Association of Gingival Crevicular Fluid Cortisol/Dehydroepiandrosterone Levels With Periodontal Status

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Background: The aim of the present study is to examine whether anxiety and depression scale scores change with regard to clinical periodontal status and to investigate the association between the levels of stress-related hormones in gingival crevicular fluid (GCF) and extent/severity of periodontal disease.

Methods: One hundred twenty participants who fulfilled the study inclusion criteria were chosen. Patients with chronic periodontitis (CP) and those with healthy periodontal tissues/mild gingivitis were included. The clinical examinations were performed on the day after the psychologic evaluations which included anxiety and depression measurements. GCF sampling was undertaken the following day. Commercially available enzyme-linked immunosorbent assay kits were used to determine GCF cortisol and dehydroepiandrosterone (DHEA) levels. Study groups were assigned as follows: group 1, non-periodontitis; group 2, localized CP; and group 3, generalized CP.

Results: There were no significant differences with respect to age, sex, education, income level, occupation, or smoking history among the groups ($P > 0.05$). There were no significant differences between the non-periodontitis and CP groups for any of the psychosocial scales ($P > 0.05$). Group 3 had significantly higher mean DHEA scores compared with group 1 ($P < 0.05$); however, the median cortisol scores showed no statistically significant differences among the three groups ($P > 0.05$).

Conclusions: Anxiety/depression scores and GCF cortisol levels did not show any difference with regard to clinical periodontal status. However, a significant association was found between elevated levels of GCF DHEA and the severity of periodontitis. *J Periodontol 2014;85:e287-e294.*

KEY WORDS

Dehydroepiandrosterone; depression; gingival crevicular fluid; hydrocortisone; periodontitis; stress, psychological.

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Environmental risk factors can alter the host response, thereby affecting disease progression, severity, and the outcome of treatment.[1] Stress, anxiety, and depression are not yet confirmed as precise risk factors for disease, but some observational studies[2,3] have identified them as potential factors that could affect periodontal disease.

Many studies have found a positive relationship between periodontal status and psychosocial factors.[4] Psychosocial stress can affect periodontal health indirectly through changes in lifestyle, such as poor oral hygiene practices, smoking heavily, and consuming more fatty foods, and directly by different biochemical mechanisms.[5] Although the biochemical mechanisms involved in this relationship have not yet been clarified, it has been suggested that psychosocial factor–related changes in the hypothalamus–pituitary–adrenal (HPA) axis may facilitate bacterial colonization, leading to periodontal tissue breakdown.[4,6] Studies also pointed to the fact that stress may impair the balance between pro-inflammatory and anti-inflammatory responses.[7,8] Sheiham and Nicolau stated that the relationship between stress and periodontal disease might be mediated by changes in GCF interleukin-1 and interleukin-6 levels, reduced polymorphonuclear leukocyte chemotaxis and phagocytosis, and reduced proliferation of lymphocytes.[7] Moreover, glucocorticoids

doi: 10.1902/jop.2014.130787

have been shown to reduce immunocompetency through inhibition of immunoglobulin G and immunoglobulin A, which results in reduced phagocytosis of periodontal pathogens by neutrophils.[6,9] Consequently, vulnerability of periodontal tissues to pathogenic microorganisms may increase by activation of cellular responses, leading to local tissue breakdown.[10]

The psychosocial stress reaction involves activation of the HPA axis, followed by corticotropin-releasing hormone secretion from the hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary gland, and glucocorticoids (including cortisol) from the adrenal cortex.[11] Chronic activation of the HPA axis may also affect periodontal status because of the dysregulation of circulating cortisol that influences the functions of the immune system.[6] Upon stimulation of the HPA axis, a physiologic response occurs toward psychosocial factors, resulting in a change in the concentration of cortisol in the body's fluids, including gingival crevicular fluid (GCF), saliva, and serum.[6,12] Acute and chronic stress play an important role in the etiology of psychiatric disorders such as depression and anxiety. The stress response system, including HPA axis activity, has been impaired in depression and anxiety.[13] Depression and anxiety are major psychologic factors that affect the stress system, including the HPA axis. Therefore, measurement of depression and anxiety may be a way of assessing psychologic stress while investigating the relationship between stress and periodontal disease. Cortisol is the primary glucocorticoid produced in the adrenal cortex. It increases blood sugar concentration and affects fat metabolism; however, cortisol has negative effects on inflammatory and immune components, inhibiting lymphocyte formation.[14] When antibody production is inhibited, the humoral immune responses decline significantly. Cortisol is antiphlogistic and has an interceptive effect on fibroblast proliferation in the inflammatory granulation tissue. In addition, it suppresses the secretion of certain proinflammatory cytokines.[15]

Stress system dysregulation also affects the levels of another ACTH-dependent hormone: dehydroepiandrosterone (DHEA).[16] One previous study claimed that DHEA levels can more properly reflect HPA axis dysregulation than cortisol levels.[17] Besides, DHEA can be measured any time during the day.[18] To the authors' knowledge, there is limited information about the relationship between clinical periodontal status and DHEA response. In addition, saliva and serum samples were used in previous studies.[10,19] There are no studies regarding the investigation of DHEA in GCF and its relationship with periodontal status.

The aim of the present cross-sectional study is to examine whether anxiety and depression scale scores change with regard to clinical periodontal status and to investigate the association between the levels of stress-related hormones (cortisol/DHEA) in GCF and the extent/severity of periodontal disease.

MATERIALS AND METHODS

Study Population

One hundred twenty participants (59 males and 61 females, aged 24 to 63 years; mean age: 38.3 years) who fulfilled the study inclusion criteria were chosen randomly from the Department of Periodontology, Faculty of Dentistry, at the University of Erciyes in Kayseri, Turkey. Patient recruitment was performed during 6 months from September 2013 to February 2014. Individuals with chronic periodontitis (CP), mild gingivitis, and healthy periodontal tissues were included. The periodontal criteria for CP were ≥1 tooth with clinical attachment loss (AL) ≥4 mm and probing depth (PD) ≥5 mm. Patients were divided into three groups according to the severity and the extent of periodontal disease to analyze the relationships among periodontal status and the GCF levels of cortisol and DHEA. The groups were assigned as follows: group 1 (non-periodontitis), patients with teeth having PD <5 mm; group 2 (localized CP), patients with <7 sites having PD ≥5 mm and AL ≥4 mm; and group 3 (generalized CP), patients with ≥7 sites having PD ≥5 mm and AL ≥4 mm. None of the patients participating were periodontally treated before the study. Periodontal treatment of the patients was initiated upon the completion of sampling and recording of clinical periodontal parameters. Exclusion criteria included: 1) periodontal treatment within the previous 6 months; 2) systemic disease; 3) self-reported psychiatric disorder; 4) antibiotic use within the previous 3 months; 5) prescribed steroids; 6) immunosuppressive and psychiatric drugs; 7) symptoms of acute illness; 8) any apparent oral infection; 9) missing questionnaire or GCF samples; 10) aged >65 or <18 years; 11) fewer than 14 teeth (excluding third molars); 12) pregnant or lactating; and 13) smoking. The study details were explained to the participants, and all patients signed a written informed consent form. The study protocol was approved by the Erciyes University Faculty of Medicine Ethics Committee (meeting date: May 3, 2011; decision no.: 2011/320).

Psychosocial Measurements

Two psychometric instruments were used to examine depression and anxiety. The Beck Depression Inventory (BDI) consists of 21 items, with each statement scored from 0 to 3.[20] The BDI scale, which was adapted and validated for the Turkish population,[21,22]

scores the intensity of depression in the Turkish population according to: 0 to 10 = minimum; 11 to 17 = mild; 18 to 29 = moderate; and 30 to 63 = severe depression. In the present study, a cutoff score of 17 identifies a patient with depression symptoms.

The State-Trait Anxiety Inventory (STAI) consists of two scales: state anxiety and trait anxiety.[23] Whereas the trait anxiety scale examines how the individual generally feels, the state anxiety scale examines how the individual feels while filling out the questionnaire. Both scales have 20 items, and each of the statements are scored from 1 to 4. Possible total scores for each of the two scales range from a minimum of 20 to a maximum of 80. This scale was also validated for the Turkish population.[24]

Clinical Periodontal Examination

The clinical examinations were performed by OC on the day after the psychologic evaluations. Full-mouth clinical periodontal measurements were recorded at six sites (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual) per tooth, including plaque index (PI),[25] gingival index (GI),[26] bleeding on probing (BOP), PD, and AL. AL is the distance from the cemento-enamel junction in an apical direction to the base of the pocket/sulcus. The BOP percentage was calculated by dividing the bleeding sites by the total sites examined for each participant. A periodontal probe¶ was used for the periodontal measurements.

GCF Sampling

Sampling was undertaken the following day to avoid the effect of irritation caused by periodontal probing during the initial examination, and at a specific time interval (between 8:00 and 10:00 am) to avoid the circadian rhythm affecting the levels of these hormones in GCF.[27] Patients were asked not to eat or drink anything, except water, before the sampling. The samples were taken at six sites on one incisor, premolar, and molar tooth of each patient. Whereas the mesio-buccal and disto-palatal sites of the test teeth were used for sampling in the non-periodontitis group, the two deepest pockets of the same teeth in the periodontitis groups were used for sampling. In some of the patients, GCF samples were taken from a mixture of diseased and apparently healthy sites to obtain two samples from the test teeth. The area was dried and isolated with cotton rolls to prevent any contamination with saliva. A curet was gently used for supragingival plaque removal, avoiding any gingival irritation. Paper strips# were placed for 30 seconds within the pocket or sulcus using the orifice method.[28] In cases of visible blood and salivary contamination, the samples were discarded and new samples were obtained at the sites, fulfilling the above criteria. All six strips were pooled in sterile tubes** and stored at –80°C until the day of laboratory analysis. Samples were taken separately for cortisol and DHEA, with 15-minute intervals.

Laboratory Assessment

Commercially available enzyme-linked immunosorbent assay (ELISA) kits†† were used to determine GCF cortisol and DHEA levels. The competitive ELISA assay was carried out separately for each hormone using standard curves, according to the recommendations of the manufacturers, at the Department of Biochemistry, Faculty of Medicine at Erciyes University.

All samples were allowed to warm to room temperature before analysis. GCF samples were eluted from the pooled strips by placing them in phosphate-buffered solution (500 μL, pH 7.4). Samples (100 and 50 μL) were added into appropriate wells for cortisol and DHEA analysis, respectively. All standards and samples were run in duplicate. The absorbance of the substrate color reaction was read on an ELISA reader‡‡ using 450 nm as the primary wavelength. The total cortisol and DHEA amount was determined in picograms. Coefficient of variation for cortisol and DHEA were 3.48% and 4.2%, respectively.

Statistical Analyses

Power analysis showed that to determine the difference in anxiety and depression among the three groups with a power of 80%, a minimum of 105 patients was required. Kruskal–Wallis *H* test was used to examine the variables because a normal distribution was not present based on the results of the Kolmogorov–Smirnov test. For the parameters that passed the normality test, comparisons among the three study groups were performed using one-way analysis of variance (ANOVA). Tukey and Tamhane tests were used for multiple comparisons of the mean groups in the one-way ANOVA. Sex, education, income level, job, and smoking status were presented by frequency distribution and compared among the groups using the χ^2 test. Correlations among the different parameters were analyzed using Spearman correlation coefficients. All variance analysis results were adjusted for age and sex, and corrected *P* values are presented in tables. All analyses were conducted using statistical software,§§ and the significance level was set at 0.05.

¶ Goldman/Fox Williams probe, Hu-Friedy, Chicago, IL.
# PerioPaper, Oroflow, Amityville, NY.
** Eppendorf, Interlab, Istanbul, Turkey.
†† Cortisol HS ELISA and DHEA ELISA, DRG Instruments, Marburg, Germany.
‡‡ Molecular Dynamics, Sunnyvale, CA.
§§ SPSS, v.15.0, IBM, Chicago, IL.

RESULTS

Demographic data of the 120 patients included in the study are shown in Table 1. There were no significant differences with respect to age, sex, education, income level, occupation, or smoking history among the groups (P >0.05).

All groups showed statistically significant differences in mean and median values of the periodontal clinical parameters (GI, BOP, PD, AL) (P <0.001). The median PI values in groups 2 and 3 were statistically higher compared with group 1 (P <0.001) (Table 2).

Table 3 summarizes the STAI and BDI scale scores of the groups. There were no significant differences among the groups for any of the psychosocial scales (P >0.05).

There was a significant difference among the groups for the GCF DHEA levels (Table 4). Group 3 had significantly higher mean DHEA scores compared with group 1 (P <0.05); however, median cortisol scores showed no statistically significant differences among the three groups (P >0.05).

Correlation analysis revealed that although the GCF levels of cortisol were positively correlated with all of the clinical parameters (P<0.05), the GCF DHEA level was correlated with PD and BOP (P<0.05). The GCF cortisol and DHEA levels correlated with each other (P<0.05), and a statistically significant negative correlation existed between the GCF DHEA levels and age (P <0.05) (Table 5).

DISCUSSION

The aim of the present cross-sectional study is to examine whether anxiety and depression scores show differences with regard to clinical periodontal status and to investigate the presence of cortisol and DHEA in GCF and its association with periodontal health.

Similar distributions were seen for the study groups regarding demographic variables, and the results of the variance analysis were adjusted for age and sex. Therefore, the variability in the GCF hormone concentrations in the study groups could be due to different disease severities at the time of GCF sampling. Smoking is well known to be a major risk factor for periodontitis,[6] and it also increases serum cortisol levels.[29] For this reason, patients who smoked were excluded from the study. Only 19 of the patients were former smokers, and they were uniformly dispersed among the groups, thus eliminating the possible effects of smoking on the parameters investigated.

Table 1.

Demographic Variables of the Study Groups

Parameter	Group 1 Non-Periodontitis (n = 40)	Group 2 Localized CP (n = 41)	Group 3 Generalized CP (n = 39)	P*
Age (years, median [min to max])	35 (25 to 59)	36 (25 to 63)	41 (24 to 60)	0.132
Sex, n (%)				0.153
Male	15 (37.5)	21 (51.2)	23 (59)	
Female	25 (62.5)	20 (48.8)	16 (41)	
Occupation, n (%)				0.973
Non-worker	19 (47.5)	20 (48.8)	18 (46.2)	
Worker	21 (52.5)	21 (51.2)	21 (53.8)	
Education level, n (%)				0.094
Primary (4 years)	14 (35)	22 (53.7)	20 (51.3)	
Secondary (8 years)	9 (22.5)	11 (26.8)	12 (30.8)	
High school	17 (42.5)	8 (19.5)	7 (17.9)	
Monthly income level (Turkish liras, n [%])†				0.830
≤1,000	13 (32.5)	16 (39)	13 (33.3)	
1,000 to 2000	13 (32.5)	13 (31.7)	16 (41)	
≥2000	14 (35)	12 (29.3)	10 (25.7)	
Smoking, n (%)				0.774
Non-smoker	35 (87.5)	34 (82.9)	32 (82.1)	
Former smoker	5 (12.5)	7 (17.1)	7 (17.9)	

* P >0.05 = no significant difference.
† USD$1 = 2.19 Turkish liras; 1 Turkish lira = USD$0.456; average annual income per person is USD$15,137 for Turkey.

Table 2.

Clinical Periodontal Parameters of the Study Groups

Parameter	Group 1 Non-Periodontitis (n = 40)	Group 2 Localized CP (n = 41)	Group 3 Generalized CP (n = 39)	*P*
PI, median (min to max)	0.78 (0.09 to 2.53)*	1.83 (0.45 to 2.69)†	2.11 (0.2 to 3)†	<0.001
GI (mean ± SD)	0.89 ± 0.37*	1.39 ± 0.34†	1.67 ± 0.39‡	<0.001
PD (mm), median (min to max)	1.72 (1.1 to 2.3)*	2.24 (1.68 to 2.72)†	2.83 (2.21 to 4.56)‡	<0.001
BOP (%), median (min to max)	13.4 (0 to 76.2)*	51.5 (15.7 to 91.6)†	64.7 (21.9 to 100)‡	<0.001
AL (mm [mean ± SD])	1.11 ± 0.83*	2.16 ± 0.67†	3.01 ± 0.87‡	<0.001

Data with different symbols are statistically significantly different.

Table 3.

Psychosocial Scale Scores of the Groups

Psychosocial Scale	Group 1 Non-Periodontitis (n = 40)	Group 2 Localized CP (n = 41)	Group 3 Generalized CP (n = 39)	*P**
STAI, scale 1 (mean ± SD)	37.1 ± 10.25	34.9 ± 8.9	38.13 ± 8.8	0.158
STAI, scale 2 (mean ± SD)	42.9 ± 7.92	41.66 ± 8.93	41.18 ± 6.42	0.772
BDI, median (min to max)	6.5 (0 to 27)	5 (0 to 41)	6 (1 to 32)	0.659

* $P > 0.05$ = no significant difference.

Table 4.

GCF Cortisol and DHEA Values of the Study Groups

Hormone Studied in GCF	Group 1 Non-Periodontitis (n = 40)	Group 2 Localized CP (n = 41)	Group 3 Generalized CP (n = 39)	*P**
Cortisol (pg/mL), median (min to max)	107 (0 to 1,492)	192 (1 to 1,517)	212 (15 to 1,162)	0.064
DHEA (pg/mL [mean ± SD])	59.2 ± 22.57†	64.07 ± 30.87†‡	78.17 ± 38.66‡	0.005

Data with different symbols are statistically significantly different.
* $P > 0.05$ = no significant difference.

Most of the previous studies that have assessed the relationship between clinical periodontal status and psychosocial factors with various psychometric scales were cross-sectional in design, and the results were conflicting.[30-33] The STAI and BDI values showed no statistically significant differences among the groups in the present study. Only 10% of the study population consisted of individuals with depression symptoms, and the anxiety levels were compatible with the mean scores determined for the Turkish population.[24,34] Patients with undiagnosed psychiatric disorders were particularly excluded in the study exclusion criteria, since any psychotic medication could have altered GCF cortisol and DHEA levels. Moreover, the distribution of the 12 patients with depression among the groups was equal. The findings of the current study are consistent with those of some previous reports,[30,31,35,36] but inconsistent with others.[2,32,33,37] Conflicting results might be attributable to the differences in the diagnostic criteria of periodontal disease, number of patients, study population, and different psychometric scales used. Another fact that should be considered is the substantiation of the psychometric scales by biochemical hormone findings. Therefore, examination of stress hormones such as cortisol and DHEA in body fluids (especially in GCF) may provide objective data in the determination of this relationship. This would also preclude any inappropriate

Table 5.

Non-Parametric Correlations of the Parameters

Correlation	*r*	*P**
GCF DHEA and age	–0.222	0.015
GCF cortisol and PI	0.326	0.000
GCF cortisol and GI	0.360	0.000
GCF cortisol and PD	0.292	0.001
GCF cortisol and AL	0.310	0.001
GCF cortisol and BOP	0.415	0.000
GCF DHEA and PD	0.216	0.018
GCF DHEA and BOP	0.245	0.007
GCF cortisol and GCF DHEA	0.251	0.006

* $P < 0.05$ = statistically significant difference.

evaluation of the patient by incorrectly filling out the questionnaire.

Psychosocial stress has a profound effect on the immune system.[38] Although earlier studies emphasized the depressive effects of stressful conditions in immunity, new evidence points toward a different vision: activation of the innate and adaptive immune system by acute, short-term, low-intensity stressors or chronic mild stressors results in increased gene expression of inflammatory cytokines, increased macrophage numbers, and increased T-cell recruitment and activation.[39] Chronic psychologic stress may be associated with a state of chronic low-grade inflammation and increased susceptibility to infectious illnesses.[40] Bearing in mind the factors above, local periodontal tissues may become more vulnerable to pathogenic microorganism attacks, and local tissue breakdown may occur in CP.

Upon activation of the HPA axis, cortisol and DHEA are secreted from the adrenal cortex, and their activity is regulated by ACTH. Although the level of stress hormones in the saliva was investigated in the vast majority of studies,[41,42] very few reports[12,43,44] examine these hormone levels in GCF. This may be particularly important in examining the relationship between local hormone levels and periodontitis, which is a local inflammatory condition. The presence of cortisol in the GCF was revealed for the first time by Axtelius et al., and they suggested that the GCF cortisol levels were higher in participants with poor oral health.[12] According to the results of the present study, the GCF levels of cortisol did not differ significantly among the groups. There are two studies on this issue by the same authors, with conflicting results.[43,44] In the first study, the authors reported that patients with depression had higher GCF cortisol levels compared with those of healthy individuals.[43] In the second study, surprisingly lower cortisol levels in the GCF in patients with deep periodontal pockets were evident, which the authors had difficulty interpreting.[44] A comparison of the findings of the present study with those of these two studies is not possible, due to the differences in the methodology of the study and patient inclusion criteria; while Johannsen et al. performed GCF sampling using the intracrevicular washing technique, Rüdin et al.'s orifice method was used in the present study.[28] In addition, although both studies are cross-sectional, the study population of those authors differed from that of the present study, in which individuals with the diagnosis of depression were included. It should also be kept in mind that variations in GCF levels of any hormone or inflammatory mediators may be extremely high due to the differences in GCF collection methods, sampling time, sensitivity of kits used, patient populations, and periodontal status of patients. GCF DHEA levels were statistically significantly different among the groups. The generalized CP group had significantly higher GCF DHEA levels compared with the non-periodontitis group. To the authors' knowledge, this is the first study to examine the relationship between periodontal status and GCF DHEA levels, and this fact precluded the comparison of the present GCF DHEA results with those of the other studies. However, in the authors' opinion, the presence of higher GCF DHEA levels in patients with generalized CP compared with individuals without periodontitis points to an association between periodontal status and psychosocial stress.

One of the limitations of the present study was that a causal relationship could not be clarified due to the cross-sectional design of the study. Prospective, controlled clinical studies are needed in which both psychologic and periodontal treatments are included. A second limitation was that in the present study population, only 10% of the patients had depression, and anxiety levels were comparable with those of Turkish population in general.[24,34] Inclusion of individuals with a diagnosis of anxiety and depression, rather than a general population, could better reflect the changes in biochemical stress hormone values. The third limitation was that the authors could not use a psychometric scale to assess stress in the present study because no stress scale has been adapted and validated for the Turkish population.

The results of the correlation analysis have shown that the GCF DHEA levels were negatively correlated with age. This finding was consistent with that

of the studies by Ishisaka et al.[10,19] and Ahn et al.,[45] who examined salivary and serum DHEA levels and reported a gradual decrease of the DHEA basal levels with age.[46] Surprisingly, there was no such correlation for the GCF cortisol levels. However, the findings of the studies examining this issue are conflicting. Very few studies have investigated GCF cortisol levels;[12,43,44] however, many others have investigated salivary and serum cortisol levels.[41,42,47] The basal cortisol level was found to be similar in elderly and young people,[48] but its increase[49] or decrease with age[50] was also reported. A limited number of studies did not investigate the relationship between GCF cortisol levels and age at all.[12,43,44]

The results of the present study revealed a positive correlation among all of the clinical periodontal parameters examined and the GCF cortisol levels. GCF DHEA levels were positively correlated with PD and BOP. Upon literature review, the authors believe that this is the first study to investigate the correlation between clinical periodontal parameters and GCF cortisol/DHEA levels and demonstrate its presence.

CONCLUSIONS

Within the limitations of the present study, the authors conclude that there was no difference among the groups regarding the demographic parameters, psychosocial scale scores, and GCF cortisol levels. However, a significant association was found between elevated levels of GCF DHEA and the extent/severity of periodontitis. This finding should be interpreted with caution because further prospective controlled studies are required to reveal the causal relationship.

ACKNOWLEDGMENTS

This study was supported by a grant from the Scientific Research Projects of Erciyes University (grant number: 2011/320). The authors report no conflicts of interest related to this study.

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Submitted December 31, 2013; accepted for publication March 4, 2014.