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SALIVARY AND SERUM CORTISOL IN PATIENTS WITH PERIODONTAL DISEASE AND ORAL LICHEN PLANUS

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Abstract

Introduction. Periodontitis and oral lichen planus are two oral diseases that affect the adult population worldwide.
Aim: A positive relationship had been proposed between psychological stress, periodontitis and oral lichen planus. The general aim of our study was to investigate the levels of "cortisol", the stress- related hormone in patients with periodontal disease and oral lichen planus.
Methodology. Our study included 20 patients with chronic periodontitis, 20 with oral lichen planus and 20 healthy subjects. We collected non-stimulated whole saliva and serum from patients and healthy subjects. Serum and salivary cortisol was measured using the ELISA method.
Results. In both patients with chronic periodontitis and oral lichen planus, we obtained, in statistical terms, increased levels of salivary and serum cortisol versus the control group $p<0.05$.
Discussions. Psychological stress may influence the evolution and progression of this two oral diseases. Cortisol can be regarded as the most useful biomarker to evaluate stress.
Conclusions. Psychological stress may be regarded as risk factor for periodontal disease and oral lichen planus progression.

Keywords: psychological stress, oral diseases, cortisol, biomarker

Introduction

Periodontitis, is a nonreversible inflammatory disease affecting the tooth supporting tissues. This chronic disease is caused by pathogenic microorganisms, three organisms in particular, Tanerella forsythensis, Porphyromonas gingivalis, and Treponema denticola, which have been directly associated with chronic periodontitis (1-3). Others factors can influence the appearance and evolution of this disease are oxidative stress and psychological stress (4-8). Psychological stress may be involved in the inflammatory responses and changes in the composition of dental biofilm (9). Oral lichen planus (OLP), a chronic inflammatory mucutaneous disease of unknown etiology, affects mainly the adult population (approximately 0.02-4%). In the pathogenesis of OLP genetic, infectious, autoimmune factors (10-12) seem to be involved.
Cortisol, the "stress hormone" is the most widely-used biomarker in stress evaluation studies. Among other fluids the free fraction or the biological active form can be readily detected in saliva (13). Saliva is currently used as a diagnostic tool for systemic and oral diseases, because of its numerous advantages: easy to collect, non-invasive technique and no special equipment for collection (14, 15).
The aim of our study was to detect salivary and serum levels of cortisol in patients with chronic periodontitis and OLP. To the best of our knowledge this is the first study to assess salivary cortisol levels in both periodontitis and oral lichen planus patients.

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Materials and methods

Periodontal disease patients

The study included twenty patients with chronic periodontitis (5 males and 15 females, with an average age of 51.26±7.4). The ethics board of "Carol Davila" University of Medicine and Pharmacy, Faculty of Dental Medicine, reviewed and approved our study. All clinical examinations were performed by one qualified examiner from the Department of Periodontology. Classical clinical parameters were measured: PD (probing depth), plaque index (PI), bleeding index (GI). GI and PI are expressed as percentage (%).

Oral Lichen Planus patients:

20 patients with OLP (clinical type: Keratosis and Atrophic/erosive lesions) including 15 males; 5 females aged between 18–68 years. The clinical examinations were performed at the Oral Pathology Department, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest. Twenty healthy subjects with no gingival inflammation, good oral hygiene and no history of periodontal disease and OLP made up the control group.

Saliva and blood collection:

Non-stimulated whole saliva has been collected between 9 and 10 a.m, (1.0–2.0 ml). Saliva collected in sterile test tube was centrifuged at 3000 rpm for 10 min. At the same time 5 ml of blood were collected and the serum was immediately obtained. Both saliva and serum cortisol were detected using the ELISA method.

Salivary and serum cortisol assays: A microtitre plate is coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase (HRP) for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme with the substrate tetramethylbenzidine (TMB). This reaction generates a blue color. After stopping the reaction with sulfuric acid, a yellow color is generated. We read the optical density on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is measured by the intensity of its color and is inversely proportional to the amount of cortisol present.

Statistical analysis

Data distributions were expressed as means, standard deviations (SD), ranges, and percentages, as appropriate. The Pearson's correlation coefficient and ANOVA test were used. A p-value < 0.05 was considered statistically significant.

Results

Our results reflect statistically increased salivary levels in patients with periodontal disease and OLP compared with controls.

Increased serum levels for cortisol were obtained in patients with periodontal disease and OLP

Table 1. Mean values for salivary cortisol in patients with periodontal disease

Parameter / Cortisol	Patients	Control group	P
Periodontal disease patients (ng/mL)	10,1±1,03	3,83±0,87	<0.0001
OLP patients (ng/mL)	10,13±1,056	3,83±0,87	<0.0001

Table 2. Mean values for serum cortisol in patients with periodontal and OLP versus controls

Parameter / Cortisol	Patients	Control group	P
Periodontal disease patients (ng/mL)	9,25±1,97	3,17±0,63	<0.001
OLP patients (ng/mL)	9,08±1,056	3,17±0,63	<0.001

versus healthy subjects.

Discussion

Saliva is an excellent natural ultrafiltrate that contains local substances as well as others derived from the blood. In saliva we can detect a myriad markers such as: cytokines (TNF-α, IL-6, IFN-γ, MIP-1β), C Reactive Protein (CRP), antioxidant biomarkers (uric acid, malondialdehyde, antioxidant enzymes, total antioxidant capacity) insulin resistance markes (adipokines) or cortisol (13, 14,16).

Steroids, such as cortisol, not bound by carrier proteins, can diffuse freely into saliva, being the free fraction. The concentration of cortisol in saliva is independent of the salivary flow rate and strongly correlated with circulating cortisol concentration. In the reference literature, cortisol is the most common used indicator of stress, released by the hypothalamo-pituitary-adrenal (HPA) axis, under the influence of several factors such as chronic inflammation (13,17).

The most important effects of cortisol release are: supression of the inflammatory response, modifying cytokine profiles, elevation of blood glucose levels and alteration of certain growth factors levels(18, 19). Low levels of cortisol were detected in patients with primary adrenal insufficiency (Addison's disease) and ACTH deficiency (20,21). Higher levels of cortisol were found in patients with systemic (Cushing's disease, malignancy) and oral diseases (22-34).

In healthy subjects, cortisol is higher in the morning (0.20-1.41 μg/mL), compared to the afternoon values (0.04-0.41 μg/mL). Salivary concentrations reflect the activity of HPA axis (35) very well. Salivary cortisol levels reflect endocrine abnormalities, insulin resistance, hypertension, dyslipidemia and type 2 diabete (13).

Previous studies reported higher levels of this biomarker in patients' saliva. Salivary cortisol was detected from stimulated, unstimulated whole saliva and from gingival crevicular fluid. The results were the same: higher levels of cortisol in all different forms of periodontitis (aggressive, chronic periodontitis). Anxious patients with chronic periodontitis present higher levels of cortisol and anxiety. Anxiety may be regarded as an important factor in the progression of periodontal diseases (8, 9, 25-30).

In our study we detected cortisol from un-stimulated whole saliva and serum and detected increased levels with patients with chronic periontitis versus healthy subjects.

During periods of stress it is believed that oral hygiene is neglected and is associated with attachment loss and missing teeth. Cortisol exercise inhibitory effects on the inflammatory immune response, because IL-12 is inhibited and IL-10 is stimulated by macrophages. These changes have major effects on the immune and inflammatory response and conduce to increased

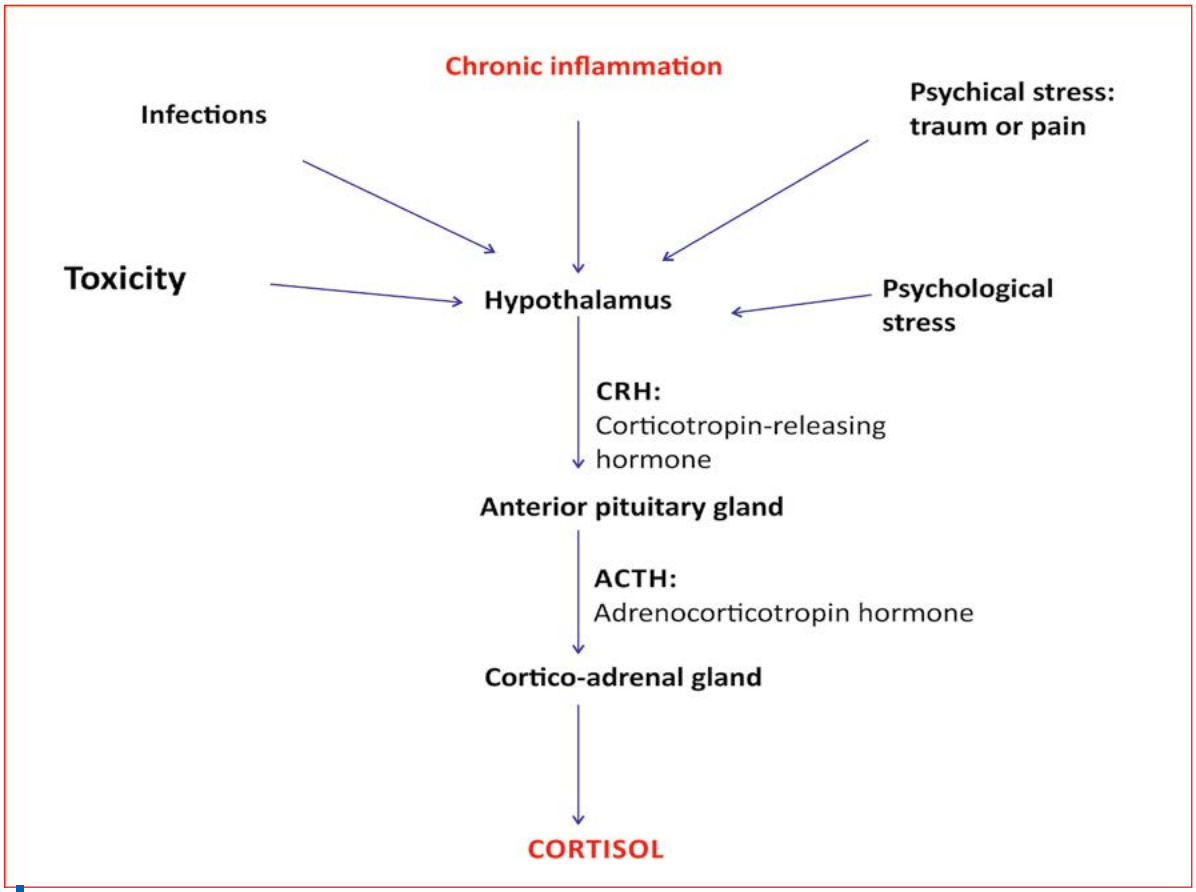

Figure 1. Cortisol release factors by HPA axis (17)

susceptibility to pathogenic microorganisms (18, 19, 36).
Positive correlation between stress and cortisol, stress and clinical parameters, and cortisol levels and patients with chronic periodontitis had been observed by Goyal S et al (30). At the same time Shah et all, observed a positive correlation between psychological factors and salivary cortisol levels in the OLP patients (34). Giardini and co-workers, indicated an association between OLP and anxiety, but salivary cortisol levels did not differ between patients with OLP and control group (33).
In our study, salivary and serum levels of cortisol were statistically increased in OLP patients versus healthy subjects. Until now there are no studies regarding the serum level of cortisol at patients with OLP.
The World Health Organisation (WHO) classifies OLP as a "potentially malignant disorder" and suggests that OLP patients should be under close monitoring. OLP patients present higher levels of anxiety, depression because there are concern about the malignant transformation of this oral diseases (37).
Chronic inflammation leads to cortisol release that counteracts inflammatory reactions and even immunological functions, so this stress hormone can be involved in the pathogenesis of OLP and periodontal disease. In conclusion psychological stress may influence the progression of these two oral diseases.

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CV

Her PhD, focused on the study oxidative stress as a new pathological biochemical mechanism in oral diseases, using saliva as a new diagnostic fluid in oral diseases.
In the spring of 2011, during an exchange program, worked at Università Politecnica delle Marche, Italia, under the direct supervision of Maurizio Battino, PhD, DSc, MS, MD (Hon), focusing on the biochemical mechanisms of oxidative stress and antioxidants.
Her current research interests are, oxidative stress, oral stem cells properties, nanoparticles, and their possible interaction with the oral stem cells and generation of oxidative stress.

Questions

Regarding this study:

❑ a. The general aim of our study was to investigate the levels of cortisol in stimulated whole saliva
❑ b. 50 patients were included in our study
❑ c. Psychological stress may influence the evolution and progression of periodontal disease and oral lichen planus
❑ d. Salivary cortisol was measured using RT-PCR method

Regarding the patients included in our study:

❑ a. 1.0-2.0 of non-stimulated whole saliva and 5 ml of blood were collected from patients
❑ b. Optical density was read at 550 nm
❑ c. 40 patients with OLP with the age between 61–68 years
❑ d. 15 males and 5 females formed the patients with periodontal disease

Regarding the salivary levels:

❑ a. All the results were statistically decreased
❑ b. The cortisol at patients with periodontal disease was 10.1±1.03
❑ c. The cortisol at patients with oral lichen planus was 9.08±1.056
❑ d. The cortisol at patients with periodontal disease was 3.17±0.63

Regarding the serum levels:

❑ a. Levels for cortisol were not statistically significant
❑ b. The cortisol at patients with periodontal disease was 3.17±0.63
❑ c. The cortisol at patients with oral lichen planus was 9.25±1.97
❑ d. The cortisol at patients with oral lichen planus was 9.08±1.056