Salivary Biomarkers Associated with Academic Assessment Stress Among Dental Undergraduates

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Abstract: This study aims to assess the difference in self-perceived stress before and after an academic assessment and its association with the marks scored and to study the association of salivary cortisol, Immunoglobulin A (IgA), and chromogranin A (CgA) with stress levels before and after the assessment. Thirty-one third-year dental undergraduates indicated how stressed they felt on a 5-point rating scale just prior to and immediately after a one-hour written test. Five-minute unstimulated whole saliva samples were also collected at those times to measure salivary cortisol, IgA, and CgA. Students rated significantly higher stress scores before (median [inter quartile range]: 3[1]), compared to after the test (2[2]) (p=0.015). Higher cortisol levels (mean: 6.32 nmol/l) were observed before than after the exam (mean: 5.16 nmol/l) (p=0.015). No significant differences were noted between the pre- and post-test saliva samples for salivary IgA and CgA levels. A negative correlation was seen between post-test stress scores and test marks (Spearman’s r = -0.325, p=0.07). Dental students perceived a higher level of stress prior to the test, which declined after that. Pre-test stress scores were associated with raised salivary cortisol, but not IgA or CgA. Students who perceived higher levels of stress after the test generally had poorer results.

Examinations are the bane of students. However, they have an important role in evaluating students’ learning outcomes and their mastery of a subject. Passing or failing usually has strong consequences for one’s career development, so examinations cause psychological stress for most students.1 Academic examinations have been considered as one of the most acute stresses experienced by students.2,7

Acute stress has been reported to increase the activity of the hypothalamus-pituitary adrenal (HPA) axis with subsequent rise in cortisol level.4 Mucosal immunity, as reflected by salivary immunoglobulin A (IgA) levels,9 is also influenced by psychological stress. With chronic stress, the production of immunoglobulin is suppressed, whereas acute psychological challenge increases IgA levels.10 Recently, Nakane et al.11 and Kanon et al.12 demonstrated that salivary chromogranin A (CgA) can be a quantitative index for monitoring the activity of the sympathetic nervous system. This constitutes the initial alarm reaction phase in stress response.

Previous studies have utilized oral presentations as acute stress situations to study the stress paradigm.3,5,7 The intensity and nature of the stress encountered in an oral presentation might be different from the stress in a written examination. This study of dental undergraduate students investigated the use of salivary cortisol, IgA, and CgA to assess stress before and after a written term test. Other objectives of the study were to assess if there was a difference in self-perceived stress before and after a test and to study the association of self-perceived stress with performance on the test.

Materials and Methods

This cross-sectional study was conducted at the Faculty of Dentistry, National University of Singapore during a written term test in pediatric dentistry. This test is part of the continuous assessment process that constitutes a small proportion of the students’ final examination marks. Students were re-
quired to answer two essay-type questions in one hour. Among the topics covered were traumatic injuries to anterior teeth in children and management of problems in the developing occlusion. The test scripts were all graded by the same staff member. Ethical approval for the study was obtained from the Institutional Review Board of National University Hospital, and informed consent was obtained from each student. Thirty-one of thirty-two (96.9 percent) third-year undergraduate dental students participated in the study. One student was on sick leave. There were twenty-one males and ten females, aged twenty-one to twenty-three years (Mean ± SD: 22.7 ± 1.0).

Students were asked to indicate how stressed they felt on a 5-point scale prior to entry for the written test at 11:00 a.m. and again just after they completed the examination one hour later. The responses for the question were as follows: 1 = not stressed at all, 2 = a little stressed, 3 = moderately stressed, 4 = quite stressed, and 5 = extremely stressed.

In addition, students were requested in advance not to eat or drink except for water one hour before saliva collection to minimize possible food debris and stimulation of salivation. Because this study was part of a prospective study on salivary biomarkers and major life events of dental students, students were familiar with the saliva collection method. Thus, sample collection should not have imposed any additional stress on the students.

A five-minute unstimulated whole saliva sample was collected immediately before and after the test. The volume of the saliva was measured and divided by five minutes to obtain the salivary flow rate, expressed in ml/min. After the collection, the samples were kept in an icebox and immediately transported to the laboratory, where they were stored at -70˚C until analysis.

Salivary IgA (µg/ml) and cortisol concentrations (nmol/ml) were determined using the Salimetrics HS-IgA Kit and HS-Cort Kit, respectively. Both kits were manufactured by Salimetrics LLC, USA. The concentration of CgA (pmol/ml) was measured using the YK070: Chromogranin A (Human) EIA kit (Yanaihara Institute Inc., Japan). The Bio-Rad Protein assay kit was used to determine the protein concentration (mg/ml) in the saliva samples. Salivary IgA secretion rate (µg/min) was computed by multiplying the IgA concentration with salivary flow rate. Because salivary cortisol is not affected by salivary flow rate, only salivary concentrations were reported. CgA levels were corrected by protein concentration and expressed as pmol/mg protein.

Data analysis was performed using SPSS 11.0 for Windows. Paired sample t-tests and, where appropriate, non-parametric tests were performed. All p values and confidence intervals are two-sided, and the level of statistical significance was set at p<0.05.

Results

The median and inter-quartile ranges of stress scores before the test were 3 and 1 respectively. After the test, the median score was 2, and the inter-quartile range was 1. The differences were statistically significant (p=0.015).

The mean cortisol level before the test was 6.32 nmol/l. This was significantly higher than the mean value of 5.16 nmol/l after the test (p=0.015). Mean levels for IgA secretion rate and CgA concentration per total protein were generally higher after than before the test. However, the differences were not statistically significant (Table 1).

Generally, stress scores were negatively correlated with test marks. The inverse relationship was stronger between the post-test self-perceived stress and test marks (Spearman’s r = -0.325, p=0.07). There

<table>
<thead>
<tr>
<th>Salivary Biomarkers</th>
<th>Before</th>
<th>After</th>
<th>Before-After 95% CI*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisol</strong> — Concentration (nmol/ml)</td>
<td>6.32</td>
<td>5.16</td>
<td>1.01 to 1.50</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>IgA</strong> — Secretion rate (µg/min)</td>
<td>107.6</td>
<td>119.5</td>
<td>-32.6 to 8.9</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>CgA</strong> — Concentration (pmol/mg protein)</td>
<td>0.43</td>
<td>0.45</td>
<td>-0.12 to 0.07</td>
<td>0.69</td>
</tr>
</tbody>
</table>

*Before-After 95% CI refers to the 95% Confidence Interval of difference between before and after test readings.
was a poor relationship between pre-examination perceived stress and examination marks (Spearman’s r = -0.135, p=0.47).

Discussion

The dental undergraduates perceived a higher level of stress before than after a written test. This finding is similar to that of other studies. The perceived stress of an examination/test may be influenced by the anticipation of difficult examination questions, feelings, or beliefs regarding mastery in the subject, relative importance of the exam, and other factors.

No correlation was observed between the self-perceived stress scores and any of the salivary biomarkers (data not shown). A possible explanation is that the single question used to indicate self-perceived stress was perhaps too crude a measure to assess stress. Respondents may underreport or exaggerate the nature of their stress. While an in-depth questionnaire may be useful, it could be argued that this may impose additional stress on the students.

Cortisol is a major glucocorticoid in humans that reflects adrenocortical activity. Activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent release of cortisol are major components of the physiological stress response. Salivary cortisol accurately reflects serum cortisol, the physiologically active component.

Salivary cortisol levels were noted to be higher prior to the written test. Anticipation of the written examination is a stressor. Studies have reported increased cortisol levels during anticipation of stressful experiences such as oral academic examinations, cardiac surgery, and dental treatment procedures. The psychological stress can increase the activity of the HPA with subsequent rise in cortisol level, including salivary cortisol.

The concentration and secretion rate of secretory Immunoglobulin A in saliva are good indicators of the functional status of the mucosal immune system. Recent studies on acute stressors such as oral presentations, public speaking, and active mental stress tasks showed significant differences in salivary IgA before and after performing the tasks. However, no significant difference in salivary IgA secretion rates before and after the test was observed in this study.

It should be noted that the grades for the midterm written test in this instance constitute only a small proportion of the total marks for the subject. It could therefore be considered a relatively mild stressor in comparison to the final examination faced by the students. We are aware that the final examination would be a stronger stressor, which could result in more significant findings. However, as the final examination is a very stressful event to students, many of the students might not wish to participate in the study, possibly resulting in a poorer response rate.

A study conducted by Lowe et al. on ten final-year students also showed no significant change in the salivary IgA levels before and after a written examination. In contrast, for the same group of students, significantly higher levels of salivary IgA were detected after a thirty-minute oral presentation on their own academic research project, in front of the whole class and tutors/assessors. As different types of examinations may induce different intensities of stress to individuals, changes in salivary IgA may be sensitive to the intensity of the stressor.

Chromogranin A (CgA) is a 48 kilodalton acidic glucoprotein that is stored and co-released by exocytosis with catecholamines from the adrenal medulla and sympathetic nerve endings. Its levels are correlated with catecholamine release.

There was no marked difference found in CgA before and after the test, contrary to the findings of Nakane et al. who observed a significant elevation in salivary CgA immediately before an oral presentation in a group of male volunteers. Dimsdale et al. suggested that within the normal physiological range, which includes situations of mild mental stress, CgA is stable and slow to respond. This could partially explain the lack of an elevated response in this study, as the midterm test presumably would have a mild to moderate impact on the stress level of the students.

An interesting finding was that perception of higher levels of stress after the examination was marginally associated with poorer marks for that exam. This may be due to the awareness of a poor performance by the student, resulting in an increase in perceived stress afterwards.

However, both animal and human studies have indicated that stress and glucocorticoids may impair memory and cognitive function. These salivary biomarkers have the potential of being assessment tools to identify those students who are consistently stressed during examinations and who may perform poorly because of inability to cope with stress. Such students may benefit from stress awareness education and learning of effective coping strategies dur-
ing the preparation for examinations. However, the question of how successful such interventions may be has yet to be answered.

Conclusions

Dental students perceived a level of stress that was higher before than after the academic assessment. This perception was associated with raised pretest salivary cortisol levels but not IgA or CgA. These findings may suggest that salivary cortisol could be considered as an indicator for assessing anticipatory stress. Students who perceived higher levels of stress after the test generally had poorer results.

Acknowledgments

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REFERENCES