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Saliva pH as a biomarker of exam stress and a predictor of exam performance

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ABSTRACT

Background: Salivary pH is regulated by the sympathetic and parasympathetic nervous system; therefore, it may serve as a biomarker of stress.
Aims: To assess the associations between the cognitive and emotional dimensions of exam stress and pH levels, and the predictability of salivary pH in relation to test performance.
Methods: A prospective study. Eighty-three nursing students answered a questionnaire on stress appraisals, experienced stress, test anxiety (including worry and emotionality subscales) and health behaviors, and gave a saliva sample for measuring pH on the morning of their first term exam and three months later. Their performance on the test (grades) was also recorded.
Results: Levels of pH in saliva were higher (levels of acidity were lower) in the post exam compared to the exam period, in parallel to lower threat appraisal, experienced stress, and test anxiety levels post exam. Controlling for smoking, physical activity and working hours per week, pH levels at both time points were predicted by appraised threat regarding the exam situation, experienced stress, and the emotionality dimension of test anxiety. pH at Time 1 predicted performance on the exams and mediated the associations of experienced stress and emotionality subscale with test performance.
Conclusions: the present study indicates that pH levels may serve as a reliable, accessible and inexpensive means by which to assess the degree of physiological reactions to exams and other naturalistic stressors.

Introduction

Stress is an inherent part of human life and penetrates almost every human experience [1]. Stressful encounters, as well as one's psychological reactions to them, activate the hypothalamic–pituitary–adrenocortical (HPA) axis and the sympathetic nervous system, resulting in the secretion of excessive levels of stress hormones, mainly cortisol and catecholamines [2]. Stress hormones may lead to the dysregulation of bodily functions, including the immune, cardiovascular and metabolic functions [2].

The outcomes of these effects may be measured as stress response biomarkers [3,4]. A wide array of stress biomarkers has been proposed and empirically assessed to different extents (e.g., cortisol natural killer activity, *in-vivo* or *in-vitro* levels of pro- or anti-inflammatory cytokines [2,4,5]. The development of new stress biomarkers was recently recognized as an important aim in stress research [3,6]. The use of salivary biomarkers, in particular, has gained increased interest over the past few years [6], due to the richness of soluble proteins and enzymes in saliva, and the relative easiness of collecting samples [7,8].

Saliva is secreted from the three major salivary glands and from the minor salivary glands in the oral mucosa [9]. It consists of 99.5% water and 0.5% electrolytes, glycoproteins, enzymes and secretory antibodies, such as secretory immunoglobulin A (sIgA). These components perform multiple roles in the process of digestion, oral health, and initial protection against bacterial or viral factors [9]. Salivary secretion is regulated by a reflex arc that consists of afferent receptors and nerves carrying impulses that are induced by the actions of gustation and mastication, salivation center, and an efferent part that consists of parasympathetic and sympathetic autonomic nerves [9]. Therefore, the secretion of saliva, as well as its composition and functions, are controlled by the sympathetic and parasympathetic autonomic nervous systems. It has been suggested that the parasympathetic nerves mainly regulate fluid secretion, while the sympathetic nerves mainly regulate salivary protein secretion. However, more recent findings suggest that the two systems work together to evoke salivary secretions comprised of both fluid and protein [10].

The most frequently-studied saliva biomarkers are levels of cortisol [7,11], sIgA [12,13] and alpha-amylase [8,14]. Recently, the pH level in saliva has been suggested as a possible useful non-expensive biomarker indicating psychological stress levels [15]. Saliva's pH represents its

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degree of acidity, whose balance (pH = 7) is needed for the optimal functioning of its various components [9]. The regulation of saliva volume and its composition by the sympathetic and parasympathetic nervous systems under stress may lead to a lower rate of secretion from the saliva glands in the mouth, often expressed by dry mouth (xerostomia) in reaction to stress [16]. This lowered rate of saliva secretion leads to a decrease in the bicarbonate secreted in saliva (alkaline), which results in increased acidity and a decrease in oral pH [9]. Indeed, salivary flow rate was found to be significantly lower during stressful periods [16].

A decrease in oral pH may be responsible for the dysregulation of other saliva biomarkers of stress, due to its effect on dysregulation of saliva components such as cortisol [17], sIgA [18] and alpha-amylase [18]. Therefore, pH may be an antecedent of stress-induced dysregulation in levels of components of the saliva previously measured as biomarkers of stress. In addition, saliva pH was found to reliably reflect skin or urinary pH [19].

An extensive search of databases revealed that only three prior studies have assessed the associations of psychological stress and pH levels [15,19,20]. Sandin and Chorot [19] examined changes in levels of skin, salivary, and urinary pH in reaction to academic oral examination stress in 32 women. During the stress situation, statistically significant reductions in the skin, salivary, and urinary pH levels were produced, and these were associated with anxiety levels. Morse et al. [20] showed that practicing meditation reduced anxiety significantly, increased salivary volume, and raised salivary pH. Recently, pH levels were examined among spouses caring for cancer patients and age-matched individuals in the community. Lower levels of pH saliva were found among caregivers of cancer patients than in the comparison group. Controlling for background variables, being a caregiver of a cancer patient, experiencing depression, and having a lower level of perceived mastery predicted lower pH levels [14].

Therefore, the use of salivary pH as a stress biomarker may constitute an answer to the need to establish non-invasive and feasible methods with which to measure the physiological indicators of stress [3,6]. However, this subject should be studied further, preferably using established models of human stress, such as academic exams.

Academic exams are an example of naturalistic stressors, which are time-limited and typically perceived as aversive, and are often studied as a model of psychological and physiological reactions to stressful encounters. Therefore, they may be a useful model for assessing novel biomarkers. Based on cognitive theories of stress (e.g., [21]), the present study assessed the effects of cognitive appraisals of the stress inherent in the exam situation, test anxiety, and levels of experienced stress on saliva's pH levels. According to the cognitive approach, stressful encounters, such as academic exams, are subjected to cognitive processing in which individuals appraise the threat or challenge embodied in the stressful situation, the coping resources available to cope with it [21]. These appraisals affect the coping strategies individuals employ in response to the stress situation, and shape the psychological (e.g., experienced stress, test anxiety) and physiological outcomes of the stress encounter.

Test anxiety consists of the specific cognitive, emotional and physiological reactions evoked by the stimuli of testing, and includes cognitive aspects (i.e., *worry*) and emotional and physiological arousal (i.e., *emotionality*) components [22–24].

Previous studies reported that academic exams are related to neuroendocrine and immune alterations [15,25,26]. In addition, studies found that heightened physiological stress levels, as measured by biomarkers, are related to lower performance on exams [24, 27,28], suggesting that heightened physiological stress might influence cognitive/emotional processes related to performance. Only one study described above [19] researched the effect of exams on salivary pH and found a decrease in pH in relation to exam stress.

Physiological responses to stress may be buffered by health behaviors and health status [2,29,30]. Low physical activity, cigarette smoking, or poorer nutrition were found to be related to the degree of activation of the HPA and sympathetic nervous system with consequent effects on stress hormones, immune parameters, as well as saliva biomarkers [2, 29–31]. For example, saliva pH was lower in smokers than in non-smokers [31].

The aim of the present study was to determine whether salivary pH can be a biomarker of stress, using stressful exams as a model for naturalistic stressors. More specifically, based on the cognitive model of stress and coping, the study attempted to determine 1. The relations between challenge and threat appraisals, experienced stress and test anxiety and levels of pH; 2. The predictive value of pH on exam performance; and 3. Whether levels of salivary pH change from exam measurement to the 3 months post-exam measurement.

The main hypotheses were as follows:

Hypothesis 1. a) levels of threat appraisal, experienced stress and test anxiety will be lower and b) challenge appraisal and c) pH levels will be higher at Time 2 (post exam) compared to Time 1 (exam).

Hypothesis 2. At both time points, a) threat appraisal, experienced stress and test anxiety scores will be negatively associated with pH levels, while b) challenge appraisal will be positively associated with pH levels, c) experienced stress and test anxiety will mediate the effect of threat appraisals on pH.

Hypothesis 3. a) pH levels at Time 1 will be negatively associated with exam performance (exam grades) and will predict performance on the examination. b) pH levels at Time 1 will mediate the effects of experienced stress and test anxiety on exam performance.

Method

Participants

The study was approved by the university ethics board. Participants included 83 students from first and second-year nursing studies for a Bachelor degree in a Northern Israeli college. The students who participated in the study did final exams (T1) in two major courses: microbiology in the first year and pharmacology in the second year. These were the students' first term semester exams (13 exams in total for the first-year students and 8 exams in total for the second-year students); the mandatory minimum passing grade was 60 and 65 (out of 100), respectively. Therefore, these exams were perceived as very difficult and stressful. A total of 121 students (71 from first-year studies and 50 from second-year studies) were asked to participate. The research assistant explained the experiment and assured students that participation would be on a voluntary basis and that confidentiality would be preserved. Eighty-three students (49 and 34, respectively) agreed and signed an informed consent form. At T1, one hour before the beginning of the exam, the participants answered the questionnaires and gave saliva samples (after a 2-hour fast) before beginning the exam. All participants were then approached three months later, during a non-exam period, for the T2 measure. An SMS message was sent to all students who had participated in T1, inviting them to participate in the T2 measure. The data was collected in college about an hour before the beginning of the study day, in the morning under fasting conditions. Sixty-eight students agreed to participate in the T2 part of the study. These students were asked to complete the questionnaire and give a saliva sample. As in T1, the data collection took about 15 min for each student. Therefore, the participation rates were 68.6% and 81.9%, respectively. T2 participants and those that dropped out were not different in background characteristics.

Measures

Saliva pH level represents the level of acidity in the saliva. A saliva specimen given by participants prior to the interview was deposited