The Effect of Examination Stress Conditions on the Cortisol Content of Saliva – A Study of Students from Clinical Semesters

Th. Krahwinkel, S. Nastali, B. Azrak, B. Willershausen

Department for Operative Dentistry, Johannes-Gutenberg University Mainz, Germany

Abstract

Purpose: Psychological stress factors can lead to changes in the immune system, the nervous system, and to psychosomatic diseases, besides releasing typical stress metabolites. This study on hand was to record the reliability of stress self assessment of students in various stress periods, and to be compared with the cortisol value of the saliva.

Materials and Methods: A total of 38 healthy students (18 women and 20 men), with an average age of 26.9 participated. The saliva of the participants was always tested between 9 AM and 10 AM, and always in various stress periods – during a typical semester internship, and immediately following the oral final examination. This was followed by a questionnaire concerning daily life styles (nutrition, part-time employment, sleeping pattern). The evaluation of the respective cortisol concentration of the total saliva resulted in a statistically high significant difference of values, relevant to stress levels ($p = 0.0001$).

Results: An average value for cortisol of 0.085 µg/dl total saliva was measured in the non-stress period, and in the stress period of 0.315 µg/dl total saliva. The comparison of the questionnaire evaluation to life styles did not show anything significant, the comparison to the cortisol concentration of the saliva showed significant deviations from the individual stress assessments by the participants.

Conclusion: The results cover a significant correlation of particular stress factors to changes in cortisol values. It is important for dental therapy to use stress reducing measures, or reduce them as much as possible in order to avoid factors that pre-operatively can affect the patient in a stressful way.

Key words: stress; cortisol; saliva

Introduction

Increased psychological stress factors, and increased permanent stress levels can lead to pathological organ changes, psychological alterations as well as psychosomatic diseases (Buddeberg et al. 1998), besides releasing typical stress metabolites, characteristic enzymes and hormones. The term "stress" today, in contrast to early history of man, does not signify a life-threatening situation, but is mostly used for everyday occurrences, as well as for particularly common, and health related stress situations. When defining stress, it must be differentiated between the "stress inducers", which trigger the stress levels on the victims, and the "stress reactions" as response to this trigger. Furthermore, the terms "distress" as decompensation, and "eustress" as normal pressure without harmful results, are being used in the area of psychosomatic medicine (Buddeberg et al. 1998).

For example, a clear significant correlation between the risk of heart disease and various stress factors in the work place, which has been suspected for a long time, seems to be scientifically confirmed (Vrijkotte et al. 1999). Next to pathological organ changes, negative stress inducers, e.g. anticipated or existing unemployment, not only influence the psychological well being in a particular way, but also the general well being (Ockenfels et al. 1995). The normally present balance between pressures and rest, stress and recuperation is significantly upset and changed by outside influences. If the amount of stress factors exceed the biological, psychological and social potentialities of the victims, this can lead to diverse reactions- and behavioral patterns with significant inter-individual variation ranges. The individual processing- and managing possibilities of incoming stress inducers ("appraisals" Bastine 1998), which take on special significance, are decisive for the respective behavior reaction.

The objective of the current study was to record the reliability of self-assessing by persons dealing with extreme stress, and to evaluate it with an objective measuring index. Dental students, who have to handle the usual daily course loads, as well as the stress of special examinations, were chosen for the current study. The subjective data of the participants, concerning possible stress factors, should also be compared with the recorded cortisol concentration in the total saliva.

Material and Methods

38 dental students of the Johannes Gutenberg University took part in this study to examine possible serious reactions to stress. The volunteers consisted of

18 women and 20 men with an average age of 26.9 years. All dental students were questioned about their history with former diseases, they neither had general or systemic, nor periodontal diseases, and were not taking any kind of permanent medication. Furthermore, pregnant women were excluded. All participants appeared to be normal, and had at least 20 natural teeth. The participants were attending the last clinical internship of dental studies, and all had comparable, study related pressures, daily routines and diets.

To objectively evaluate the stress level, total saliva samples were taken from these students during an internship (NS) free of stress, and while taking a civil service examination (S). Because of the circadian rhythm, changes of the saliva composition can be expected, and particularly the cortisol concentration. To determine the cortisol distribution, only a certain defined time can be evaluated, and the saliva samples were therefore taken at the same time every day, between 9 AM and 10 AM. The last intake of food and drink had to have been an hour earlier. The first saliva sample was taken during a typical non-stress period, and took place during a clinical semester internship, with no imminent testing situation (time NS). The second saliva sample (time S) was taken immediately following an oral civil service examination, and represented an acute stress situation.

For the samples, the participants were asked to chew a normal wad of cotton for 5 minutes, until it was thoroughly moistened. The cotton wads were then put in test tubes, marked and stored at -20 °C until analytical processing (Thomas, 1998). The saliva samples were centrifuged (800 rpm, 15 min) to determine the cortisol content, and the supernatant was examined for its cortisol content via chemical luminescence process (ACS:180; Bayer Vital Corp.), according to the standard procedure of the Institute for Clinical Chemistry of the University.

To evaluate the self-assessment by the participants concerning individual stress behavior, all participants filled out a questionnaire about diet, sleeping habits, quality of sleep, as well as stress levels. Additional questions included more stress factors, such as working and studying at the same time, general self evaluation concerning stress, and questions concerning concentration and productivity. The average sleep time, and daily hours of study had to be stated in hours, and the participants themselves added the data of the current stress level on a scale of "not stressed" up to "very stressed", (scale 0-11). Quality of sleep was also evaluated by the participants on a scale from "very good" to "very bad" (scale 0-11). The data on the questionnaire received the same markings as the relevant saliva sample, and was evaluated statistically upon conclusion of the study. To guarantee the anonymity of the examined participants, the questionnaires, as well as the saliva samples were coded.

The statistical evaluation of the data was conducted at the Institute for Medical Biometry, Epidemiology und Statistics of the Johannes Gutenberg-University Mainz, using the program SAS (SAS Institute Inc., Cary, NC, USA; release 6.12). To com-pare the non-stress situation with the stress situation, for quantitive variables, the Wilcoxon test was used for connected random samples, and the McNemar test for alternative variables. Similarities between categorical variables were examined with the help of contingency tables and Fishers exact test (two-sided). As a measure for the strong cohesion between quantitative variables, Spearman rank correlation coefficients were calculated. All statistical tests were used in terms of descriptive data analysis. P-values were viewed as statistically significant.

RESULTS

Of the total of 38 studied and questioned dental students, the data from 5 participants in stressful situations could not be evaluated. According to their own statements, the students did not feel capable of evaluating themselves during the stress period. During the non-stress period, 11% (n = 4) of the participants stated, that they had part-time employment at the same time as attending classes, but in the stress period only 9% (n = 3) still had a side job. According to the participants' own data, they had an average of 3.8 hours leisure time per day in the non-stress period, however in the stress period this statistically decreased ($p \leq 0.05$) to 1.6 hours. During the time without exams 42% (n = 16) of the students ate a balanced diet, whereas in the stress period only 18% (n = 6) followed this ($p \leq 0.05$). Duration of sleep at night was an average of 6.0 hours during the non-stress period, and decreased significantly ($p \leq 0.05$) in the stress period to an average of 4.2 hours. According to the participants, quality of sleep decreased from the NS period, with an evaluation of an average of 5.1 on the 11 cm scale (0 = very good, 11=very bad) to 6.6 at the S period ($p \leq 0.05$). The detailed questioning concerning quality of sleep showed, that 44.7% (n = 17) of participants slept restlessly, in the stress period this increased to 63.6% (n = 21) ($p \leq 0.05$). 21.1% (n = 8) of the people questioned admitted having problems falling asleep during non-stress periods, and 36.4% (n = 12) during testing. The question concerning how soundly they slept showed no significant differences between non-stress periods and high-stressed periods. According to this, the participants stated that in the non-stress period 65.8% (n = 25), and in the stress period 75.8% (n = 25), they did not sleep well, and did not feel rested (Fig.1).

The average evaluation concerning stress intensity had a value of 6.6 on the 11cm scale (0 = not stressed, 11= very stressed) during the non-stress period, whereas this value rose to an average of 9.2 in the stress period. Significant differences showed up between the examination times ($p \leq 0.05$). 23.7% of the participants (n = 9) assessed themselves to be less productive during the non-stress period; 42.4% (n = 13) of the persons questioned gave this assessment ($p \leq 0.05$) in the stress period. Concerning stress, 42.1% (n = 16) of the participants assessed themselves as non-stressed, and 54.5% (n = 18) with less intensity of stress. 28.9% (n = 11) of the persons questioned stated, that they were less able to concen-
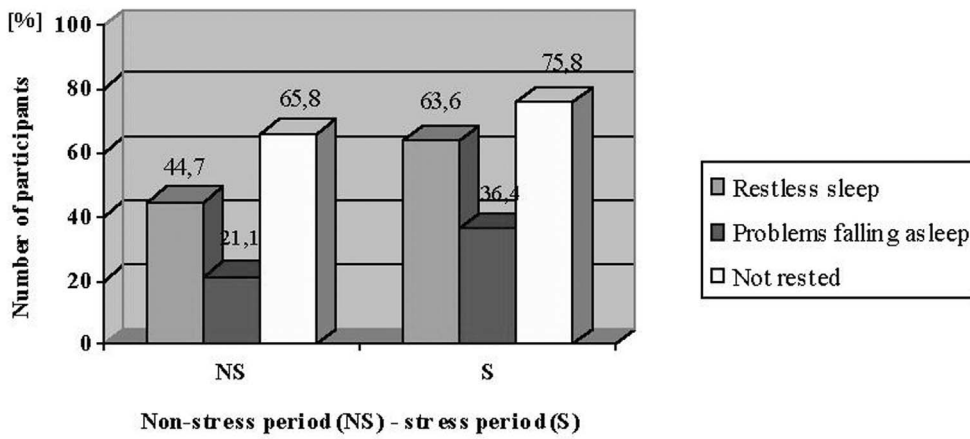
Fig. 1. Frequency distribution of the participants with restless sleep, problems falling asleep, as well as a period of interrupted sleep during the NS- and S-phase in percentage.
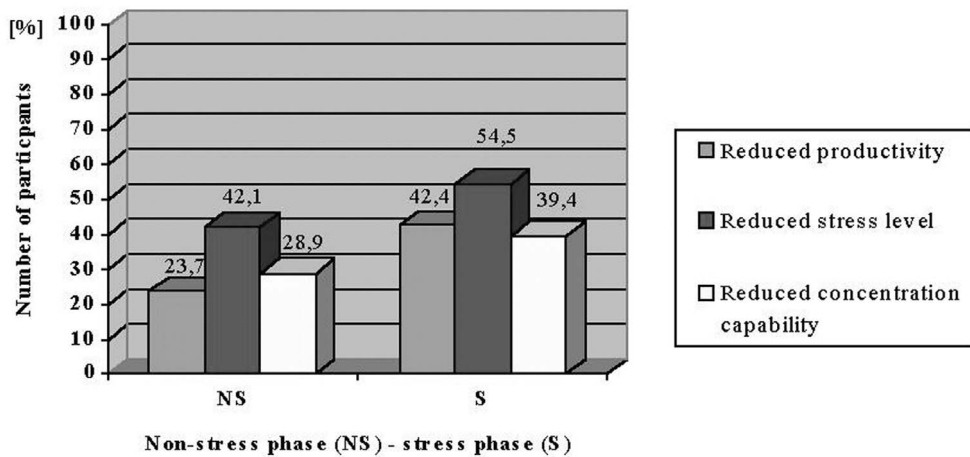

Fig. 2. Frequency distribution of the participants with a reduction in productivity, stress level, and concentration capability in the non-stress phase and the stress phase in percentage.
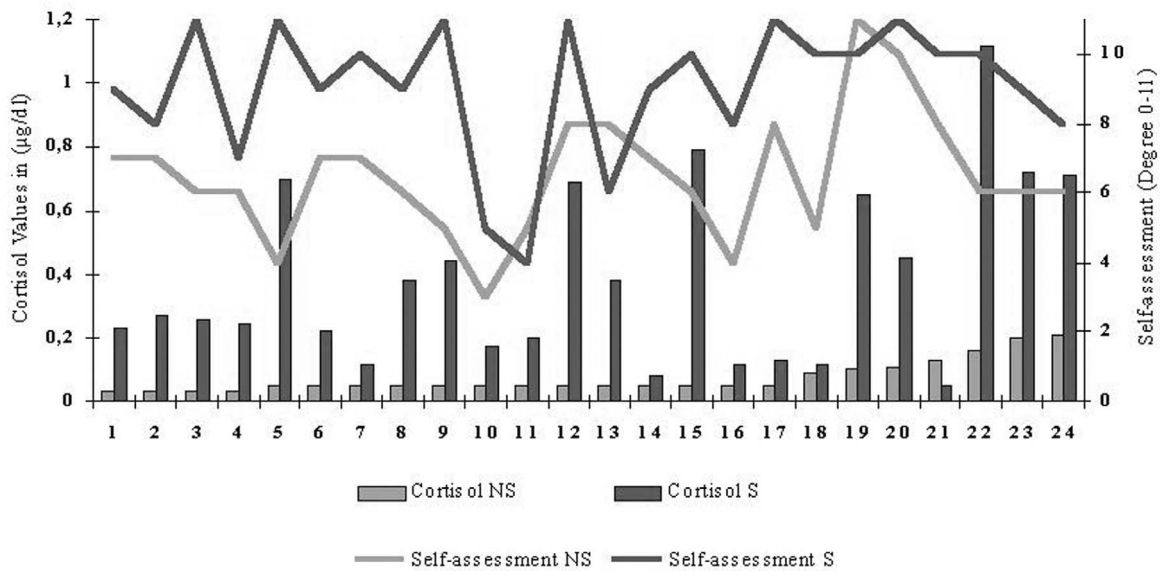

Fig. 3. Individual assessment of stress level on a 11-cm-scale (0cm = no stress, 11cm = a lot of stress) compared with the cortisol concentration in μg/dl total saliva in participants 1-24, that showed varying concentrations at examination times NS and S. The result of the cortisol content of participants 25-33 showed very slight differences during the stress period, as well as in the no-stress period.

trate in a non-stress period, on the other hand 39.4% (n = 13) said the same in a stress period. The differences concerning pressure and concentration were not significant (Fig. 2).

The evaluation of the respective cortisol concentration of the total saliva resulted in a statistically high significant difference of the values to the respective stress periods (p = 0.0001). For the non-stress period, a mean value for cortisol of 0.085 mg/dl total saliva was determined, and for the stress period of 0.315 mg/dl total saliva. The measured cortisol concentrations were compared with the participants' data concerning daily diet, quality of sleep, reduced productivity and concentration, as well as decreased capability of handling pressure. However no significant correlation was found. Furthermore, no gender differences were noted.

The results show that participants who had identical or minimally deviating cortisol values during non-stress- and stress periods, had significantly higher stress levels at examination time. An objective stress load could not be verified based on the measured cortisol values. The values for stress assessment were already above average at non-stress (NS) periods for the majority of these participants, when compared with standard values from the general population. At the time of the stress period (S), all recorded values were in the top third of the scale (degree of stress scale: 0=non stressed, 11 = very stressed). This evaluation confirmed the individuality and/or unreliability of self-assessments with perceived stress periods, as illustrated by the special situation of a civil service examination (Fig.3).

DISCUSSION

Primary factors of psychological stress situations, possible reactions, and recognizable, symptomatic organic changes show multi-factorial appearances. The individual perception and self-assessment, as well as coping with stress situations play an important role. Only with reliable, subjective self-assessment, and objective evaluation of stress inducing factors, can a stressful situation be addressed, so that meaningful measures for corrective treatment and remedy can be taken.

In the study on hand, to record the stress levels of students during special testing conditions, the saliva cortisol values of the participants were examined, among others. Cortisol in total saliva or plasma has already been used as a reliable indicator in numerous scientific tests concerning stress levels. The results by Schreinicke (1990), and the stress related changes of the cortisol values of the saliva in 77 healthy participants showed a significant increase of the values already 30 minutes after the stress effect. Steptoe (2000) examined the morning cortisol values of 105 teachers (41 men and 64 women). The results of this study also showed a significant increase of the measured cortisol values when occupationally related stress situations arose. It furthermore could be shown, that the cortisol values of the women between 8AM and 8:30AM were higher than those of the men tested. The results of the study on hand resulted in negligible higher cortisol concentrations during the non-stress period, whereas at the stress period, the cortisol concentration of the women was below that of the men, but without pointing to any statistical oddity.

Pruessner (1999) examined 66 teachers (42 women, 24 men) for possible effects from long-term stress and found that the morning cortisol values of the total saliva of the participants increased significantly under stress conditions. The results of the study showed that women, compared to men, had higher cortisol values. Pruessner (1999) had chosen teachers as the test group to demonstrate that long-term stress, together with the "burn-out syndrome", occurs mainly in occupational groups that perform nurturing and teaching activities.

In the study concerning stress behavior, advanced dental students were chosen, since they generally are healthy and have a similar daily routine, besides being approximately the same age, and balanced in gender. A comparison study with 35 medical students, who at the time of the study, and sampling, were under stress with exams, reached similar results. The cortisol concentration of the blood tests performed also resulted in a significant increase under stress conditions (Whitehouse et al. 1996). It can be assumed, that increased cortisol values can occur as physiological constants in stress situations (Scherbaum 1999). Since the concentration of this hormone in the total saliva correlates with the plasma values, this parameter can be valued as a reliable indicator of the free cortisol in the plasma (Van Eck et al. 1996).

In the Whitehouse study (1996) the subjective evaluation of the sleep quality of all participants was also requested. Here, a statistical significant reduction of the given sleep duration under stress conditions appeared. A study with students taking exams, concerning sleep quality and quantity, also reached similar results (Bosch et al. 1999). This study confirmed a significant change for the worse of these parameters under stress conditions. In our study, the students were also questioned about the duration of sleep. The evaluation of the answers resulted in highly significant differences between the stress periods and the non-stressed periods.

The questions concerning dietary habits also showed statistically significant differences. Similar results were achieved by a study, which was based on a questionnaire that was given to administrative personnel regarding their dietary habits (Van Eck et al. 1996). A significant rise in the consumption of coffee and alcohol under stress conditions was established, as well as irregular meals. In connection with the measured cortisol values of the participants, a correlation of the hormone concentration with an unbalanced diet in a stress period could be established.

A reliable and quick inclusion and evaluation of the actual intensity of a stress level, and the correlated effects on the human organism, would be advisable before medical examinations, and particularly before serious, invasive treatment. Not only a dental treatment can influence the total awareness, includ-

ing the way of driving a car (competent communication) by the patient with the physical intervention, but also possibly anxiety (oralophoby) before dental treatments. Corresponding stress inducing factors should always be recognized and established before the respective treatment, in order to relieve and/or avoid possible additional complications due to anxiety or stress.

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Address for correspondence:
Prof. Dr. B. Willershausen
Department for Operative Dentistry,
Johannes-Gutenberg University Mainz
D-55131 Mainz, Germany
Tel.:   +49 – 6131 – 177247
Fax: +49 – 6131 – 173406
e-mail: willersh@mail.uni-mainz.de