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The influence of academic stress on gingival inflammation

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Abstract: *Objective:* The aim of the present study was to investigate the effects of academic stress on periodontal health, in relation to inflammatory markers in gingival crevicular fluid (GCF) and cortisol in saliva. *Materials and methods:* The study included 20 healthy dental hygienists (females: mean age 29.3 ± 8.5 SD) and was conducted during a major exam period and 4 weeks later after the exams. A clinical examination was performed and GCF was collected from four sites in each subject on these two occasions. Interleukin (IL)-1β, IL-4, IL-6, IL-10 levels were determined using Luminex 100 and cortisol amounts by radioimmunoassay (RIA ^{125}I). Students registered their perceived stress on a visual analogue scale (VAS). Significance of the findings was determined using paired *t*-test, Wilcoxon-matched pair and Spearman's rank correlations. *Results:* Students had higher amounts of dental plaque ($P < 0.007$) and gingival inflammation ($P < 0.001$) during the exam period compared with after the exams. The amounts of IL-6 and IL-10 in GCF were significantly increased during the time of examinations. The median level of cortisol in saliva was also significantly raised during the exam period compared with after the exams, 20.52 nmol/l (range: 11.91–27.34) and 16.41 nmol/l (range: 10.91–24.17) respectively, $P < 001$. The results from the VAS registration revealed a significant difference ($P < 001$) between the two occasions. *Conclusion:* Academic stress appears to affect periodontal health, shown by more plaque accumulation, gingival inflammation and increased amounts of IL-6, IL-10 in GCF and cortisol in saliva.

Key words: academic stress; cortisol; dental plaque; inflammatory markers; periodontal diseases

Introduction

Stress is an ambiguous concept, but in research terms it usually refers to physiological and psychological reactions that mobilize

an organism's defence against external or internal threats to stressors. Different kinds of stress have been defined, such as work related, negative life experiences and socioeconomic status (1–3). An acute stress response causes no physiological problems, as long as the stress can be shut down when the reason for the stress activation terminates. When stress activation is prolonged, such problems may occur. The stress reaction includes activation of the hypothalamus-pituitary-adrenal cortex (HPA) axis, with release of corticotropin-releasing hormone from the hypothalamus, and glucocorticoids, including cortisol, from the adrenal cortex (4). To study the function of the HPA-axis during stress, several techniques have been used to measure cortisol levels in blood, saliva or gingival crevicular fluid (GCF) (5–7).

Over the past decade, it has become more apparent that stress can negatively influence the oral health status, which can lead to increased amounts of dental plaque, gingival inflammation and more severe periodontitis (8, 9). Some studies have shown that the levels of pro-inflammatory cytokines, interleukin (IL)-1β and IL-6, are increased in patients with depression (7, 9, 10); however, contradictory results have also been described (11).

An academic exam can be considered a psychological stressor, consisting of a period of preparation, anticipation and then the exam itself. Furthermore, an academic exam plays an important role in evaluating student learning outcomes and their mastery of a subject, and has as such become one of the most acute stressors experienced by students. Recent studies have reported high levels of anxiety among dental and medical students (12, 13). Students who participated in a major exam had significantly more dental plaque, and more gingival inflammation (14) compared with students who did not participate in any exam. Furthermore, academic stress has been associated with increased levels of IL-1β, IL-6 and IL-10 in serum and GCF (15–17), but the data regarding IL-1β alterations have been inconsistent (18, 19). Additional reports have also stated that academic stress increases the production of cortisol in saliva before an exam (20); however, other studies found no differences (21).

The aim of the present study was to investigate the effects of academic stress on periodontal health in relation to inflammatory markers in GCF and cortisol in saliva.

Materials and methods

Twenty-two female students in their 3rd year of the Dental Hygienist Program, at Karolinska Institutet, Huddinge in Sweden were recruited for the study. Students were recruited during a seminar, and the research plan and aims were presented to the class by one of the authors, inviting the students to participate in the study. Throughout the recruitment process and research, it was emphasized that participation in the study was voluntary and each student was free to withdraw at any time. Exclusion criteria for the study were self-reported psychiatric disorders, use of psychotropic medication, pregnancy, use of oral contraceptives or oestrogens, and current dental or orthodontic treatments. One student was excluded due to pregnancy and one student did not attend the clinical examination. The remaining students were in good general health as assessed by a health questionnaire. None had received antibiotics during the previous 3 months. The final student group comprised 20 subjects (mean age 29.3 ± 8.5 SD). The students registered their perceived stress on a visual analogue scale (VAS; 100 mm), ranging from no stress at all, to the worse stress imagined. The VAS is a simple-to-administer, reliable and a valid measurement tool for evaluating discomfort such as dental pain and stress (22, 23).

Ethics

This study was approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden. The subjects gave their informed consent to participate in the study.

Procedure

The students participated in a major exam period for 3 weeks, during which they took two written exams (3 h each) and three oral exams (30 min each). All participants underwent a clinical examination and the collection of GCF and saliva was performed at the beginning of the exam and after the exam period 4 weeks later. The participants continued with their usual adjuncts to oral hygiene, such as flossing.

Saliva collection procedures

Saliva samples were taken in the classroom, at the beginning of a lecture. All samples were collected between 8.30 and 9.00 a.m. Furthermore, in advance the students were requested not to eat or drink except water, 1 h before saliva collection minimizing possible food debris. To reduce the risk of blood contamination, tooth brushing was not allowed during the 60 min preceding saliva collection. During collection, the subjects were instructed to swallow, first prior to saliva sampling and then to spit all saliva produced during a 5 min period into a test tube. The samples were immediately centrifuged at 8000 *g* for 5 min and the supernatants frozen at −70°C pending analysis.

Clinical examination

Clinical examination included assessment of dental plaque (plaque/no plaque), six sites: mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual and mid-lingual, gingival index (24), and the number of remaining teeth, excluding third molars. Probing pocket depth (PPD) and clinical attachment level (CAL) were measured to the nearest millimetre, using a standard probe (Hu-Friedy, Chicago, JL, USA) graded at 2 mm intervals and with a tip diameter of 0.5 mm. All teeth were probed at six sites: mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual and mid-lingual. CAL was measured with a probe from the cemento-enamel junction. Bleeding on probing (BOP) was assessed by probing intracrevicularly, using a probe with a tip diameter of 0.5 mm (Hu-Friedy). Bleeding within 60 s was recorded as 'BOP'. One examiner (NB) performed all measurements.

Gingival crevicular fluid sampling

Gingival crevicular fluid was collected distobuccally at the first molar in each quadrant. Prior to GCF sampling, the respective tooth was dried by isolation with a cotton roll and a gentle air stream for 5 s. A paper strip (Periopaper; Oraflow Inc., Planiview, NY, USA) was inserted 1 mm into the gingival crevice until resistance was felt, and this was kept in place for 30 s. The absorbed fluid volume was determined using a Periotron^{TM} 8000 meter (Oraflow Inc) (25). Immediately after collection, the white section of the Periopaper was removed and the four quadrant samples were pooled in 1 ml of phosphate buffered saline. The samples were evaluated with algorithm for 30 min, centrifuged (8000 *g*) for 5 min and the supernatants frozen at −70°C pending analysis. One examiner (AJ) performed all sampling.

Assays

The IL-1β, IL-4, IL-6 and IL-10 levels were determined by Multiplex bead analysis using a Luminex 100 (Luminex Corp., Austin, TX, USA) and commercial immunoassays (Lincoplex, High sensitivity human cytokine panel; Linco Research Inc., St Charles, MO, USA) (26). Levels of the cytokines were determined as total amount per site (pg/site).

Cortisol in saliva

Cortisol was measured using sensitive radioimmunoassay (RIA Kit Orion Diagnostica AB, Espoo, Finland) (5), according to the manufacturer's instructions and the levels of cortisol were determined as concentration (nmol/l).

Statistical analysis

The clinical data were displayed as mean and standard deviation, whereas the biochemical markers and VAS data were displayed as median and quartile range. A comparison between the exam time and the non-exam period, regarding the clinical data (full-mouth and site assessments), was made by means of paired *t*-test. Differences between data sets with a probability of <0.05 were regarded as significant. The biochemical markers and VAS data were analysed with a Wilcoxon-matched pair test. Spearman's rank correlations were used to determine association between cortisol and VAS. Analyses were performed using the software package Statistica (StatSoft, Inc., Scandinavia, Uppsala, Sweden 2005) version 7.1.

Results

The mean age of the 20 students were 29.3 (±8.5), 15 were non-smokers and five smokers, who had smoked an average of 12 cigarettes per day, over a 13-year period. The clinical measurements including dental plaque, gingival inflammation, BOP and PPD differed significantly ($P < 0.001$) between the exam and non-exam period (Table 1). There were no

Table 1. **Mean (SD) of dental plaque, gingival inflammation, bleeding on probing, pocket depth, clinical attachment level and number of teeth in 20 female students**

	Exam period (A)	After exam period (B)	
Parameters	Mean (±SD)	Mean (±SD)	*P*-value
Dental plaque (%)	25.8 (14.1)	10.1 (5.6)	0.001*
Dental plaque, site (%)	45.5 (18.9)	37.0 (15.1)	0.007*
Gingival inflammation	0.6 (0.3)	0.3 (0.2)	0.001*
Gingival inflammation, site	0.85 (0.5)	0.63 (0.4)	0.001*
Bleeding on probing (%)	26.3 (11.8)	11.5 (7.8)	0.001*
Bleeding on probing, site (%)	53.7 (12.7)	38.3 (16.2)	0.001*
Probing depth (mm)	1.87 (0.33)	1.76 (0.33)	0.001*
Probing depth, site (mm)	2.12 (0.38)	2.09 (0.37)	NS
Clinical attachment level (mm)	1.58 (0.19)	NR	
Number of teeth	27.4 (1.3)	NR	

'Site' indicates clinical condition at sites sampled for gingival crevicular fluid.
NS, non-significant; NR, not registrated.
*Significant between the exam period (A) and after exam period (B).

differences in any of the clinical parameters between smokers and non-smokers (no data shown).

The sites sampled for GCF showed higher amount of dental plaque, gingival inflammation and BOP during the exam period compared with after the exams (Table 1). The mean GCF volumes during and after the exams were 116.49 (±31.1) and 90.12 (±21.1) respectively, $P < 0.001$. The amounts of IL-6 and IL-10 were significantly higher ($P < 0.05$) during the exam period than after the exams (Table 2), while the amount of IL-1β did not differ between the two periods. IL-4 was not detectable in any of the samples.

The median level of cortisol in saliva was significantly increased during the exam period compared with after the exams; 20.52 nmol/l (range: 11.91–27.34) and 16.41 nmol/l (range: 10.91–24.17) respectively, $P < 0.001$ (Table 2). The results from the VAS score showed a significantly higher number during the exam period than after the exams, $P < 0.001$ (Table 2), and a correlation between cortisol and VAS during the exam period ($r_s = -0.48$, $P < 0.036$) was observed.

Discussion

In the present study, students were found to have more plaque accumulation and gingival inflammation during an exam period, suggesting that academic stress might influence periodontal health. These results confirm the findings of Deinzer *et al.* (14), who found increased dental plaque and gingival inflammation in students who experienced academic stress. The increased levels of dental plaque and gingival inflammation in the present study may be explained by behavioural changes in the stressed students, for example, oral hygiene might be less effective and/or reduced in frequency during this time of stress. After the exam period, a reduced amount of dental plaque was found and this may partly be explained by the Hawthorne effect (27), meaning that panelists involved in clinical trials might be affected because of attention and interest. Regarding gingival inflammation, two possible explanations might be considered. The increase could be explained either by the direct influence of stress on the immune system, through release of stress hormones or by an influence of plaque accumulation leading to gingival inflammation, both resulting in increased susceptibility to periodontal diseases.

In the present study, the students revealed significantly higher levels of cortisol during the exam period compared with after the exams. Increased levels of cortisol in saliva have also been observed in a study of dental students before an exam period compared with afterwards (28). Repeated exposure to stress, such as that in exam periods, may result in the overactive release of glucocorticoids, including cortisol from the adrenal cortex. In the study by Ng *et al.* (28), the students were asked how stressed they felt on a 5-point scale prior to and after the exam. The response alternatives to the questions were as follows; not stressed at all (1), a little stressed (2), moderately stressed (3), quite stressed (4) and extremely stressed (5). The median stress scores before the test was 3 and after the test 2 ($P < 0.001$). This is consistent with our study where the VAS scores were significantly higher during the exam period compared with after the exams.

In the present study, we also assessed GCF levels of two inflammatory related cytokines, IL-1β and IL-6. The level of IL-6 was significantly higher during the exam period compared with after the exams. Similar levels have also been found in depressed patients in earlier studies (7, 9). It is known that IL-6 stimulates the HPA-axis activity (29), and is associated with activation of the immune system and with inflammatory response. In comparison, the amount of IL-1β in our study showed no significant differences between the two time periods. Contradictory results have been reported from Deinzer *et al.* (30), who found higher amounts of IL-1β in GCF during stress and Marques-Deak *et al.* (31) who reported similarities

Table 2. **Median (quartile range) for IL-1β, IL-6 and IL-10 in gingival crevilcular fluid, cortisol in saliva and visual analogue scale (VAS) during exam period (A) and no exam period (B) in 20 female students**

	Unit	Median	Minimum	Maximum	Lower quartile	Upper quartile	*P*-value
IL-1β A	pg/site	2.455	0.00	56.57	1.225	5.925	0.5023
IL-1β B		1.730	0.00	24.52	1.18	5.03	
IL-6 A	pg/site	1.725	0.33	14.27	0.910	4.185	0.05*
IL-6 B		0.770	0.00	5.91	0.285	1.575	
IL-10 A	pg/site	1.38	0.00	3.79	0.73	2.165	0.05*
IL-10 B		0.37	0.00	3.86	0.00	0.765	
Cortisol A	nmol/1	20.52	8.07	54.57	11.91	27.34	0.001*
Cortisol B		16.41	7.65	35.65	10.91	24.17	
VAS A		7.00	2.20	10.00	5.25	8.00	0.001*
VAS B		4.20	2.00	9.00	3.45	6.00	

Nonparametric *t*-test was used in all parameters.
*Significance of the differences between exam period (A) and after exam period (B).

between IL-1β and IL-6 in both stressed and non-stressed individuals. One problem in stress studies in general could be the difficulty to know when the influence on the biochemical markers by a stress period is over. An explanation to why we did not find high IL-1 β levels in GCF, despite a high degree of inflammation could be that stress inhibits the IL-1β response to stress (4, 6, 32, 33).

Another study by Paik *et al.* (16) which confirms our results showed in 42 college students that stress from the academic exams significantly increased the levels of IL-6 and IL-10. This study supports the notion that stress alters immune function and affects different immune cells (15).

Conclusion

In conclusion, academic stress appears to affect periodontal health, shown by more plaque accumulation, gingival inflammation and increased amounts of IL-6, IL-10 in GCF and cortisol in saliva. Therefore, the clinical implication should be to inform individuals about stress as a possible risk factor for gingivitis and periodontitis and to introduce additional preventive strategies in these individuals. Further studies in larger populations with both genders are required to explore the relationship between biochemical mediators of stress, immune function and behavioural changes.

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