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# Evaluation of psychological stress and cortisol levels in males with and without gingivitis

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## Abstract

**Aim:** Gingivitis is a prevalent health problem seen most commonly in males. Psychological stress is also associated with periodontal disease. Thus, to evaluate the possible role of stress in gingivitis, this study investigated the psychological stress and cortisol levels in males with and without gingivitis.

**Material and Methods:** Sixty systemically healthy males between 18 and 28 years were divided into two groups, including those with generalized gingivitis (G) (n=30) and periodontally healthy controls (H) (n=30). Gingival crevicular fluid (GCF) and saliva samples were obtained for determination of cortisol levels using enzyme-linked immunosorbent assay. Plaque (PI) and gingival index (GI), bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) were noted. In addition, BECK depression inventory (BDI), Perceived Stress Scale (PSS), and Oral Health Impact Profile-14 (OHIP-14) were recorded from all participants.

**Results:** PI, GI, BOP ( $p<0.01$ ), and PD ( $p<0.05$ ) were significantly higher in group G. BDI, PSS, and OHIP-14 scores were not significantly different between groups ( $p>0.05$ ). Cortisol levels in saliva and GCF were similar between groups ( $p>0.05$ ).

**Conclusion:** Psychological stress and cortisol levels in GCF and saliva were found to be similar in males with and without gingivitis.

**Keywords:** Gingivitis; hydrocortisone; oral hygiene; saliva; stress

## INTRODUCTION

Periodontal disease is a highly prevalent inflammatory condition which is seen commonly in two types, periodontitis and gingivitis (1). Periodontitis is a chronic inflammatory situation destroying the supporting periodontal tissues around the teeth, in contrast gingivitis is characterized by restricted inflammation in superficial gingival tissues without any loss of periodontal tissues (2). Gingivitis is extremely prevalent affecting up to 90% of adults and, has been found to be more prevalent in males compared to females (3). In addition, males have been reported to have a higher risk for periodontitis development compared to females (3). This gender base difference has been reported to be because of the attitude and behavioral differences including the frequency of tooth brushing and also receiving dental check-ups between males and females (4,5). Females have been reported to be more concerned with their oral health and have better oral hygiene status compared to males (6). These oral health behaviours have been found to be also associated

with psychological situation particularly stress (7).

A positive relationship has previously been reported between psychological situation including stress, depression, and periodontal status (8). The mechanism of stress in periodontal health manifests through lifestyle changes, including poor oral hygiene, behavioural alterations, and diet changes, and/or through biochemical changes, such as proinflammatory cytokine responses (9). Upon triggering the hypothalamic–pituitary–adrenal axis (HPAA), a physiological response causes the alteration in the concentration of cortisol in the saliva, and serum and GCF. Circulating predominantly in blood, saliva cortisol levels reflected by HPAA activity are used as a stress marker to indicate psychological stress (10). Therefore, chronic HPAA activation involving cortisol circulation may affect periodontal health. Depression is another psychological factor affecting HPAA. Thus, the assessment of psychological depression may be a way of determining stress levels while evaluating the periodontal disease and psychiatric situations relationship (11).

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To determine the psychological condition well defined scales have been defined in the literature. The Beck Depression Inventory (BDI) is the most commonly used self-rating scale for measuring depression (12). Its international application, validity and internal consistency has been confirmed by literature (12). Furthermore, the Perceived Stress Scale (PSS) is among the most used psychological tests for measuring the perception of stress (13). It measures current levels of experienced stress and its degree to which situations in one's life are admitted as being stressful. Moreover, oral health impact profile index (OHIP-14) is frequently used and well-documented and includes a 14-item scale to determine the impact of oral health on the quality of life (14).

As gingivitis is a prevalent health problem and is observed at a higher frequency in males compared with females, investigating and identifying the possible disease mechanism such as psychological stress in the development of the disease in males is important. Thus, the current study aimed to investigate the psychological stress and cortisol levels in males with and without gingivitis.

## MATERIAL and METHODS

Thirty systemically healthy males with gingivitis (group G) and 30 periodontally healthy males (group H) were recruited for this study between November 2018 and January 2019. Using a questionnaire, age information, socioeconomic and educational status, duration of smoking, number of cigarettes smoked per day, daily oral hygiene maintenance protocols, and diet habits were recorded for all participants. This study was approved by Canakkale Onsekiz Mart University Clinical Research Ethics Committee (approval number #2018-19, approval date 31/10/18). Participants were informed about the study and a written informed consent form was received from all participants. The current study was also carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Inclusion criteria for all study groups included: age between 18 and 28 years, having more than 20 teeth other than wisdom teeth, a relatively high educational following attendance at least to university level, and a relatively high socio-economic status. Inclusion criteria for healthy group included: probing depth  $\leq$  3mm, bleeding on probing less than 5 %, no attachment loss, gingival index  $<1$  at all surfaces.

Exclusion criteria for all participants included: periodontal therapy and/or antibiotic usage within the last six months, any ongoing orthodontic treatment, systemic disease, self-reported psychiatric disease, prescribed psychiatric medications and steroids.

### Periodontal Examination

A full mouth periodontal examination was performed to all participants, including plaque index (PI) (15), gingival index (GI) (16), bleeding on probing (BOP) (17), probing depth (PD) (18), and clinical attachment level (CAL) (19).

Measurements were recorded by the same examiner at six sites per tooth using the Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). Intra-examiner reliability was detected by intraclass correlation coefficients was 0.79, 0.90 and 0.85 for PI, GI and PD, respectively. The BOP percentage was calculated by dividing the bleeding sites by the total sites examined for each participant. Radiological examination was conducted for all participants and the diagnosis of dental plaque biofilm-induced gingivitis was performed in accordance with new classification (20).

### GCF and Saliva Sampling

Unstimulated whole saliva and GCF samples were obtained from all participants in the same visit. Saliva samples were centrifuged at 800 x g for 10 minutes, and the supernatants were kept at  $-80^{\circ}\text{C}$  until analysis (21). Then, GCF samples were collected. Sampling areas were dried and isolated by using cotton wool rolls. Supragingival plaque was carefully removed using a sterile curette. Paper strips (Periopaper, Oraflow, Smithtown, NY) were carefully placed into the sulcus and left for 30 seconds. GCF samples obtained at 6 Ramfjord teeth of each participant were pooled in a sterile tube and kept at  $-80^{\circ}\text{C}$  until analysis.

Saliva and GCF cortisol were measured by the commercially available enzyme-linked immunosorbent assay (Enzo Life Science Human Cortisol ELISA Kit, Exeter, UK) kits according to the manufacturer's instructions. The minimum detection limit was 0.156-10 ng/ml, and the sensitivity of the kit was 0.056 ng/ml. The total cortisol amount in GCF was presented in nanograms per 30s (ng/30s), while the total cortisol amount in saliva was presented in ng/ml.

### Questionnaire

BDI consisting of 21 items scored each from 0 to 3, was used to determine the presence and level of depression (22). The BDI scale (adapted and validated for the Turkish population) scores the intensity of depression in the Turkish population according to the following: 0-10 = minimum, 11 to 17 = mild, 18 to 29 = moderate, and 30 to 63 = severe depression (23). PSS version of 14 items scored each from 0 to 4 was performed to appraise day-to-day stress. A high PSS score indicates a high level of stress (24). OHIP-14, which included 14 items scored each from 0 to 4, was conducted to determine the effect of personal oral health on quality of life and general health; a high score indicates a negative oral health impact on quality of life. BDI (23), PSS (25), and OHIP-14 (26) were adapted and validated for the Turkish population.

### Statistical Analysis

To estimate the sample size for the current study, a statistical power analysis which was calculated using cortisol levels based on a study conducted by Yarkac et al. (27) was performed by using a software (G\*Power version 3.1.7, Franz Faul, Kiel, Germany). A total of 60 individuals was estimated to be enough to find a significant difference in cortisol levels (0.50 effect size at an  $\alpha = 0.05$

significance level, actual power = 0.9677, non-centrality parameter = 15.00, critical F = 4.01, numerator df = 1, dominator df = 58). The normal distribution of data was determined using the Kolmogorov–Smirnov test. Student t test or the Mann–Whitney U test was used to detect significant differences between groups. Correlation analysis was also conducted using Spearman's or Pearson correlation coefficient tests according to the distribution of data. Statistical analysis was performed using a software (SPSS v.20.0, IBM, Chicago, IL). P values <0.05 were considered statistically significant. Data is expressed as mean ± standard deviation.

## RESULTS

The mean age between group G (22 ± 1.41) and group H (22.50 ± 1.52) were found similar ( $p > 0.05$ ). Fifteen of the participants in both groups were smokers, and the daily consumption of smoking between the groups was similar ( $p > 0.05$ ). Twenty of the participants in group G and 19 in group H were reported to consume alcohol. Seven of 30 participants in group G and 25 of 30 participants in group H regularly visited the dentist at least once a year. In group G, 19 of the participants brushed irregularly, 9 brushed once, and 2 participants brushed twice a day. In group H, 9 of the participants brushed once and 21 brushed twice a day. None of the participants in group G reported flossed or using interdental brushes regularly; in group H, 29 of the 30 participants used interdental brushes or flossed (Table 1). All participants in both groups reported a relatively high educational status. In group G, 25 of the participants had a moderate and 5 had a high socioeconomic status; in group H, 26 of the participants had moderate and 4 had high socioeconomic status. In group G, 23 of the 30 participants had no knowledge of periodontal disease; in group H, 29 of the 30 participants did report knowledge of the disease (Table 1).

**Table 1. Demographic profile of study participants**

Characteristics	Group G	Group H
Age (year) (mean ± SD)	22 ± 1.41	22.50 ± 1.52
Smoking (Y/N)	15/15	15/15
Smoking amount (daily)	7.60 ± 8.29	9.57 ± 8.24
Alcohol consumption (Y/N)	20/10	19/11
Regular dental visit (1 in a year) (Y/N)	7/23	25/5
Brushing (day) (not regular/1/2)	19/9/2	0/9/21
Flossing and interdental brushing (regularly/never)	0/30	29/1
Educational level (high school/university)	0/30	0/30
Socioeconomic status (low/moderate/high)	0/25/5	0/26/4
Knowledge about periodontal disease (Y/N)	7/23	29/1

**No differences were found in age and daily smoking amount between the two groups ( $p > 0.05$ )**

Questionnaire results showed that BDI, OHIP-14, and PSS scores were found to be similar between the two groups ( $p > 0.05$ ) (Table 2). Both groups had minimum depression according to the BDI results.

**Table 2. Demographic profile of study participants**

Scales	Group G	Group H
OHIP-14 (mean ±SD)	9.50 ± 4.12	11.14 ± 7.22
BDI (mean ±SD)	3.75 ± 1.25	8.85 ± 6.03
PSS (mean ±SD)	25.80 ± 11.12	28.25 ± 7.45

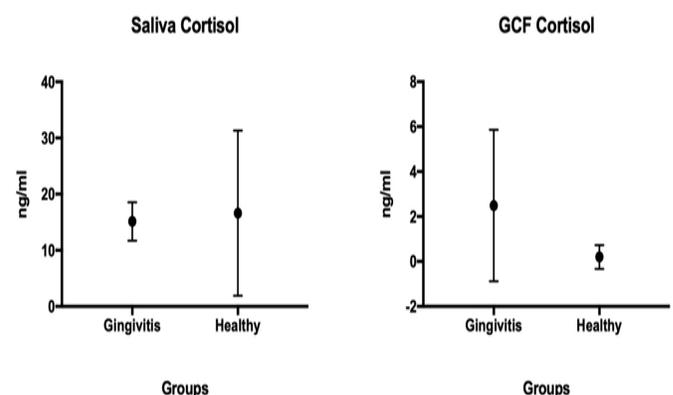
**No differences were found in OHIP-14, BDI, and PSS scores between the two groups ( $p > 0.05$ )**

Periodontal examination showed that PI, GI, BOP ( $p < 0.01$ ), and PD ( $p < 0.05$ ) were significantly higher in group G compared with group H, however no differences were found in CAL ( $p > 0.05$ ) (Table 3).

**Table 3. Clinical Periodontal Parameters of the Participants**

Parameters	Group G	Group H
PI	1.81 ± 0.15	0.51 ± 0.76
GI	1.18 ± 0.25	0.00 ± 0.00
PD	1.86 ± 0.21	1.50 ± 0.21
BOP	15.35 ± 7.47	2.70 ± 0.7
CAL	0.00 ± 0.00	0.00 ± 0.00

**PI, GI, BOP ( $p < 0.01$ ), and PD ( $p < 0.05$ ) were significantly higher in group G than in group H, but no differences were found in CAL ( $p > 0.05$ )**



**Figure 1.** No statistically significant differences were found between the two groups in saliva and GCF

Table 4. Correlation of cortisol values with periodontal parameters and scales

Correlation	Group G		Group H	
	r	p*	r	p*
GCF Cortisol and Saliva Cortisol	0.348	0.566	-0.134	0.774
GCF Cortisol and PI	<b>-0.912</b>	<b>0.03*</b>	-0.292	0.525
GCF Cortisol and GI	-0.586	0.299	-	-
GCF Cortisol and PD	-0.719	0.171	<b>0.783</b>	<b>0.037*</b>
GCF Cortisol and BOP	-0.567	0.319	-	-
Saliva Cortisol and PI	-0.90	0.866	-0.426	0.341
Saliva Cortisol and GI	0.300	0.624	-	-
Saliva Cortisol and PD	0.154	0.805	-0.184	0.693
Saliva Cortisol and BOP	0.317	0.603	-	-
GCF Cortisol and BDI	0.132	0.868	0.10	0.982
GCF Cortisol and PSS	0.498	0.393	0.515	0.236
GCF Cortisol and OHIP-14	0.566	0.434	0.419	0.350
Saliva Cortisol and BDI	0.892	0.108	-0.563	0.188
Saliva Cortisol and PSS	0.196	0.752	-0.477	0.279
Saliva Cortisol and OHIP-14	0.113	0.887	-0.506	0.247

GCF cortisol and PI were negatively correlated in group G ( $r = -0.912$ ,  $p = 0.03$ ), but no correlation was found in group H ( $p > 0.05$ ). GCF cortisol and PD were positively correlated in group H ( $r = 0.783$ ,  $p = 0.037$ ), but no correlation was observed in group G ( $p > 0.05$ )

Table 5. Correlation of BDI, PSS and OHIP-14 scores with periodontal parameters and scales

Correlation	Group G		Group H	
	r	p*	r	p*
BDI and PI	-0.132	0.868	0.338	0.459
BDI and GI	0.565	0.435	-	-
BDI and PD	0.348	0.566	-0.902	0.844
BDI and BOP	0.698	0.302	-	-
OHIP-14 and PI	-0.544	0.566	-0.158	0.736
OHIP-14 and GI	-0.156	0.823	-	-
OHIP-14 and PD	-0.039	0.951	-0.064	0.891
OHIP-14 and BOP	-0.127	0.839	-	-
PSS and PI	-0.304	0.619	-0.502	0.251
PSS and GI	0.139	0.823	-	-
PSS and PD	-0.039	0.951	0.500	0.253
PSS and BOP	-0.127	0.839	-	-

No correlation was found between BDI, PSS, and OHIP-14 and periodontal parameters in each group ( $p > 0.05$ ).

Biochemical analysis identified similarities between the two groups for saliva and GCF cortisol levels ( $p > 0.05$ ) (Figure 1). According to correlation analysis, GCF cortisol and PI were negatively correlated in group G ( $r = -0.912$ ,  $p = 0.03$ ); no correlation was found in group H ( $p > 0.05$ ). GCF cortisol and PD were positively correlated in group H ( $r = 0.783$ ,  $p = 0.037$ ), and no correlation was found in group G ( $p > 0.05$ ) (Table 4). No correlation was found in BDI, PSS, and OHIP-14 and in GCF and salivary cortisol in each group ( $p > 0.05$ ) (Table 4). No correlation was found in BDI, PSS, and OHIP-14 and in the periodontal parameters in each group ( $p > 0.05$ ) (Table 5).

## DISCUSSION

The current study was aimed to investigate the psychological stress and cortisol levels in males with and without gingivitis. Group G showed significantly higher PI, GI, BOP and PD scores compared to group H. Both groups had similar age, smoking and alcohol usage, educational level and socioeconomic status. However, group H reported more regular brushing and flossing & interdental brushing and also visited dentists more regularly once in a year. In addition, they reported to have more knowledge about periodontal disease compared to group G. Both groups had similar psychological stress conducted using questionnaires including BDI, OHIP-14 and PSS and also cortisol levels in GCF and saliva. Thus, the current study

concluded that males with or without gingivitis had similar psychological stress and cortisol levels.

As gingivitis is a prevalent health problem and is observed at higher levels in males than in females, to determine possible risk factors may be significant for prevention and early diagnosis of the disease. Males have been reported to be less informed about brushing and to have less interest in oral health hygiene and to be less aware and interested about oral health and more willing to seek dental help than males (6). However, no study in the literature has compared above mentioned parameters in males with or without gingivitis. According to current results we found that males with gingivitis had less daily brushing and flossing habits and less visited dentists yearly compared to periodontally healthy controls. In addition, the gingivitis group was less aware of periodontal disease. However, it may be suggested that psychological stress and cortisol levels may not contribute to the presence of gingivitis in males.

The relationship between psychological stress and gingivitis has been investigated previously. It has been shown that psychosocial stress may increase of plaque accumulation by inducing neglect of oral hygiene (28). In addition, depression was reported to be weakly related to elevated plaque level, and anxiety was poorly associated with gingivitis (29). However cortisol levels in saliva were not found to be associated with gingivitis (29). In the current study, cortisol levels in both GCF and saliva and psychological stress were not significant in groups G and H. Furthermore, PI levels were not associated with BDI and PSS scores although GCF cortisol levels were found to be weakly correlated with plaque levels. No differences were found between group G and H for GCF cortisol levels, and this correlation appears not to be important. Thus, the current study indicated that plaque-induced gingivitis may not be associated with stress and/or depression in males.

Smoking alone or increased age was reported to increase cortisol levels (30). Most of the studies have reported increased salivary cortisol levels in current smokers compared with never or former ones (30,31). In the current study, the ratio of smokers was high in both groups which means cortisol levels might have influenced by smoking. In addition, periodontal health status has previously been found to be related to work-related demands, marital and socioeconomic status and educational level (32). Nevertheless, both smoking status and age between the two groups were comparable. In this current study, all the participants were unmarried and had a similar socioeconomic status. However, knowledge about periodontal disease was superior in group H, as 23 of the 30 participants in group G reported some knowledge. It may be concluded that, knowledge about the disease may cause less concern about periodontal health and subsequently decrease daily oral hygiene habits. Therefore, it may be a predisposing factor for gingivitis in males.

The current study aimed to investigate GCF and salivary cortisol levels only in males to eliminate gender-based differences. However, the current study has no female population as a control, and this therefore this may be considered as a limitation. Thus, future studies should be performed to compare both genders.

## CONCLUSION

Within the limitations of this study, psychological stress and cortisol levels in gingival crevicular fluid and saliva were not different in males with or without gingivitis.

*Competing interests: The authors declare that they have no competing interest.*

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*Ethical approval: This study was approved by Canakkale Onsekiz Mart University Clinical Research Ethics Committee (approval number #2018-19, approval date 31/10/18).*

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