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The effect of scaling and root planing on salivary cortisol levels in patients with moderate chronic periodontitis

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Abstract

Periodontitis is known as one of the most prevalent inflammatory oral diseases. Glucocorticoid hormones such as cortisol, produced by the hypothalamus–pituitary–adrenal axis can influence all processes and tissues in human body homeostasis. Typically, the glucocorticoids have been regarded as anti-inflammatory substances and have pro-inflammatory effect in the body. The aim of this study was to evaluate the effect of non-surgical periodontal treatment (NPT) on cortisol levels in patients with moderate chronic periodontitis (MCP). Saliva samples were collected from 42 participants with MCP before and two months after scaling and root planing (SRP). Salivary cortisol levels (SCLs) were measured by electrochemiluminescence immunoassay method. The relationship between clinical periodontal parameters such as gingival index (GI) and probing pocket depth (PPD) with cortisol levels was assessed and analyzed by statistical analysis. was statistically analyzed The SCLs were significantly lower after treatment than before treatment ($p=0.02$). The GI and PPD showed significant differences ($p<0.001$, $p=0.001$, respectively). There was a positive relationship between these periodontal parameters and cortisol levels, the correlation between GI and cortisol levels was not statistically significant ($p = 0.19$) and there was a borderline significant correlation between PPD and SCLs ($p = 0.06$). In the present study, the SCLs were related to NPT. The treatment improved clinical periodontal parameters.

Keywords: glucocorticoid, cortisol, scaling and root planing, chronic periodontitis, saliva

1. Introduction

Periodontitis is a chronic multifactorial inflammatory response caused by an interaction between the host system and polymicrobial biofilms, leading to the destruction of the supporting structures of the oral dentition (1). Generally, the periodontitis is classified into two major categories of chronic and aggressive (2). Chronic periodontitis is defined as a relatively slow destruction of the periodontium, characterized by the presence of abundant calculus and plaque (2, 3). It initiates in older age and affects approximately 40% of adults (4) while aggressive periodontitis which is usually asymptomatic and unrelated to calculus and plaque is a rapid destruction of the tissues, supporting the tooth (2, 5). It affects only 5% and is more prevalent in young population, indicating genetic predisposition (4, 5). Periodontitis is associated with systemic diseases and conditions such as diabetes, preterm low birth weights, stroke, rheumatoid arthritis, obesity, alcoholism, cardiovascular disease, inflammatory bowel diseases, pancreatic cancer, dementia and respiratory diseases (1, 6). Poor glycemic control (7), genetic polymorphisms (8), poor oral hygiene, smoking, systemic diseases such as diabetes (9), lifestyle and psychosocial factors can increase the risk of periodontitis (10). Evidence demonstrates that the development of periodontitis may be influenced by the patient's psychological conditions including depression, stress and anxiety (11, 12). Declined cell-mediated immune function has been observed in patients with depression and distress (13).

Cortisol, a glucocorticoid hormone, which is known as a stress biomarker is secreted by the hypothalamic-pituitary-adrenal (HPA) system from the adrenal glands (14, 15). Glucocorticoid actions including pro-inflammatory and anti-inflammatory effects are very complex. However, a study indicates that the type of exposure to glucocorticoids is one of the important factors influencing on them. Chronic exposure to glucocorticoids seems to be immunosuppressive, but acute exposure enhances the peripheral immune response of the body (16). The glucocorticoids can suppress the immune system through decreasing the number of monocytes, eosinophils and

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lymphocytes as well as inhibiting the production of cytokines and accumulation of eosinophils, neutrophils and macrophages at inflammatory sites (14). Addison's disease (glucocorticoid deficiency) and Cushing's syndrome (glucocorticoid excess) are two pathological conditions of imbalanced glucocorticoids (16). Enhanced cortisol levels are related to obesity which increases the risk of osteoporosis, cardiovascular disease and diabetes (17). The cortisol can also help in bone resorption (2).

The cortisol levels can be measured in various body fluids like saliva, gingival crevicular fluid (GCF) and serum (14). Salivary cortisol is reliably free serum cortisol index. Due to the strong association between salivary cortisol levels (SCLs) and unbounded cortisol in the serum, the salivary cortisol is a biomarker used to study the HPA function during stress (8, 9). Moreover, saliva is a practical assessment tool used as a potential source for analysis of biomarkers in periodontitis as well as stress (8). In a study, the relationship between some inflammatory mediators in saliva and periodontal disease was confirmed (10).

Increased glucocorticoid during a long period can suppress immunity via reducing the secretion of immunoglobulin A (IgA) and immunoglobulin G (IgG) as well as the neutrophils function. The mentioned mechanisms may result in the development of inflammation and periodontal disease (18, 19).

Scaling and root planing (SRP) which is effective for periodontal diseases is considered as a non-surgical periodontal treatment (NPT) (20). To our best knowledge, there are only two studies that have examined the effect of NPT on cortisol levels. Cakmak et al. observed the decrease in cortisol levels after the treatment (21). In contrast, Jabali et al. reported increased cortisol levels following the treatment (22). Considering these controversial studies, a few human studies about the effect of periodontal treatment on cortisol levels and importance of biomarkers in periodontal disease; therefore, the aim of this study was to investigate the changes of SCLs following NPT in patients with moderate chronic periodontitis (MCP) in order to prevent obesity and risk of related diseases through decreasing the SCLs after the treatment.

2. Materials and methods

2.1. Patient selection

The ethical approval of this semi-experimental study was obtained from the Ethics Committee of Babol University of Medical Sciences (No.: 1398.004). From September 2019 to February 2020, 42 patients (18 males and 24 females; age range: 25-65; mean age: 44.31), referred to the Department of Periodontology, Faculty of Dentistry, Babol University of Medical Sciences were selected through availability based on the presence of MCP.

Inclusion criteria are as following:

- Age range from 25 to 65 years old (Body mass index between 21-25),
- Absence of systemic disease,
- No history of receiving periodontal treatment within the previous 6 months,
- No history of antibiotic intake during the past 3 months or at the time of study,
- No history of chronic use of corticosteroids or immunosuppressive drugs,
- Absence of visible oral infection and acute illness,
- Having more than 18 teeth (except third molars),
- No smoking and alcohol consumption, and
- No pregnancy and lactation.

Exclusion criteria are as following:

- Being under treatment for psychiatric disorders,
- Existence of psychiatric disorders (anxiety or other psychiatric disorders mentioned by the patient), and (9, 21)
- Existence of any systemic disease, metabolic syndrome, etc.
- Plaque index (PI) more than 20%.

According to the classification of the American Academy of Periodontology (AAP), the patients with MCP were selected based on the presence of at least one tooth with 3-4-mm clinical attachment loss (CAL) (23).

2.2. Periodontal examination

The necessary explanations on the aims of the current study were represented for all participants, the informed written consent was obtained from them before the periodontal examinations and their salivary samples were collected. Patients' demographic information such as age, gender, occupation and frequency of daily brushing was recorded. Clinical periodontal measurements and periodontal treatment were performed by one examiner for all the participants.

The clinical periodontal measurements including the gingival index (GI; Löe & Silness) (24), probing pocket depth (PPD), distance from free gingival margin to the base of the gingival sulcus were recorded 30 minutes before the initiation of NPT. The PPD was recorded at six sites (mid-buccal, mesiobuccal, distobuccal, distolingual mesiolingual and mid-lingual) using the periodontal probe (Goldman/Fox Williams probe, Hu-Friedy, Chicago, IL) (21). The mean score was calculated for the whole mouth of the patients.

After the diagnosis of MCP was confirmed, the unstimulated saliva was collected through spitting method from 8 to 10 a.m. It was preferred to collect the saliva sample in the morning hours in order to decrease any circadian rhythm effects of cortisol (14, 25).

2.3. Saliva sample collection

The amount of the collected saliva was 0.5-1 ml using sterile tubes. All participants were informed not to chew gum, brush, drink or eat for 1 hour before the sample collection in order to avoid salivary contamination. All samples were sent to the Biochemistry Department of Babol University of Medical

Sciences for biochemical testing and measurement of cortisol levels (while preserving cold conditions). The samples were centrifuged at 4°C for 3 minutes at 2500 rpm, and the supernatants were kept in the separate microtubes at -20°C in the freezer until the day of analysis.

2.4. Periodontal treatment

After collecting the primary samples, the NPT including SRP was carried out using Ultrasonic Scaler (DTE D5 LED) with 50 KHZ speed or, if necessary, by manual. Oral hygiene instruction and brushing method (Modified BASS) were presented to all patients twice a day. Periodontal treatment included 2-3 (SRP) sessions at 2-week intervals to ensure periodontal condition has been improved. Periodontal measurements and collection of saliva samples were repeated two months after the periodontal treatment.

2.5. Laboratory assessment

The SCLs were measured in nanomol/liter (nmol/L) by electrochemiluminescence immunoassay method (ECLIA) using Cobas-e-411 autoanalyzer (Roche, USA, REF NO: 06687733, LOT NO: 41946601) based on the standard performance protocol.

2.6. Statistical analysis

The collected data were analyzed using SPSS 24. The pair T-test, independent T-test, covariance analysis and linear regression were used to analyze the data. P value less than 0.05 was considered as significant levels.

3. Results

The study was performed on 42 patients with MCP. Their demographic variables are described in table 1. They were 25-65 years old.

Table 1. Demographic data of patients with MCP

Demographic Variables	Age (years), mean ± SD	Gender, n (%)		Employment status, n (%)		Frequency of daily brushing, n (%)		
		Males	Females	Employment	Unemployment	Once	Twice	Three times
MCP patients	44.31±12.66	18 (42.85%)	24 (57.15%)	20 (47.62%)	22 (52.38%)	9 (21.43%)	27 (64.28%)	6 (14.29%)

SD: Standard deviation

Table 2 summarizes the periodontal status of the patients before and after the treatment. Comparison of clinical periodontal parameters in periodontitis patients illustrated that the pretreatment GI and PPD were higher at baseline, which significantly reduced following the treatment ($p<0.001$ and $p = 0.001$, respectively).

Table 2. Clinical parameters of periodontitis patients before and after the therapy

Parameters	Before treatment	After treatment	p-value*
GI	1.65±0.23	1.41±0.33	<0.001
PPD (mm)	3.38±0.37	3.20±0.34	0.001

*Wilcoxon test

The mean (SD) of SCLs in periodontitis patients before and after treatment was 10.38 (6.17) and 8.02 (5.56), respectively. There was a significant difference in SCLs before and after treatment ($p = 0.02$) (Fig. 1).

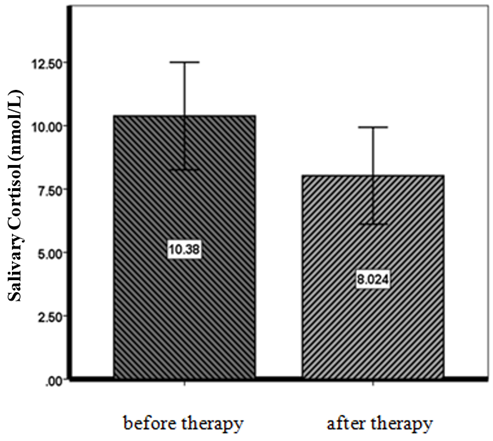

Fig. 1. Salivary cortisol levels before and after treatment; Data are expressed as mean, $p = 0.02$

According to fig. 2 and 3, the clinical parameters (GI and PPD) have a positive correlation with cortisol levels. No statistically significant correlation was found between GI and SCLs ($p=0.19$), but there was a borderline significant correlation between PPD and SCLs ($p=0.06$).

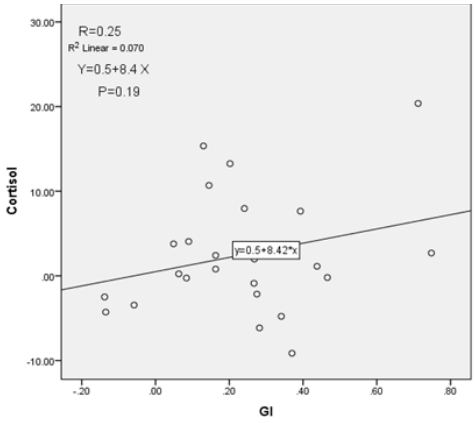

Fig. 2. Correlation between GI and SCLs

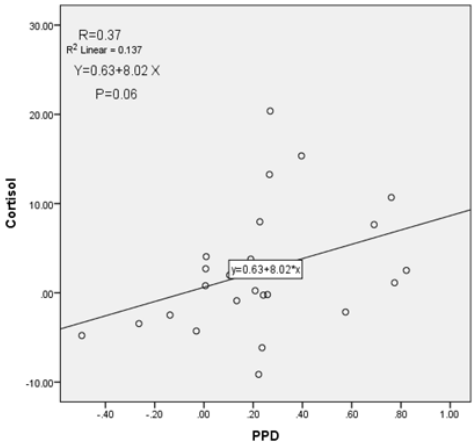

Fig. 3. Correlation between PPD and SCLs

4. Discussion

The present study was conducted to evaluate the effect of NPT on SCLs in MCP patients. It was found that the SCLs were different before and after treatment. Periodontal disease which influences on the hard and soft tissues surrounding the tooth is considered as an inflammatory disease. It accounts for a major proportion of important oral diseases for the World Health Organization (WHO) (26). Some conditions that can reduce the host immune responses may have an effect on the progression of periodontitis so that the cortisol is an immunosuppressive hormone in chronic exposure (9, 16). The cortisol inhibits the increased vascular permeability and vasodilation in inflammatory conditions. Therefore, the leukocytes' migration into the inflamed areas is reduced (27). Furthermore, the cortisol directly affects the expression of genes associated with oral microbes as well as causes periodontal disease and its progression (28). Although destructive effects of cortisol in inflammatory conditions have been reported in some studies, the beneficial and modifying effects of this factor in maintaining homeostasis and augmenting the immune response in acute exposure should not be underestimated (16).

The results indicated that the SCLs in MCP patients significantly decreased following non-surgical treatment. Comparison of clinical periodontal parameters such as PPD and GI before and after the SRP exhibited significant improvement in all these indicators. The ongoing study is one of a few studies that compared cortisol levels before and after treatment in patients with periodontitis. According to the mentioned literature, the present study, for the first time, demonstrated the reduction of SCLs in periodontitis patients owing to periodontal treatment.

Like the current study, Cakmak et al. (21) in 2018 investigated the effect of NPT on GCF cortisol levels in patients with chronic periodontitis. Decreased GCF cortisol levels and clinical parameters such as PI, GI, PPD and bleeding on probing (BOP) in periodontitis patients were found. Though it was difficult to compare the results of the present study with those of other studies due to a few articles, it could be stated that a successful periodontal treatment may lead to a significant reduction in the pathogenic factors and in the release of pro-inflammatory mediators, related to the SCLs reduction. In their study (21), the patients with chronic periodontitis were studied regardless of the severity of periodontitis whereas in the ongoing study, the MCP patients were examined to obtain more reliable results. Moreover, the former study used enzyme-linked immunosorbent assay (ELISA) method that had lower sensitivity than ECLIA, but the latter study (present study) applied ECLIA method for laboratory assessment.

Evidence indicated that the long-term increase of circulating cortisol levels may be associated with chronic low-grade inflammation, because the glucocorticoids lose their inhibition ability to inflammatory responses initiated by the immune system (29). The combination of tendency to inflammation of cortisol and immune system suppression may be the reasons for progression of many chronic diseases such as periodontitis (30). However, the involved biological mechanisms are not yet well realized because of the few available studies on the relationship between cortisol level changes and periodontal treatment.

Jabali et al. (22) compared the concentrations of salivary biomarkers such as cortisol in patients with generalized moderate to severe chronic periodontitis before and after NPT. They reported the increase in SCLs after treatment, which is inconsistent with our results. The sample size of their study was only 18 patients while in the current study, it was larger, and 42 patients were evaluated for changes in SCLs to obtain general results. In their study the ELISA method was applied for laboratory assessment, too. The controversy between the results of the mentioned study and ongoing study can be due to several reasons such as small sample size, temperature of stored samples, storage time of samples before laboratory assessment and centrifuge considerations. Moreover, the existence of anxiety or other psychiatric disorders was not among exclusion criteria in their study; however, the SCLs could be affected by psychiatric disorders.

Interleukin 1 (IL-1) and interleukin 6 (IL-6) are two cytokines that can enhance the secretion of glucocorticoids through the preparation of synthesis as well as the release of corticotropin-releasing hormone (CRH) and adreno-corticotropin hormone (ACTH) in hypothalamus and pituitary gland. They can also elevate the cortisol secretion in adrenal cortex (31). The IL-6 is generated rapidly and temporarily in response to tissue damages and infections. This cytokine plays a supportive role in host defense via stimulating the acute phase responses, hematopoiesis and immune processes (32). The IL-1 mainly regulates inflammation through controlling a variety of innate immune reactions. This cytokine has many biological functions like acting as a leukocyte pyrogenic, fever and leukocyte endogenous mediator, inducer of several components of the acute-phase response and lymphocyte-activating factor (LAF) (33).

According to the above explanations, the decline of inflammation with successful periodontal treatment reduces IL-1 and IL-6 levels, which can decrease the secretion of glucocorticoids such as cortisol. Decreased cortisol levels after the treatment may be due to this mechanism.

The limitations of the present study were sample size and lack of control group. This study can be expanded to include a larger sample size with more parameters and control groups in the future to obtain more reliable results in order to prove the relationship achieved in the present study.

Within the limits of the current study, the SCLs may have been associated with periodontal inflammation. According to the obtained findings, the SCLs were lower in patients after treatment than before treatment. Non-surgical treatment causes

beneficial changes in pocket depth and periodontal ecosystem, eventually leading to the reduction of inflammation and inflammatory factors. Salivary cortisol may be a sign of risk for the severity of periodontal disease.

Although the analysis of the present study demonstrates this relationship, cortisol has a complex nature that many disruptive factors may have affected on its levels. The knowledge about the possibility of periodontal disease and the effect of its treatment on cortisol levels is low, and the details of the relationship have not been obtained yet. Nevertheless, the periodontal treatment appears to create conditions to decrease the secretion and production of cortisol in the body. The pathogenesis mechanism of the periodontal disease in relation to the cortisol levels is unknown. Hence, further studies are needed to clarify the exact mechanism of this relationship and to achieve adequate statistical power.

Ethical Statement

The ethical approval of this semi-experimental study was obtained from the Ethics Committee of Babol University of Medical Sciences (No.: 1398.004; Date: 13.05.2019). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflict of interest

Authors declares that there is no conflict of interest.

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Authors' contributions

Concept: M.A., S.H., M.F., D.Q., M.K., N.N., H.G., Design: M.A., S.H., M.F., D.Q., M.K., N.N., H.G., Data Collection or Processing: H.G., M.F., M.K., D.Q., Analysis or Interpretation: H.G., N.N., Literature Search: N.N., Writing: N.N., D.Q.

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