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ORIGINAL PAPER

Evaluation of salivary pH and flow rate among exam going students of Karpaga Vinayaga Institute of Dental Sciences

Raghupathy [1], Sasirekha Balasubramanian [2], Gokulapriya Subramaniam [2], Ishwariya Krishnan [3], Maheshwari Shanmugam [2], Kalaiselvi Santhosh [1]

[1] Department of Oral Medicine and Radiology, Karpaga Vinayaga Institute of Dental Science, Chinna Kolambakam, India
[2] Department of Oral Medicine and Radiology JKKN Dental College and Hospital, Komarapalayam, India
[3] Department of Oral Pathology and Microbiology Nandha Dental College and Hospital, Erode, India

ABSTRACT

Introduction and aim. The central nervous systems control salivary pH and Flow; hence it is considered as stress biomarker. To correlate the relationships linking test performance and the cognitive along with affective aspects for the stress of the exams, flow rate and pH levels of saliva.

Material and methods. Cross-sectional research was carried out on the day of their final exam and after three months. 90 dental college students provided saliva samples for measuring pH and salivary flow rate. The saliva was collected to measure stress, anxiety, and wellbeing.

Results. Salivary flow and pH were increased in the after-exam period. This is mostly due to less threat, stress, and anxiety perception.

Conclusion. According to this study, perceived stress can affect salivary flow rate and pH levels, which can be used to gauge the degree of physiological reactions accurately, conveniently, and affordably to tests and variable realistic stresses.

Keywords. exam stress, psychological stress, salivary pH, salivary flow perception

Introduction

Stress is defined as a physiological response either good or negative.[1] which is body's reaction to any change-related demand.[2] In general, stress is divided into two categories: eustress and distress; as a result, it can either be beneficial to an individual's health, performance, and behaviour, or it may be harmful since it puts more strain on their physical, mental, and emotional resources.[3] More than 50% of students studying medicine and dentistry said they were stressed.[4] Stress is experienced differently by each person and is influenced by a few inter- and intrapersonal, intellectual, and environmental factors. It may be harmful since it puts more strain on their physical, mental, and emotional resources. More than 50% of students studying medicine and dentistry said they were stressed.[4] Stress is experienced differently by each person and is influenced by a few inter- and intrapersonal, and intellectual.

It chronologically decreases with increase in year of study, and it is more than twice as high in girls as in boys.[2] Stressful conditions and emotional reactions are responsible to affect central nervous system.[2] The immune system, the heart, and the metabolism have all been shown to be dysregulated by stress hormones.[2] Saliva is necessary for maintaining healthy teeth and gums. These effects could lead to the development of stress re-

Corresponding author: Kalaiselvi Santhosh, e-mail: dr.sks13@gmail.com

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sponse biomarkers. Due to the central nervous systems' control over saliva, salivary glands in the mouth may produce less saliva in response to stress.[5] Dry mouth is a prevalent symptom of this (xerostomia). Less bicarbonate, an alkaline chemical, is created as a result of the slower salivary secretion rate, which boosts acidity and decreases oral pH.[6] The salivary flow rate at rest, ranges from 0.29 to 0.41 ml/min.[7] Hypo-salivation can be explained by less than 0.1 ml/min, which indicates underactive salivary glands.[8] Exams are a common example of a time-limited, unfavourable naturalistic stressor that is used to study the physiological and psychological effects of stressful conditions. They might therefore serve as a good model for evaluating novel biomarkers. Based on cognition the current study linked the anxiety and stress levels on salivary pH.[9] These assessments have an impact on the coping mechanisms people employ in order to deal with stressful situations as well as the physiological and psychological (stress, test anxiety, etc.) impacts of the stressful scenario.

Aim

Here we sought in determining the crosslinking between stress, flow, and salivary pH under the pressure of year-end exams at universities.

Material and methods

The participants were 90 exam going students who were enrolled in dental program in their last year at the Karpaga Vinayaga Institute of Dental Sciences from December 2021 to March 2022. Information was acquired from those who attended the oral exam on the day of the final exam. The questionnaire and samples were collected from the participants when they arrived for the oral exam. Elimination criteria included those who were under the age of 18, patients who have not completed all the necessary examinations and tests, had a systemic ailment, and had taken medication within the previous six months. Order IEC/KIDS/012/2021, issued by Institutional Ethical Committee and it is a crucial thing to initiate the study, was issued to acquire ethical permission.

Data collection

Saliva was sampled after participants had finished the demographic questionnaire and form. The perceived stress scale (PSS) is a frequently utilized validated questionnaire for determining how stressful one feels about various life events. The survey consisted of ten items on a Likert scale with five possible answers. Greater perceived stress is correlated with higher scores. The low and high stress levels were divided on scores recorded.[10] Individuals with low levels of stress had scores below the median, whereas those with high levels of stress had scores at or above the median. At T1, an hour prior to the exam, students filled out the questionnaire and provided samples (in a two-hour fast). Students were called after three months to complete the T2 measure. To avoid eating, drinking, brushing their teeth, and smoking approximately 60 minutes within a day and these types of data sets are acquired for the flow rate assessment.[8] For standardizing the methodology and reduce circadian rhythm-related fluctuations in saliva secretion, all saliva samples were taken between 8 and 10 am.[9] Every participant was required to sit still while providing a saliva sample in a calm area. The draining technique was used to collect unstimulated saliva, which was then placed in a plastic container that had been previously weighed. After being instructed to swallow once to clear the mouth of any remaining saliva, the subjects prevented from doing again until the researcher instructed them to. The individual was instructed that a bell would sound after five minutes to tell them to stop drooling into the container. A timer was used to time the initial swallow. The subject maintained a partially open mouth with a slight inclination of the head until saliva began to accumulate, at which point the person began drooling into the container. After the collecting period was through, the container was checked once more. The weight before and after saliva collection was subtracted to get the weight of the saliva in gram.[11] Based on the classification of the quantity of saliva within the time of collection, the unstimulated flow of salivary was measured. All participants gave adequate saliva for pH tests without the need for extra water, with saliva samples ranging in size from 3 to 5 ml. As a legal digital device, the Cyber Scan pH 501 set of multifunctional sensors from Israel's El-Hamma Instruments Ltd (Kibbutz, Israel). was used.[12] The pH level was taken right away to reduce the impact of the environment on the measurement. The measurement was complete when the buzzer was heard a short while after the sensor attached to the device was dipped into the saliva collection container.[13] The pH of the saliva was then written on the survey form after being noted on the device's screen. The pH of saliva should be between 6.5 and 7.5, and it is kept at this level throughout the day (pH=7), making it equally acidic and alkaline throughout the body. While saliva with a pH below 7 is basic (alkaline), saliva with a pH over 7 is alkaline.[14,15]

Data analysis

To analyze the data, statistics were reported as frequency and percentage. Paired t-tests were done to calculate the conflicts between T1 (exam) and T 2 (post-exam). Pearson correlations were used. In terms of the multiple regression models, it is mainly utilized to make the connections between study variables and pH as well and it is performed between pH and exam performance (controlling for background and study variables). The pH

distribution was stable. Test performance Z-scores were acquired. The data were examined using SPSS, version 23.0. (IBM Corp, Armonk, NY, USA). The p value was set at 0.05 for each test.

Results

Approximately 87 participants – with a response rate of 96% – who fulfill the required inclusion and exclusion criteria, completed the three validated questionnaires, and fulfilled inclusion and exclusion criteria had their data included in the study. The features of the study population are outlined (Table 1).

Table1. Mean±SD and differences between T1 (exam) and T2 (post exam) study variables[a]

	T1	T2	t (67)
Threat appraisal	4.91±2.45	3.3±2	4.33**
Challenge appraisal	6.12±2.15	5.09±1.8	1.88
Experienced stress	5±0.8	4.32±0.38	1*
Worry	3.18±1.68	1.09±1.7	2
Emotionality	1.39±1.7	3.35±1.64	0.8
Test anxiety (total score)	3.56±1.65	1.30±1.73	1.55*
pH	5.75±1.55	6.2±1.84	-3.53**
Test performance	59.73±13.01		

[a] *p<0.06; **p<0.002

The means regarding threat (scale range 1–9) were around typical for all of the time point measures, however the means for challenge appraisal were higher. Exam-related stress was also experienced at a significant level (scale range 1–9). The scale's midpoint corresponded to the subscales and overall scores for test anxiety (scale range 1–4). The pH values were within the typical 6.5–7.5 range.

Table 2 presents the correlation of the pH levels with threat and stress along with anxiety at T1 and T2.

Table 2. The correlation of the pH levels with threat and stress along with anxiety at T1 and T2

	pH (T1) stress	pH (T2) anxiety	pH (T3) stress	pH (T4) exam
Work hours	-0.12	-0.13	-0.07	-0.02
Physical activity	0.17	0.17	0.037	0.04
Smoking	-0.24**	-0.21*	-0.28*	-0.24*
Threat appraisal (T2)	-0.11	-0.25*	-0.53***	-0.54***
Stress (T2)	-0.38***	–	-0.14*	–
Anxiety (T2)	–	0. 10	–	-0.16
Emotional stability (T2)	–	-0.32*	–	-0.03
pH (T1)	–	–	-.08	0.05
R2 (modified R2)	0.38 (0.3)	0.31 (0.25)	0.31 (0.25)	0.31 (0.27)
F (df)	9.36 (64)***	5.57 (64)***	4.30 (60)	4.19** (60)

Correlations between salivary flow rate and biochemical parameters in exam stress situation and nonstres situation are presented in Table 3 and 4.

Table 3. Correlations between salivary flow rate and biochemical parameters in exam stress situation

Parameter	Rate of flow (ml/min)	Ca^{2+} (mmol/L)	Quantity of protein (g/100ml)	Uric acid (mmol/L)	Albumin (mg/100ml)
Rate of flow (ml/min)	–				
Ca^{2+} (mmol/L)	-0.186	–			
Amounts of protein (g/100ml)	-0.260	0.121	–		
Uric acid (mmol/L)	-0.245	-0.311	-0.041		
Albumin (mg/100ml)	-0.057	0.185	-0.330	-0.081	–

Table 4. Correlations between flow rate and biochemical variables in non-stress situation

Parameter	Rate of flow (ml/min)	Ca^{2+} (mmol/L)	Amounts of protein (g/100ml)	Uric acid (mmol/L)	Albumin (mg/100ml)
Rate of flow (ml/min)	–				
Ca^{2+} (mmol/L)	-0.05	–			
Amounts of protein (g/100ml)	-0.188	0.036	–		
Uric acid (mmol/L)	0.48	0.106	0.311	–	
Albumin (mg/100ml)	-0.05	0.074	0.251	0.135	–

Discussion

The current results show that, compared to Time 1, saliva pH levels at Time 2 were higher (acidity levels were lower). A decrease in perceived danger, tension, and test anxiety explained the difference between the exam and post-exam period which was better explained in the current than the earlier studies which relates the association between stress and pH: the participants' perception of stress increases as pH decreases.[15-18] Stressful events cause pH levels, which indicate saliva acidity, to decrease because the central nervous system is activated in reaction.[2,19] Since measuring pH levels may be a reliable, useful, and practical technique to determine the strength of physiological responses to stresses, the current work adds to the body of evidence supporting this idea. The stress, as demonstrated by the parameters of perceived stress and emotional stability, was a mediator of the impact of threat has a drastic impact on physiological determination of pH, according to the cognitive approach.[20,21] These results support the hypothesis that assessments significantly affect health, mostly indirectly through the induction of physiological stress responses by emotions.[5] pH levels, powerfully predicted exam performance, attenuated the connection between stress, anxiety, and performance. These results are included in the paucity of earlier studies (such as studying connections between test performance and biomarkers.[14-22] Turner and Sugiya claim that sympathetic stimulation causes reduced saliva with increased protein concentration, parasympathetic stimulation gives increased salivary flow and decreased protein content, which may create a feeling of dryness.[19] As a defense response, the hypothalamic-pituitary-adrenal axis may become active, releasing cortisol into the sa-

liva, and increasing the amount of total protein and secretory immunoglobulin A. Increased sympathetic tone and catecholamine output and stimulation of the hypothalamic-pituitary-adrenal axis and salivary cortisol redemption are these two potential explanations of this spike.[2,4,25-27] Changes in the concentration of specific salivary ingredients have an impact on the kinetics of the mechanisms that aid in creating a new equilibrium between tooth demineralization and remineralization.[6,28,29] Recent studies have emphasized how different salivary glands create different types of saliva and how flow velocity affects the concentration of various salivary constituents.[17,18] The present study's edge over previous studies is defines that potential design along with the evaluation of performance is considered a prominent outcome variable. Researchers typically stress the requirement to utilize accurate metrics that are less likely to introduce bias into research than questionnaires.[30,31] One of the major flaws of the study is extremely small sample size, which limits the generalizability of the results. Additional pH and flow rate measurements may provide fresh information on the relationships between pH and the stress of the exam. An important notable restriction is the one-time pH readings in saliva. As pH levels vary with time mainly in reaction to various stimuli and an associated flow index, variation is present in the value of pH, and it may be predicted. In this study, all assessments were made between 8 and 10 am, at least two hours after ceasing to eat or drink, and without smoking. However, it is possible that multiple assessments made on a day or on some other days in a row could provide a better control for pH variable effects.[10,32]

Conclusion

The final test of this study illustrates how stress affects numerous important salivary components. The results suggest that acute psychological stress affects salivary pH and composition. Furthermore, each group experienced a shift in salivary pH towards acidity which in turn raises the possibility of caries and related problems. This highlights the significance of saliva as most important dynamic biological fluid which helps in preserving the process for oral health.

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Declarations

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Authors have clearly stated that they do not have any commercial interest and financial interest. The research costs were easily covered by the researchers.

Author contributions

Conceptualization, L.P.R and S.B.; Methodology, L.P.R, S.B., G.S., I.K S.M and K.S.; Validation, I.K. and K.S.; Resources, G.S and S.M.; Writing – Original Draft Preparation, L.P.R, S.B and G.S ; Writing—Review & Editing, I.K, S.M and K.S.; Supervision, L.P.R., S.B. and K.S.; Project Administration, L.P.R. and K.S.

Conflicts of interest

All authors clearly stated that they do not have any conflicts of interests.

Data availability

Usually, the sets of data are created during and/or analyzed throughout the entire study and are available from the corresponding author on reasonable request.

Ethics approval

The ethical approval was acquired from institutional ethical committee of Karpaga Vinayaga Institute of Dental Sciences KIDS/012/2021/II.

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