety perceptions may differ based on individual characteristics such as gender, age, education, income, smoking status, and oral health [17]. In addition, it also differs depending on the type of procedures involved, which can also make it procedure specific [17].

Klages et. al.[18] showed that pain experienced during restorative procedures due to failure of anesthesia occurs in 5-15% of patients, and that personality traits can contribute to the pain experience. These patients experience physiological arousal similar to anxiety symptoms (sweating, palpitations, muscle tension, and rapid breathing) [18]. According to Reis's theory in regards to perceived pain, "The danger expectancy and sensitivity towards fear predisposes people to acquire anxiety disorders" [18]. It was also demonstrated that highly anxious and sensitive patients suffer from multiple pain experiences. In addition, the patients' expectations of pain cause anxiety like symptoms and therefore avoidance of the painful stimuli. In this study, the extent to which anxiety sensitivity increases expected and experienced pain by patients with different levels of dental fear was assessed and concluded that higher dental fear predicted higher pain experience [18].

vi. The Physiological Impact of Dental Anxiety

Another important aspect concerning dental anxiety is the physiologic impact it has on body responses. Stress is one of the physiologic responses to any threatening situation,

and its defined as the physiological and psychological reaction that mobilizes an organism's defense against internal or external stressors or threats [19].

Stressors usually affect the body physiologically by activating the Autonomic Nervous System (ANS) and the Hypothalamic-Pituitary-Adrenal (HPA) axis[20]. Since dental treatment is considered stressful and anxiety provoking, it can affect the physiological stress response by activating the ANS and the HPA axis. Different dental procedures produce different amounts of stress depending on the severity and patient factors[21]. In a study by Georgelin-Gurgel et. al.[22] the authors considered stress-inducing procedures in endodontics and compared the neurophysiologic consequences; changes in blood pressure, heart rate, and pulse between surgical and non-surgical endodontics. Since endodontic procedures are considered stressful, the authors found it important to consider the psychological and physiological aspects that are correlated with the stress of the procedure. It was concluded that in both surgical and non-surgical endodontics, heart rate and systolic blood pressure are increased especially after local anesthesia [22].

a) The Autonomic Nervous System response and Salivary Alpha Amylase

When the autonomic nervous system becomes activated, it stimulates the secretion of epinephrine and norepinephrine from the adrenal medulla. These catecholamines, in turn, activate the sympathetic and parasympathetic nervous systems and have variable effects on body responses. The ANS stress response is characterized by being rapid and prepares the body for the fight or flight reaction [20]. In the mouth, this is reflected by the increased production and secretion of alpha amylase from the acinar cells of salivary glands. Alpha amylase is a salivary protein that is secreted by the parotid and submandibular salivary glands. It accounts for 20% of salivary secretions[23] and has a

calcium containing metalloenzyme that acts on alpha 1,4 linkages of starch, and hydrolyzes it to glucose and maltose. Studies by Nator et. al.[23] have shown that its level reflects ANS activity, since its secretion is stimulated by norepinephrine's effect on β-adrenergic receptors in the salivary glands. Measuring sAA levels in the saliva proved to be an easy and non-invasive way of measuring the activity of the autonomic nervous system, as its level reflects the level of the catecholamines in plasma [20]. Stimuli such as exercise, cold exposure, and hypertension were shown to increase its level [23]. In addition, its level was also found to increase in response to psychological stress [23].

Nator et al.[24] also demonstrated that alpha amylase has a definite circadian rhythm where its levels fluctuate within a 24 hr period. This study has shown that sAA levels were highest in the morning upon awakening, with a sharp decrease 30-60 minutes afterwards, followed by a constant increase throughout the day. No significant differences were noted among variations in gender, BMI, smoking status, eating, and drinking of caffeinated beverages. Stress, however, caused an increase in its secretion. According to this study, the geometric mean of sAA levels upon awakening was found to be 106.14 U/ml, which was followed by a sharp decrease noted to be, on average, as low as 46.73 U/ml 39 minutes after awaking. A 17% increase in its level was noted each hour afterwards with an average peak level of 156.87 U/ml at around 4:25pm [24].

The suggestion that sAA levels increase in stress situations came out in the late 1970s. Brown (1970) suggested that changes in salivary parameters were regarded as an index state of psychopathology[25].

In regards to the effect of flow rate on sAA levels, it was suggested that, since the ANS has sympathetic and parasympathetic components that oppose each other's actions, that

measuring the level of alpha amylase may not be a useful marker of sympathetic activation [26]. The reason was due to the assumption that sympathetic activity decreases flow rate and that the protein level remains the same, thus it measures higher and may not be representative of an actual increase [26]. However in a study done by Rohleder et al.[26] that compared sAA in 2 saliva collection methods, it was shown that flow rate does respond to stress with a small increase, only with the passive drooling method, and that this contradicts the criticism stating that stress results in a decrease in flow rate. Nevertheless, a correction for the flow rate is usually recommended before analysis, where sAA levels are usually represented as an outcome rate in U/min. (Salimetrics [TM]).

b) Hypothalamic-Pituitary-Adrenal (HPA) axis and Salivary Cortisol

When the hypothalamic-pituitary- adrenal axis (HPA) gets activated, an increase in the secretion of cortisol occurs from the adrenal cortex into all body fluids, including saliva [20]. Cortisol is a twenty-one-carbon glucocorticoid that regulates carbohydrates, proteins, fats, and water metabolism. It also maintains vascular reactivity, affects the sensitivity of the nervous system, regulates blood cell numbers and affects the human stress response [21]. The ACTH hormone that is secreted by the pituitary gland regulates the production of cortisol. Stress and diurnal rhythms are modulating factors that can upregulate its production. The HPA axis response is usually slower than the ANS response. This time lag is explained by the fact that cortisol is lipid soluble and has low molecular weight, therefore, it diffuses into the salivary mucous membranes and then depending on the concentration gradient it gets excreted by osmosis. This way it has to pass through the basal and luminal membranes of the salivary glands twice, which may cause this delay in the response[21].

Many studies observed an increase in salivary cortisol levels in response to stress. In a study by Shah et. al[27], patients with oral lichen planus were found to have higher salivary cortisol levels compared to patients without it, as these patients have a higher tendency to be depressed and stressed. The study concluded that measuring cortisol levels in the saliva is a good way to assess stress, as it is easy and non-stressful to perform [27]. In another study, Hill et. al.[28] assessed salivary cortisol levels in patients undergoing wisdom teeth extractions where it was concluded as well that its an easy, non-invasive, and a good way to collect samples to measure physiologic stress. Kanegane et. al.[12] measured salivary cortisol levels prior to urgent dental care. Pain caused by acute tissue injury causes an increase in the secretion of cortisol due to activation of the HPA axis. Litt et al[12] suggested that in situations of acute anxiety, anxiety and pain maybe indistinguishable, therefore, both produce a stress physiologic response, hence the increase in secretion of cortisol in all body fluids. The cortisol that is measured usually represents the molecules that are not bound to globulin in serum, and its level is irrespective of salivary flow rate[12].

It is important to mention that salivary cortisol has a diurnal rhythm and its levels fluctuate depending on the time of day. Levels usually peak around 6am in the morning, where its around 400 pg/100μl, decreasing to 150-200pg/μl around noon, dropping further to 100pg/μl in the evening [20].

vii. The Dental Anxiety Scale

Multiple scales have been devised over the years to quantify dental anxiety. The Dental Anxiety Scale (DAS), devised by Norman Corah in 1969, is the most commonly used scale and has proved to have high validity and stability over time [29]. It has been used as a tool in many studies as a standard scale for dental anxiety scoring. It consist of four multiple choice questions relating to the patients feelings and experience prior to a dental appointment, while waiting in the waiting room to be seated, while in the dental chair, and while waiting to get a cleaning. The scoring can range from 4-20, where a score range of 4-8 indicates no to mild anxiety, 9-12 indicates moderate anxiety, 13-14 indicates high anxiety, and 15-20 indicates severe anxiety[29]. Due to the simplicity and ease of administration of this questionnaire, and to make this study comparable to other studies, as far as anxiety scoring is concerned, the scale was adapted for use in this study.

viii. Significance of Research

As discussed above, there are multiple parameters and impacts of dental anxiety on the body's physiologic responses. It is important to study these parameters and their impact on different groups of people in terms of age, gender, and race. Multiple studies have correlated between dental anxiety and these parameters achieving variable results, but with a definite female predilection, and younger age group involvement, as mentioned previously[1, 3, 30, 31]. As far as the correlation between salivary cortisol levels and dental anxiety, some studies showed results in favor of the correlation while others failed to show any significant correlation. Nonetheless, there is a general agreement that dental anxiety causes stress, which has physiologic manifestations on body responses. Alpha

amylase correlation with stress has been studied extensively in bio-behavioral, and medical research with results indicating a positive increase in its level in correlation with stressful situations. In the study by Takaia et. al.[20], it was suggested that measurement of alpha amylase is a better measure of physiologic stress response compared to salivary cortisol level as it produces a more rapid reaction in the body. To our knowledge, however, no data is available in the dental literature of any correlation between dental anxiety and salivary alpha amylase levels.

Due to the location and diverse population of patients presenting to Tufts University School of Dental Medicine seeking dental care, there is a vast amount of data that can be collected to allow us to better understand the physiological responses of dental anxiety, and the effect that it has on the outcome of dental treatment. Comparisons can be made between different groups based on patients' age, gender, and race. This study can serve as a first step in assessing the physiological impact of dental anxiety on the patient population presenting to the clinic, and baseline correlations can be made between patients with different backgrounds, dental anxiety scale scores, salivary cortisol, and alpha amylase levels. Future research can assess the effect of different interventions on reducing the anxiety scores in general and the effect that this reduction in anxiety scoring will have on these physiological parameters.

II- Specific Aims

Because dental anxiety is a significant issue, this research aimed at conducting a preliminary observational study on patients presenting to Tufts University School of Dental Medicine as new patients. The patients' anxiety scoring, using the Dental Anxiety Scale, was determined initially, then that was correlated to the salivary cortisol and alpha amylase levels using the method of saliva sampling. The aim was to observe if there is any correlation between the two physiologic biomarkers; cortisol or alpha amylase, and dental anxiety. In addition, due to the diverse population that presents to the clinic, we observed if there are any differences between age, gender, and race and determined any correlation between those variables and the Dental Anxiety Scale. Finally, we observed whether patients who present with pain and/or have had a previous traumatic dental experience show higher anxiety levels.

III- Hypotheses

Four hypotheses were tested in this study. First, the Dental Anxiety Scale Score is positively correlated with an increase in salivary cortisol levels. Second, the Dental Anxiety Scale Score is positively correlated with an increase in salivary alpha amylase levels. Third, age, gender, and race, are associated with the Dental Anxiety Scale. Finally, patients who report pain symptoms and who report a previous traumatic experience at the dentist will show a greater Dental Anxiety Scale score.

IV-Research Design and Methods

The study took place at Tufts University School of Dental Medicine's General Practice Residency (GPR) clinic. It followed an observational cross-sectional design. The Institutional Review Board at Tufts University approved all procedures and protocols. Patients selected for the study were given informed consent for their participation and all protocols and requirements for enrollment were explained prior to beginning of the study. Patient's confidentiality was kept at all times.

i. Patient Selection

New patients, who had no prior dental history at Tufts University School of Dental Medicine, were selected. Inclusion in the study entailed patients' prior agreement to participate by signing informed consent. All protocols were explained and all questions answered prior to enrollment. Adult patients, with age range of 18-80 years, who are in good systemic health, were included.

The exclusion criteria included: First, patients with any preexisting health conditions affecting cortisol levels such as adrenal gland disease or insufficiency or pregnant women. Second, women taking oral contraceptives or hormone replacement therapy, asthmatic patients on steroid inhalers, or any patient requiring corticosteroid therapy. Third, patients taking any medication or have conditions that affect the salivary flow rate, such as xerostomia and patients on beta-blockers. Lastly, patients with psychological problems who are on antipsychotic medication were excluded due to the effect that these medication have on their mood and mental status.

Considering the effects of cigarette smoking on salivary flow rate and saliva composition, smokers were included on the provision that they agreed not to smoke at least 2 hours prior to sample collection, otherwise they were excluded. Furthermore, general recommendations prior to saliva sample collection for more accurate results, considered time since eating the last meal, caffeine consumption, as well as alcohol consumption. Patients were instructed not to consume meals for at least four hours prior to sample collection, as well as no caffeine or alcohol consumption for a minimal of 12 hours prior to sample collection. (Salimetrics ^{TM}, PA)

To account for the diurnal rhythms for both cortisol and alpha amylase, all samples were collected between the hours of 9-12am.

Patients were offered a free exam and cleaning as an incentive to participation. A value of 150 USD.

Sample collection took place between July 15th, 2011 and January 31st, 2012.

ii. Methods and Saliva Sample Collection

Patients requesting to become new patients at the GPR clinic at TUSDM were asked by phone or in person prior to making their appointment whether they would be interested in participating in the study. Upon their approval, and their meeting all inclusion/exclusion criteria for participation, they were given a new patient exam appointment between the hours of 9-12 am. Upon their arrival, they were seated in the dental chair and where given the study packet that included the study informed consent form to confirm their understanding of study protocol and their approval to participate, the Dental Anxiety

Scale, and background information questionnaire collecting basic demographics; age, gender, race, history of dental pain, any previous traumatic dental experience, and the type of experience, if applicable. All patients' questions were answered and upon completion of the paperwork, everything was placed in number coded, sealable envelopes, which were stored in a locked office drawer until all samples were collected. Upon completion of the paperwork, a saliva sample was collected using the method of passive drooling utilizing 2 ml number coded cryovials, provided by Salimetrics™. Patients were seated upright during the collection and they were asked to allow the saliva to pool at the floor of the mouth before ejecting it through a short piece of straw angled at 45 degrees into the cryovials. Saliva was collected over a maximum of 5 minutes to account for salivary flow rate. The research coordinator, who was present in the room with the patient, observed and documented the time of saliva collection.

Once saliva samples were collected, cryovials were disinfected and transferred to a freezer where they were stored at -80 degrees Celsius until they were ready to be shipped to Salimetrics™ for analysis.

When sample collection was completed, samples were shipped with dry ice to Salimetrics™ who confirmed that samples were still frozen and in good condition upon receipt.

At the time of analysis, all frozen samples were thawed, and then centrifuged at 1500 x g (3000 rpm) for 15 minutes, to separate the mucin components and any particulate matter as those may interfere with the assays to be used (Salimetrics™, PA).

Salimetrics method of Alpha amylase analysis utillized a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The enzymatic action between this

substrate and alpha amylase yields 2 chloro-p-nitrophenol, which can be spectrophotometrically measured at 405nm. The amount of alpha amylase present is directly proportional to the increase in substrate at 405nm. (Salimetrics ^{TM}, PA)

Salimetrics method of salivary cortisol level measurement utilized monoclonal antibodies to cortisol. The plates have existing cortisol molecules bound to horseradish peroxide where the added cortisol from the saliva competes with it at the antibody-binding site. Bound Cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine resulting in a blue color. Optical density is then read at 450nm. The amount of cortisol peroxidase detected, measured by the intensity of the color is inversely proportional to the amount of cortisol present. (Salimetrics^{TM}, PA).

iii. Sample size calculations

A sufficient sample size resulting in 80% power and type I error rate of 5% was calculated based on previous literature.

From prior literature a Pearson correlation coefficient equal to 0.535 yielded statistically significant results. Using the same value, with the aid of nQuery advisor version 7.0, aiming for 80% power and a type I error rate of 5% showed that a sample size of 23 would be required. In order to increase the power, we used a sample size of 50, which resulted in a power of over 90% while maintaining a type I error rate of 5% (nQuery Advisor version 7.0).

V- Data Analysis and Results

A) Data Analysis

All data collected were entered and analyzed using SPSS version 18 (SPSS, Inc., Chicago IL). Statistical significance was considered at p-value of < 0.05. All tests performed were two-sided.

The primary outcomes in this study were salivary alpha amylase (sAA) measured in U/ml, and salivary cortisol measured in μg/dl. The Dental Anxiety Scale (DAS) score was the primary predictor for the analyses done for the above salivary biomarkers, however, it was also the outcome variable for the secondary analyses. Predictors that were included in the secondary analyses, which allowed for comparisons between basic sample characteristics and dental anxiety were age, gender, race, presence of pain, and history of traumatic dental experience. Salivary flow rate was considered as a potential confounder for alpha amylase levels. It was adjusted for before the analysis was performed by calculating the salivary alpha amylase output in U/min; method described below.

A descriptive analysis was performed for all variables. Continuous variables (DAS, sAA, salivary cortisol, sAA output, age, and flow rate) were summarized as means and standard deviations, while all other binary variables (pain, race, gender, traumatic experience) were summarized as counts and proportions. A summary of all variables can be found in tables 1 and 2 in appendix A.

Salivary flow rate was calculated by dividing the volume of saliva collected in ml by the amount of time it took to get the sample in minutes. Average sampling time was 5 minutes. To account for the controversy about the effect of salivary flow rate on salivary alpha amylase levels, the output of salivary alpha amylase, in U/min, was calculated by multiplying the flow rate (ml/min) with the level of alpha amylase (U/ml) for each sample. Four samples had to be excluded due to evidence of blood contamination at the time of analysis, leaving final sample size of 46. Data from the excluded samples were not included in any of the analyses.

Multiple analyses were done based on the collected data. Normality of data was checked using Q-Q plots and parametric tests were utilized for the analyses. A Pearson Correlation Coefficient was done to test the correlation between the DAS score and age, salivary cortisol (ug/dl), salivary alpha amylase (U/ml) and salivary alpha amylase output (U/min). In addition, independent samples t-tests were performed to test the association between the DAS scores and gender, race, presence of pain, history of traumatic experience, and more specifically type of traumatic dental experience. Furthermore, Pearson correlation coefficient was calculated to test the correlation between alpha amylase level (U/ml) and alpha amylase output (U/min), flow rate (ml/min), and volume collected (ml).

B) Results

a) DAS and age:

The mean sample age was 46.065 (SD = 16.224), and the mean DAS score was 10.044 (SD = 3.705). Pearson's Correlation Coefficient was performed to test the correlation between

DAS and age, however, no statistically significant correlation was found ($r = -0.193$, $p = 0.199$). (See table 2 and figure 2 for summary of data)

b) DAS and gender

The sample consisted of 26 males with mean DAS score of 9.923 (SD = 2.992) and 20 females with mean DAS score of 10.200 (SD = 4.549). Independent samples t-test was performed to test the association between DAS and gender. Equal variances were tested using Levene's test, where equal variances were assumed ($p = 0.084$). No significant association was found between DAS and gender ($t = -0.249$, $p = 0.805$). (See table 1 and figure 1 for summary of data)

c) DAS and race:

Since the sample was predominantly white/Caucasian, the race variable was converted into a binary variable (whites/non-whites). There were 36 whites with a mean DAS score of 10.583 (SD = 3.652) and 10 non-whites with a mean DAS score of 8.100 (SD = 3.380). An independent samples t-test was performed to test the association between DAS and race. Equal variances were tested using Levene's test, and equal variances were assumed ($p = 0.669$). No significant association was found between DAS and race ($t = -1.931$, $p = 0.060$). (See table 1 and figure 1 for summary of data)

d) DAS and pain:

Seven subjects reported pain. Their mean DAS score was 14.000 (SD = 4.761). On the other hand, 39 subjects did not report pain and their mean DAS score was 9.333 (SD = 3.046). Independent samples t-test was performed to test the association between DAS and presence of pain. Equal variances were tested using Levene's test, and equal variances were assumed (p = 0.226). A statistically significant association was observed between DAS and presence of pain (t = -3.411, p = 0.001). (See table 1 and figure 1 for summary of data)

e) DAS and history of traumatic experience:

Twenty-one subjects reported a history of traumatic dental experience. Their mean DAS score was 11.286 (SD = 4.485). On the other hand, 25 subjects did not report a traumatic dental experience and their mean DAS score was 9.000 (SD = 2.550). Independent samples t-test was performed to test the association between DAS and history of traumatic dental experience. Equal variances were tested using Levene's test, and equal variances were not assumed (p = 0.015). A statistically significant association was observed between DAS and history of traumatic dental experience (t = -2.168, p = 0.470). (See table 1 and figure 1 for summary of data)

To investigate this further, the type of traumatic experience was analyzed as follows:

- *DAS and Painful local anesthesia injection (PLA):*

Ten subjects reported history of a painful local anesthesia injection. Their mean DAS score was 12.200 (SD = 4.467). On the other hand, 36 subjects did not report a painful local

anesthesia injection and their DAS score was 9.444 (SD = 3.290). Equal variances were tested using Levene's test, and equal variances were assumed (p = 0.256). A statistically significant association was observed between DAS and history of painful local anesthesia injection (t = -2.164, p = 0.036). (See table 1 for summary of data)

- *DAS and Pain reported while drilling tooth for a filling (PDTF)*

Thirteen subjects reported history of pain while drilling tooth for a filling. Their mean DAS score was 10.385 (SD = 4.253). On the other hand, 33 subjects did not report history of pain while drilling tooth for a filling, and their mean DAS score was 9.909 (SD = 3.530). Equal variances were tested using Levene's test, and equal variances were assumed (p = 0.257). No statistically significant association was found between DAS score and history while drilling tooth for a filling (t = -0.388, p = 0.700). (See table 1 for summary of data)

- *DAS and traumatic tooth extraction or oral surgery (TTEOS)*

Eleven subjects reported a history of traumatic tooth extraction or oral surgery procedure. Their mean DAS score was 11.546 (SD = 4.083). On the other hand, 35 subjects did not report a history of traumatic tooth extraction or oral surgery procedure, and their mean DAS score was 9.571 (SD = 3.509). Independent samples t-test was performed to test the association between DAS and history of traumatic tooth extraction or oral surgery procedure. Equal variances were tested using Levene's test, and equal variances were assumed (p = 0.709). No statistically significant association was found between these variables (t = -1.566, p= 0.125). (See table 1 for summary of data)

- *DAS and Negative Dentist Remarks (NDR)*

Seven subjects reported a history of negative dentist remarks. Their mean DAS score was 13.571 (SD = 5.159). On the other hand, 39 subjects did not report a history of negative dentist remarks, and their mean DAS score was 9.410 (SD = 3.058). Equal variances were tested using Levene's test, and equal variances were not assumed (p = 0.029). No statistically significant association was found between DAS and history of negative dentist remarks (t = -2.070, p = 0.079). (See table 1 for summary of data)

f) DAS and Salivary Alpha Amylase (U/ml)

The mean alpha amylase level was 73.730 (SD = 46.194) and the mean DAS score was 10.044 (SD = 3.705). Pearson's correlation coefficient was used to test the correlation between these variables, however, no statistically significant correlation was observed (r = -0.045, p =0.766). (See table 2 and figure3 for summary of data)

g) DAS and Salivary Alpha Amylase output U/min

The mean salivary alpha amylase output was 47.107 (SD = 45.791) and the mean DAS score was 10.044 (SD = 3.705). Pearson's correlation coefficient was performed to test the correlation between DAS and salivary alpha amylase output, however, no statistically significant correlation was observed (r = -0.162, p = 0.282). (See table 2 and figure 4 for summary of data)

h) DAS and salivary Cortisol levels in μg/dl

The mean salivary cortisol level was 0.277 (SD = 0.273) and the mean DAS score was 10.044 (SD = 3.706). Pearson's correlation coefficient was performed to test the correlation between DAS and salivary cortisol levels, however, no significant correlation was observed ($r = 0.126$, $p = 0.403$). (See table 2 and figure 5 for summary of data).

i) Salivary Alpha Amylase (U/ml) and Flow Rate (ml/min)

The mean salivary alpha amylase was 73.730 (SD = 46.194) and the mean flow rate was 0.602 (SD = 0.480). Pearson's correlation coefficient was performed to test the association between salivary alpha amylase and flow rate, however, no significant association was observed ($r = 0.126$, $p = 0.404$). (See table 3 and figure 7 for summary of data)

j) Salivary Alpha Amylase (U/ml) and Salivary Alpha Amylase Output (U/min)

The mean salivary alpha amylase was 73.730 (SD = 46.194) and the mean salivary alpha amylase output was 47.107 (SD = 45.791). Pearson's correlation coefficient was performed to test the correlation between salivary alpha amylase and salivary alpha amylase output. A statistically significant association was observed between these variables ($r = 0.630$, $p < 0.001$). (See table 3 and figure 6 for summary of data)

k) Salivary alpha amylase (U/ml) and Volume of Saliva (ml)

The mean salivary alpha amylase level was 73.730 (SD = 46.194) and the mean volume collected was 1.431 (SD = 0.467). Pearson's correlation coefficient was performed to test the correlation between salivary alpha amylase and volume. A statistically significant correlation was observed between these variables ($r = 0.394$, $p = 0.007$). (See table 3 and figure 8 for summary of data)

VI-Discussion

i. Anxiety and Pain

In agreement with previous literature, our study results do confirm a relationship between dental anxiety and pain. In their study about perceived pain in relation to a dental hygienist treatment, Hakeberg et al[32] demonstrated that even though patients generally report higher anxiety towards dental treatment, it was shown that perceived pain is correlated with higher anxiety towards different aspects of a dental hygiene treatment. Their results also showed higher anxiety reported in women. Another review by Loggia et al[17] demonstrated evidence that psychological factors influence pain perception. This is revealed by changes in activity in pain pathways with changes in attentional state, stress, positive and negative emotions via neuroimaging techniques. Furthermore, Van Wijk and Makkes[31] demonstrated that anxious patients report more perceived pain than non-anxious patients while receiving a local anesthesia injection, while Klages et al [18] showed that patients with high anxiety report and anticipate more pain when exposed to a critical situation. Our study outcomes reconfirm the results of previous literature where higher anxiety scores were reported in patients who have pain.

ii. Anxiety and Previous Traumatic Dental Experience

In regards to the effect of a prior traumatic experience on dental anxiety, Agdal et al[6] showed that anxious patients might experience intrusive recollection of earlier dental experiences similar to patients with post-traumatic stress disorder. Locker et al [10] also

suggested that a negative dental experience is the most stated single cause of dental anxiety. In addition Van Wijk and Hoogstaten[3] demonstrated that people's expectations of pain could make them susceptible to end up in a vicious circle of anxiety, fear of pain, and treatment avoidance. Our study results did reveal a significant correlation between dental anxiety and a prior traumatic dental experience. Considering the type of negative experiences reported, history of a painful local anesthesia injection reached statistical significance, while a traumatic tooth extraction, pain while drilling a tooth for a filling, and negative dentist remarks did not reach statistical significance.

iii. Anxiety, Stress, and Salivary Cortisol

Previous studies by Shah et al[27] and Hill et al[28] have demonstrated a positive correlation between stress, anxiety, and salivary cortisol levels. A study by Krueger et al[34] showed that patients who have higher anxiety showed significantly higher salivary cortisol levels in an educational session compared to those who had a low dental anxiety score. In addition, a study by Koray et al[35] found a positive association between state/trait anxiety scores and salivary cortisol in patients with oral lichen planus. Furthermore, in a study by Miller et al[21] it was demonstrated that salivary cortisol levels in dental treatment are highest in patients undergoing tooth extraction compared to other procedures such as a prophylaxis, restorative, and an exam. On the contrary to all prior literature mentioned, Brand[33] attempted to correlate between DAS scale score and salivary cortisol, however, no statistically significant correlation was observed. In comparison to all the studies mentioned, our study results did not show any significant correlation between the DAS score and salivary cortisol levels.

iv. DAS, flow rate, and Salivary Alpha Amylase

Previous literature by Nator et. al[36], Rohleder et al[26], Allwood et al[37] , and Kang et al[38] looked at the correlation between stress conditions, including psychological stress, with the levels of salivary alpha amylase. They all showed that stress causes a significant increase in sAA levels when patients were exposed to a stressful condition compared to a rest condition. In addition, Noto et al.[39]and Takai et al[20] looked at the correlation between state/trait anxiety scoring with alpha amylase levels and showed a significant correlation. Our study was the first, to our knowledge, to examine the correlation between the Dental Anxiety Scale and salivary alpha amylase, nevertheless, our results did not reveal any significant correlation, and there were no significant differences based on age, gender, or race.

Considering the effect of flow rate on salivary alpha amylase, our results were in line with other studies by Beltzer et al[40] and Rohleder et al [26] where a positive correlation was observed between the alpha amylase levels (U/ml) and the alpha amylase outputs (U/min). In the study by Rohleder et al[26], the effects of flow rate on sAA levels was examined in both baseline and stress conditions, and it was concluded that the sAA and sAA output response to stress was significantly higher in both parameters, therefore the stress response was the same irrespective of flow rate. In addition, our results showed a higher correlation between sAA and sAA output, which reconfirms previous findings by Rohleder et al[26].

In regards to the correlation between alpha amylase level and volume of saliva collected, our study results revealed a positive correlation between sAA level and volume

of saliva collected. This contradicts the results achieved by Beltzer [40] et al, who demonstrated a decrease in sAA levels with the increase in volume over collection time of 5 minutes, utilizing the method of passive drooling.

v. Limitations:

Our study design had some limitations. First, a convenience sample was utilized to achieve the sample size needed, and it consisted of new patients who have never been seen in the clinic before. We had no way of predicting their anxiety level, or previous dental history, if any. Second, the patients were presenting for a regular exam appointment and only few of them reported pain. Lastly, only one saliva sample was collected from each patient, which represented pretreatment levels of the salivary biomarkers tested. The levels could have been low because the patients were not exposed to any dental treatment yet, and they all knew that they were not getting any dental treatment done.

VII- Conclusions

Based on the results of our study we can conclude that the presence of pain or any history of traumatic dental experience does contribute to patients' dental anxiety level. A painful local anesthesia injection was found to contribute more to the anxiety experienced by patients compared to other types of traumatic experiences. Dental anxiety, nevertheless, was not found to be associated with an increase in salivary cortisol or salivary alpha amylase levels and there were no differences between genders, race, and age in terms of these biomarkers. Future research should aim at comparing baseline levels of these biomarkers with any changes due to different types of dental treatment such as cleanings, extractions, and fillings and correlate their levels to dental anxiety. In addition, the effects of volume and flow rate on the level of salivary alpha amylase will need to be looked into further in future research to determine whether the volume collected maybe a potential confounder for alpha amylase in addition to salivary flow rate.

REFERENCES

1. Thomson W, Broadbent J, Locker D, Poulton R. Trajectories of dental anxiety in a birth cohort**.**. *Community Dent Oral Epidemiol*. 2009;37(3):209-219.

2. Fuentes D, Gorenstein C, Hu L. Dental anxiety and trait anxiety: An investigation of their relationship.. *Br Dent J*. 2009;8(25):206.

3. Van Wijk A, Hoogstraten J. Experience of dental pain and fear of dental pain. *J Dent Res* 2005;84(10):947-950.

4. Oosterink F, De Jongh A, Hoogstraten J, Aartman I. The level of exposure- dental experiences questionnaire (LOE-DEQ): A measure of severity of exposure to distressing dental events. *Eur J Oral Sci*. 2008;116(4):353-361.

5. Crofts-Barnes N, Brough E, Wilson K, Beddis A, Girdler N. Anxiety and quality of life in phobic dental patients. *J Dent Res*. 2010;89(3):302-306.

6. Agdal M, Raadal M, Skaret E, Kvale G. Oral health and its influence on cognitive behavioral therapy in patients fulfilling the diagnostic and statistical manual of mental disorders IV criteria for intraoral injection phobia. Acta Odontologica Scandinavica 2010.;2(2):98-105.

7. Eitne S, Wichmann M, Paulse A, Holst S. Dental anxiety- an epidemiological study on its clinical correlation and effects on oral health. *Journal of Oral Rehabilitation*. 2006;33(8):588-593.

8. Lago-Méndez L, Diniz-Freitas M, Senra-Rivera C, Seoane-Pesqueira G, Gándara-Rey J, García-García A. Postoperative recovery after removal of a lower third molar: Role of trait and dental anxiety. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108(6):855-860.

9. Lin K. Behavior-associated self reported items in patients charts as predictors of dental appointment avoidance. *J of Dental Education*. 2009;2(73):218-224.

10. Locker D, Shapiro D, Liddell A. Negative dental experiences and their relationship to dental anxiety. *Community Dent Health* 1996;13(2):86-92.

11. Ng S, Leung W. A community study on the relationship of dental anxiety with oral health status and oral health-related quality of life. *Community Dent Oral Epidemiol*. Community Dent Oral Epidemiol 2008;36(4):347-356.

12. Kanegane K, Penha S, Munhoz C, Rocha R. Dental anxiety and salivary cortisol levels before urgent dental care. *J Oral Sci*. 2009;51(4):515-520.

13. Vermaire J, De Jongh A, Aartman I. Dental anxiety and quality of life: The effect of dental treatment. *Community Dent Oral Epidemiol*. 2008;36(5):409-416.

14. Pohjola V, Lahti S, Vehkalahti M, Tolvanen M, Hausen H. Age-specific associations between dental fear and dental condition among adults in finland. *Acto Odontologica Scandinavica*. 2008;66(5):278-285.

15. Guzeldemir E, Toygar H, Cilasun U. Pain perception and anxiety during scaling in periodontally healthy subjects. *J of Periodontology*. 2008;79(12):2247-2255.

16. Rhudy J, Meagher M. Fear and anxiety: Divergent effects on human pain thresholds. *Pain*. 2000;84(1):65-75.

17. Loggia M, Schweinhardt P, Bushnell C. Effect of psychological state on pain perception in the dental environment. *Journal of Canadian Dental Association*. 2008;74(7):651-656.

18. Klages U, Kianifard S, Ulusoy O, Wehrbein H. Anxiety sensitivity as predictor of pain in patients undergoing restorative dental procedures. *Community Dent Oral Epidemiol*. 2006;34(2):139-145.

19. Blomqvist M, Holmberg K, Lindblad F, Fernell E, Ek U, Dahllof G. Salivary cortisol levels and dental anxiety in children with attention Deficit hyperactivity disorder. *Eur J Oral Sci*. 2007;116(1):1-6.

20. Takaia N, Yamaguchib M, Aragakia T, Etoa K, Uchihashia K, Nishikawa Y. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Archives of Oral Biology* 2004;49(12):863-968.

21. Miller C, Dembo J, Falace D, Kaplan A. Salivary cortisol response to dental treatment of varying stress. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1995;79(4):436-441.

22. Georgelin-Gurgel M, Diemer F, Nicolas E, Hennequin M. Surgical and non-surgical endodontic treatment induced stress. *J Endod*. 2009;35(1):19-22.

23. Nater U, Rohleder N, Gaab J, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology* 2005;55(3):333-342.

24. Nater U, Rohleder N, Schlotze W, Ehlert U, Kirschbaum C. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*. 2007;32(4):392-401.

25. Davis E, Granger D. Developmental differences in infant salivary alpha-amylase and cortisol responses to stress . *Psychoneuroendocrinology* 2009;34(6):795-804.

26. Rohleder N, Jutta M, Wolf E, MALDONADO F, Kirschbaum C. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology*. 2006;43(6):645-652.

27. Shah B, Ashok L, Sujatha G. Evaluation of salivary cortisol and psychological factors in patients with oral lichen planus. *Indian J Dent Res*. 2009;20(3):288-292.

28. Hill C, Walker R. Salivary cortisol determination and self-rating scales in the assessment of stress in patients undergoing the extraction of wisdom teeth. *British Dental Journal*. 2001;191(9):513-515.

29. Corah N. Development of a dental anxiety scale. *J Dent Res.* 1969;48(4):596.

30. Oosterink F, De Jongh A, Hoogstraten J. Prevalence of dental fear and phobia relative to other fear and phobia subtypes. *Eur J Oral Sci 2009*. 2009;117(2):135-143.

31. Van Wijk A, Makkes P. Highly anxious dental patients report more pain during dental injections. *Br Dent J*. 2008;205(3):E7.

32. Hakeberg M, Cunha L. Dental anxiety and pain related to dental hygienist treatment. *Acta Odontol Scand*. 2008;66(6):374-379.

33. Brand HS. Anxiety and cortisol excretion correlate prior to dental treatment. *Int Dent J*. 1999;49(6):330-336.

34. Krueger T, Heller H, Hauff B, Haake P, Exton M, Schwedlowski M. The dental anxiety scale and effects of dental fear on salivary cortisol. *Parcept Mot Skills* 2005;100(1):109-117.

35. Koray M, Dulger O, Ak G, et al. The evaluation of anxiety and salivary cortisol levels in patients with oral lichen planus. *Oral Dis*. 2003;9(6):298-301.

36. Nater U, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research . *Psychoneuroendocrinology* 2009;34(4):486-496.

37. Allwood M, Handwerger K, Kivlighanc K, Granger D, Stroude L. Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment. . *Biological Psychology* 2011;88(1):57-64.

38. Kang Y. Psychological stress-induced changes in salivary alpha-amylase and adrenergic activity. *Nurs Health Sci*. 2010;12(4):477-484.

39. Noto Y, Sato T, Kudo M, Kurata K, Hirota K. The relationship between salivary biomarkers and state- trait anxiety inventory score under mental arithmetic stress: A pilot study. *Anesth Analg* 2005;101(6):1873-1876.

40. Beltzer E, Fortunato C, Guaderrama M, Peckins M, Garramone B, Granger D. Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. *Physiol Behav*. 2010;1(1):289-296.

Appendix A:

Tables

Table 1. Summary of Sample Demographics N= 46

Variable	% in Sample	Total	DAS score Mean (SD)	t	P-Value
Gender					
Males	56.52	26	9.923 (2.992)	-0.249	0.805
Females	43.48	20	10.200(4.549)		
Race					
Whites	78.26	36	10.583(3.652)	-1.931	0.060
Non-whites	21.74	10	8.100(3.380)		
Pain					
Yes	15.22	7	14.000(4.761)	-3.411	0.001*
No	84.78	39	9.333(3.046)		
Traumatic Experience					
Yes	45.65	21	11.286(4.485)	-2.168	0.047*
No	54.35	25	9.000(2.550)		
Painful LA injection					
Yes	21.74	10	12.200(4.467)	-2.164	0.036*
No	78.26	36	9.444(3.290)		
Pain while drilling tooth for filling					
Yes	28.26	13	10.385(4.253)	-0.388	0.700
No	71.74	33	9.909(3.530)		
Traumatic tooth extraction of oral surgery					
Yes	23.91	11	11.546(4.083)	-1.566	0.125
No	76.09	35	9.571(3.509)		
Negative dentist remarks					
Yes	15.22	7	13.571(5.159)	-2.070	0.079
No	84.78	39	9.410(3.058)		

t values represent outcomes of independent samples t tests

P values represent DAS associations with variables using independent samples t-tests.

* statistically significant at p< 0.05

Table 2. Summary of Continuous Variables in Sample. N=46

Variable	Mean (SD)	Range	DAS Mean(SD)	r	P value
Age (years)	46.065 (16.224)	19-76	10.044(3.705)	-0.193	0.199
sAA (U/ml)	73.730 (46.194)	3.000-171.500	10.044(3.705)	-0.045	0.766
sAA output (U/min)	47.107 (45.791)	0.600-178.90	10.044(3.705)	-0.162	0.282
Cortisol (ug/dl)	0.277 (0.273)	0.001-1.779	10.044(3.705)	0.126	0.403

r= Pearson's correlation coefficient; outcome of correlation test between DAS and variables.

p-values represent results of correlation test between DAS and variables using Pearson's correlation coefficient.

Table 3. Summary of sAA correlations with Salivary Variables. N=46

Variable	Mean (SD)	Range	sAA mean (SD)	r	p-value
sAA output (U/min)	47.107 (45.791)	0.60-178.90	73.730 (46.194)	0.630	0.000*
Flow rate (ml/min)	0.602 (0.480)	0.05-1.80	73.730 (46.194)	0.126	0.404
Volume (ml)	1.431 (0.467)	0.25-2.0	73.730 (46.194)	0.394	0.007*

r= Pearson's correlation coefficient; outcomes of correlation tests between sAA and variables

p values represent results of correlation between sAA and variables using Pearson's Correlation Coefficient

*statistically significant at p <0.05

Appendix B:

Figures

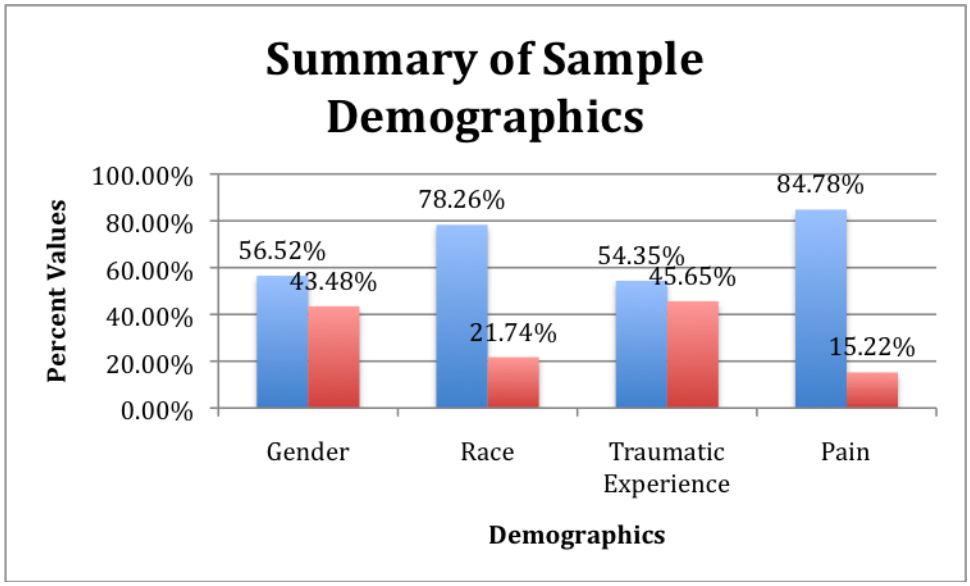

Figure 1. Basic Demographics of Sample. Gender (Blue=M, Red= F), Race (Blue= Whites, Red= Non-whites), Traumatic Experience (Blue= No , Red= Yes), Pain (Blue= No , Red= Yes)

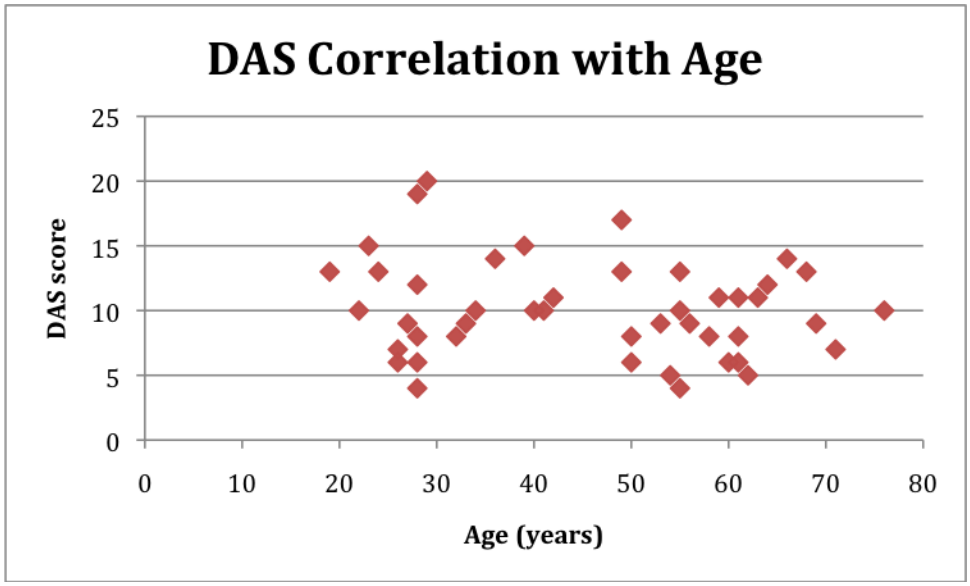

Figure 2 Scatter plot showing DAS correlation with age (years). r= -0.193, p= 0.199

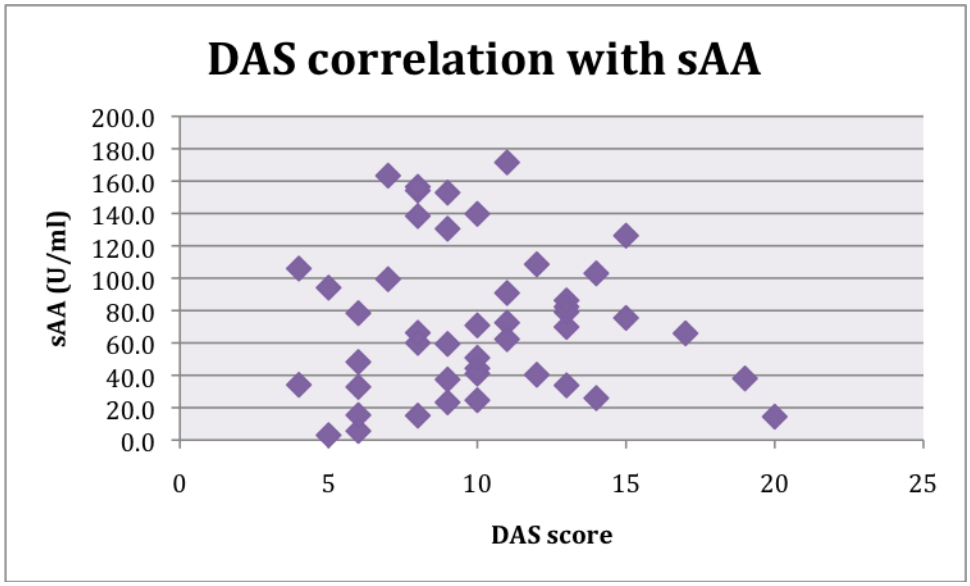

Figure 3. Scatter plot showing DAS correlation with sAA (U/ml). r= -0.045, p= 0.766

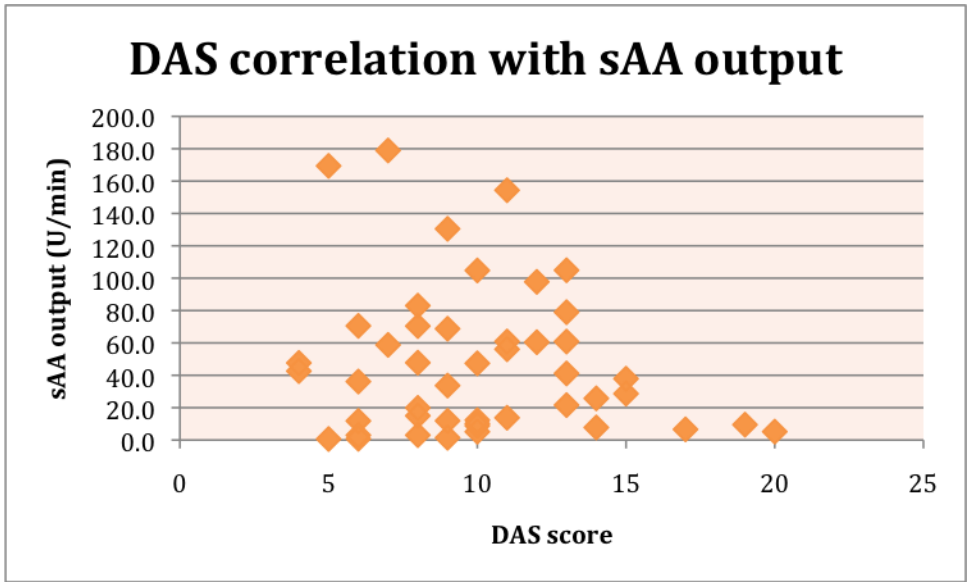

Figure 4. Scatter plot showing DAS correlation with sAA output (U/min). r= -0.162, p= 0.282

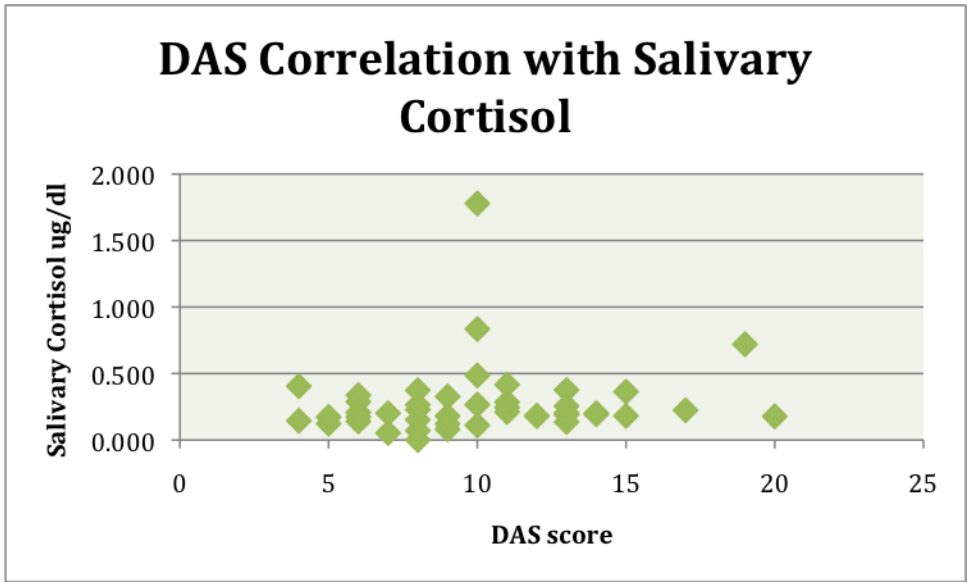

Figure 5. Scatter plot showing DAS correlation with Salivary Cortisol (ug/dl). r= 0.126, p= 0.403

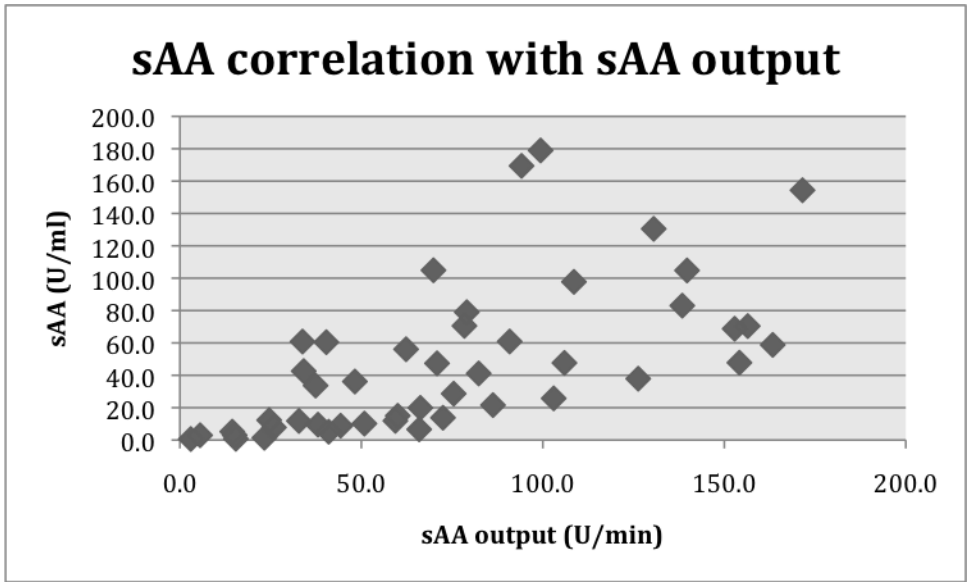

Figure 6. Scatter plot showing sAA correlation (U/ml) with sAA output (U/min). r= 0.630, p< 0.001

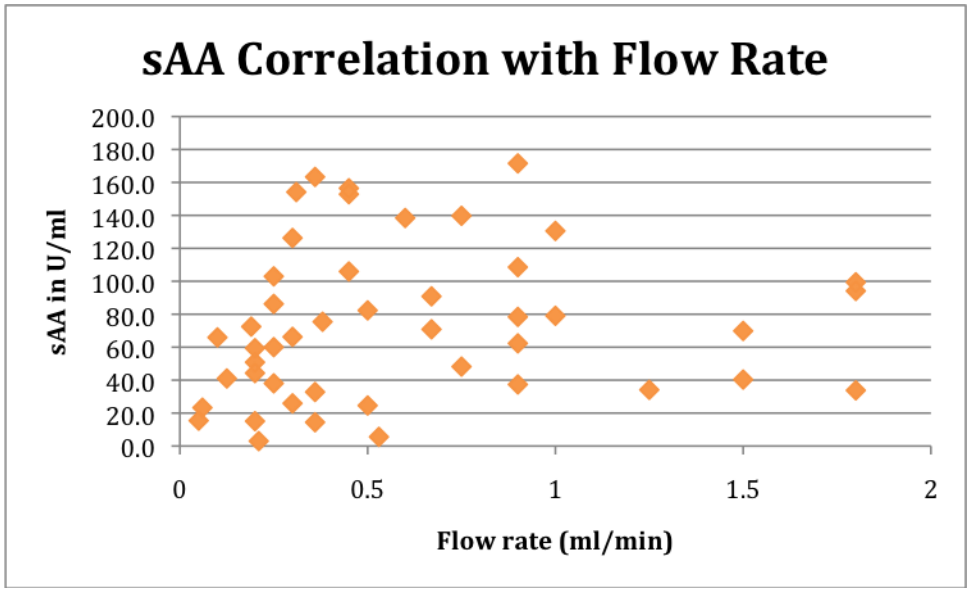

Figure 7. Scatter plot showing correlation between sAA (U/ml) and flow rate (ml/min). r= 0.126, p= 0.404

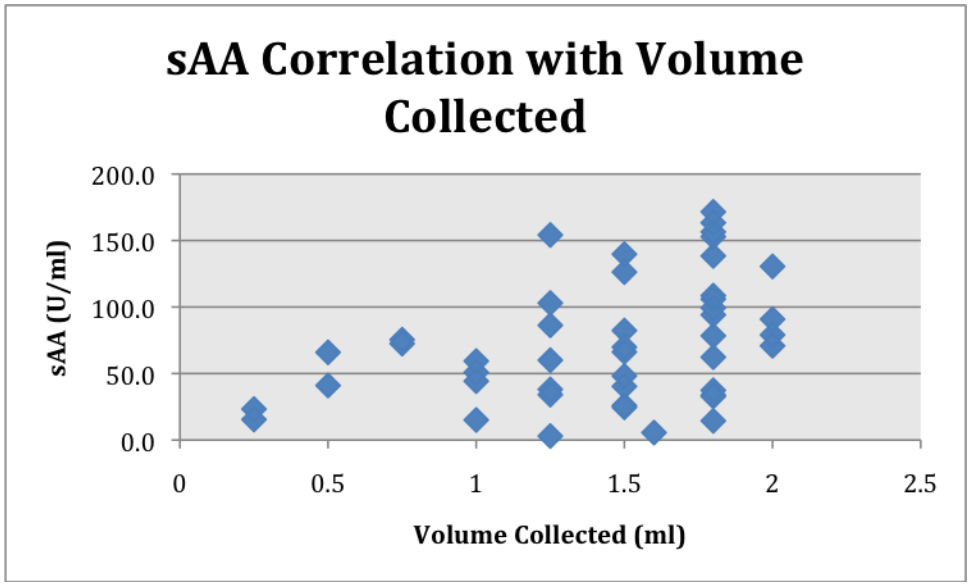

Figure 8. Scatter plot showing sAA (U/ml) correlation with volume collected (ml), r= 0.394, p= 0.007

Appendix C:

Copy of survey instruments

Sample Patient letter

Dear Patient,

Do you get anxious before going to the dentist?

Do you have pain or had a past negative dental experience?

We are conducting a study at the GPR clinic/ Tufts University School of Dental Medicine that will allow us to observe if there is any relationship between dental anxiety and the release of certain hormones (biomarkers) in the saliva. To enroll, all you need to do is respond to 2 simple questionnaires and supply us with a saliva sample. As an incentive to participation you will get a free exam and cleaning (A value of $150).

To verify that you are eligible to participate please read the following:

1) Are you between the ages of 18-75?
2) Are you in good systemic health?
3) Do you take coticosteriods, hormone replacement therapy, or oral contraceptives (women only)?
4) Are you currently being treated for anxiety or depression?
5) Are you willing to present for an appointment between the hours of 9-11am?
6) If you are a smoker, do you agree not to smoke for 2 hours before the appointment?
7) Will you agree not to eat a major meal at least 4 hours prior to the appointment?

If you are in good systemic health, and don't take any of the types of medication mentioned above, and are willing to agree to items 5,6, and 7 you will be eligible to participate. If you choose to participate in the study and your appointment was not scheduled between the hours of 9-11, please call 617-636-4067 to have it rescheduled.

All necessary paperwork will be presented to you on the day of your appointment. You will have the opportunity to ask questions regarding any portion of the study prior to responding to any questionnaires and supplying us with the saliva sample.

Please note that participating in the study is a one-time appointment. It will not affect your dental care in any way and it will commence normally after the first appointment.

Best Regards,

Research team/ GPR clinic

Date:

Code#

Basic Demographic Survey

Please check all that applies:

1) What is your age: _____

2) What is your gender:
   - Male
   - Female

3) How would you describe yourself:
   - Asian/Pacific Islander
   - African-American
   - Caucasian/White
   - Hispanic
   - Other (Please Specify): _____

4) Have you ever had a bad or traumatic experience at the dentist:
   - Yes
   - No

   If Yes: Did it include any of the following:

   - Painful Local Anesthesia Injection
   - Pain while drilling a tooth for a filling
   - Traumatic tooth extraction or surgical experience
   - Negative dentist remarks
   - Other (please specify): _____

5) Are you currently in pain:
   - Yes
   - No

Corah’s Dental Anxiety Scale, Revised (DAS-R)

Name _____ Date _____

Norman Corah's Dental Questionnaire

1. If you had to go to the dentist tomorrow for a check-up, how would you feel about it?

a. I would look forward to it as a reasonably enjoyable experience.

b. I wouldn't care one way or the other.

c. I would be a little uneasy about it.

d. I would be afraid that it would be unpleasant and painful.

e. I would be very frightened of what the dentist would do.

2. When you are waiting in the dentist's office for your turn in the chair, how do you feel?

a. Relaxed.

b. A little uneasy.

c. Tense.

d. Anxious.

e. So anxious that I sometimes break out in a sweat or almost feel physically sick.

3. When you are in the dentist's chair waiting while the dentist gets the drill ready to begin working on your teeth, how do you feel?

a. Relaxed.

b. A little uneasy.

c. Tense.

d. Anxious.

e. So anxious that I sometimes break out in a sweat or almost feel physically sick.

4. Imagine you are in the dentist's chair to have your teeth cleaned. While you are waiting and the dentist or hygienist is getting out the instruments which will be used to scrape your teeth around the gums, how do you feel?

a. Relaxed.

b. A little uneasy.

c. Tense.

d. Anxious.

e. So anxious that I sometimes break out in a sweat or almost feel physically sick.