Relationship of psychological factors with salivary flow rate and cortisol levels in individuals with oral lichen planus: A case-control study

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Objective. The objective of this study was to analyze the relationship between psychological factors and salivary flow rate and cortisol levels in individuals with oral lichen planus.

Study Design. A case-control study of 21 individuals with clinical and histopathological diagnosis of oral lichen planus and 21 individuals without the disease (controls), matched by sex and age, was performed. The Beck Anxiety and Depression Inventories and Perceived Stress Scale were administered, and the salivary flow rate and salivary cortisol secretion pattern were determined. Data were statistically analyzed using Mann-Whitney *U*, McNemar's χ^2, and Student's *t* tests and the Spearman correlation coefficient. The significance level adopted was 5%.

Results. Individuals with oral lichen planus had higher anxiety ($P = .001$), depression ($P = .005$), and perceived stress ($P = .026$) scores, but no association was found between the disease and salivary flow rate ($P = .29$) or with the pattern of salivary cortisol secretion (at awakening: $P = .98$; 30 min post-awakening: $P = .95$; at bedtime: $P = .97$).

Conclusion. An association was observed between oral lichen planus and anxiety, depression, and stress scores through psychological tests but not in relation to salivary flow rate and the pattern of salivary cortisol secretion. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;130:675−680)

The oral cavity may reflect the general state of health of an individual because many systemic diseases, physical or psychological, manifest in the mouth.[1] Studies in the area of psychoneuroimmunology point to the effect of acute and chronic psychological stressors on the immune system and their role in the onset and progression of immune-mediated disorders.[2]

Oral lichen planus (OLP) is considered a chronic immune-mediated disease, with manifestation in the oral cavity. Its etiology is unclear and psychological factors, such as anxiety, depression, and stress, seem to play an important role in the development and/or progression of this condition.[3]

Some authors suggest that individuals with OLP present a higher level of anxiety, depression, and stress[4] and more frequently report the development or exacerbation of the lesions during a period of emotional tension.[5] In contrast, others point to controversies in the relationship between OLP and psychological factors.[6]

To elucidate the role of psychological factors in the etiopathogenesis of OLP, some authors have investigated the activity of the hypothalamic-pituitary-adrenocortical axis (HPA) in individuals with this lesion by quantifying salivary cortisol. However, results were divergent.[6-8]

The cortisol present in saliva reflects the free fraction of plasma cortisol. Its collection is noninvasive and fast and can be done by volunteers at home, facilitating the evaluation of its levels at different times throughout the day.[9] The collection of saliva also allows quantification of salivary flow, a physiologic variable that would theoretically be decreased in stress situations due to the increase and decrease, respectively, of sympathetic and parasympathetic tonus on the salivary glands.[10]

Thus, the objective of this study was to analyze the relationship between psychological factors (anxiety, depression, and stress) and salivary flow rate and cortisol levels in individuals with OLP.

MATERIAL AND METHODS

Study Design

This matched case-control study was reviewed and approved by the institutional review board of the State University of Feira de Santana (Protocol Number: 114.132). All participants read and signed an informed

Statement of Clinical Relevance

Oral lichen planus was associated with anxiety, depression, and stress but not with salivary flow rate and cortisol levels.

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consent form, and the study was performed in accordance with the Declaration of Helsinki.

Efforts were made to minimize bias typical of case-control studies. Bias due to case patient selection was reduced using an acknowledged case definition and specific eligibility criteria. Bias due to selection of controls was reduced by accurate examination to exclude OLP. Cases were matched to controls based on age and sex to eliminate possible confounding due to age-related decline in immune function or any hormonal variation.

Participants

A total of 820 patients were referred to the Reference Center for Oral Lesions in the Department of Health Sciences, State University of Feira de Santana, by dentists who detected changes in their oral mucosa, and patients were examined between 2016 and 2017 by 3 examiners (J.C., J.S., V.F.). Of these, 23 patients had the clinical diagnosis of OLP.

All data were collected between 6 months and 1 year after the biopsy. On the days of collection, patients were free of pain. OLP diagnosis was established by a clinician and oral pathologist (V.S.) based on typical clinical features and histopathological examination, according to criteria established by Van der Meij and Van der Wall.[11] Histologic diagnosis was confirmed in 21 patients.

A convenience sample was formed including 42 patients who completed follow-up and were analyzed, divided as follows: (1) OLP group: 21 patients with histopathological confirmation and who had no painful symptoms; (2) control group: 21 patients recruited from the dentistry clinics at the State University of Feira de Santana with a similar socioeconomic level. Controls were matched with cases by age (with a variation of ±5 years) and sex in a 1:1 proportion.

Exclusion criteria included (1) other oral mucosal disorders such as burning mouth syndrome; (2) any history of malignant neoplasm or autoimmune diseases, such as lupus erythematous, rheumatoid arthritis, and Sjögren's syndrome, or other systemic disease, including diseases of the endocrine or metabolic systems, especially those related to cortisol production, Cushing's disease and Addison's disease; (3) taking any anabolic steroids, corticosteroids or immunosuppressants, antidepressants, anxiolytics, oral contraceptives, or xerogenic medications in the previous 30 days; (4) pregnancy or currently on hormone replacement therapy for menopause, and smoking and alcoholism.

Investigation of Psychological Factors

The presence and intensity of anxiety and depression were determined using the Beck Anxiety Inventory (BAI)[12] and Beck Depression Inventory (BDI).[13] The Perceived Stress Scale (PSS 14)[14] was used to evaluate the presence of stress.

The BAI and BDI scores were computed by adding the responses given by the participant for each of the 21 items. Each item consisted of a group of affirmations with response choices from 0 to 3, with the maximum total score of 63. According to this score, anxiety and depression were classified as minimal (0 to 9), mild (10 to 16), moderate (17 to 29), or severe (30 to 63). The PSS 14 included 14 questions, with scores ranging from 0 to 4 and a maximum score of 56. Higher scores indicated higher levels of stress, with no cut off point.

The BAI and BDI scores were dichotomized as the absence (minimal and mild levels) or presence (moderate and severe levels) of anxiety and depression, constituting the variable denominated an anxiety-depressive component. For the PSS, the mean was computed for each group and values equal to or above the mean were considered to indicate the presence of stress.

Determination of Salivary Flow Rate and Secretory Pattern of Cortisol

Unstimulated saliva samples were collected between 8 AM and 10 AM. Upon arrival, participants were advised regarding collection. In summary, they were instructed to abstain from drinking, eating, chewing gum, and brushing their teeth for an hour before determining salivary flow rate. They rinsed their mouths with 20 mL of distilled water and were seated in ordinary chairs, with their elbows resting on their knees and head lowered without movement, allowing the saliva to drain in a preweighed plastic container for 15 min. Each container was then weighed again and, by discounting the container weight and dividing by the collection time, the salivary flow rate (mL/min) was obtained. Salivary density was considered equal to 1 g/mL, and a normal flow for unstimulated saliva ranged between 0.25 and 0.35 mL/min.[15]

For the cortisol dosage, participants performed 3 daily saliva collections: at awakening, 30 min post-awakening, and at bedtime, using appropriate devices for this procedure (Salivettes; Sarstedst, Nümbrecht, Germany). Volunteers kept a cotton roller under the tongue for 3 min at each collection time and then immediately inserted the roller into the polyethylene tube, which was closed and kept under refrigeration in the freezer until delivery to the responsible researcher (A.P). Volunteers were instructed not to drink alcohol or caffeine for 12 h before collection, not to ingest or place any substance in their mouth or brush their teeth for 1 h before saliva collection, and not to perform strenuous physical activity before collection. In the laboratory, the materials were centrifuged (1000 × g, 4°C, 2 min) so that the saliva was removed from the cotton roller, and the saliva was then aliquoted into microtubes and frozen (−20°C) until analysis.

Salivary cortisol analysis was performed by enzyme-linked immunosorbent assay using a specific kit (Salimetrics, State College, PA, USA). The concentration of

cortisol (μg/dL) was calculated in each saliva sample, based on the absorbance values of the calibration curve using Galapagos software for EZ read microplate readers (Biochrom, Waterbeach Cambridge, UK).

Data Analysis

Data are presented as means and standard deviation or, if they did not satisfy the assumption of normality, as medians and interquartile ranges (25th and 75th percentiles). Evaluation of the data distribution was assessed by the Shapiro-Wilk test.

The Mann-Whitney *U* test was used to compare both groups on anxiety, depression, and stress scores and the mean of salivary cortisol secretion pattern at awakening, 30 min post-awakening, and at bedtime. McNemar's χ^2 test was used to compare groups regarding the anxiety-depression component and perceived stress. The Friedman test evaluated the cortisol secretion pattern throughout the day.

Cortisol levels were quantified by 2 indices: the cortisol awakening response (CAR), which was defined by subtracting 30 min post-awakening cortisol levels from cortisol levels at awakening. Diurnal decline was defined by subtracting cortisol levels 30 min post-awakening from bedtime cortisol levels. Student's *t* test was used to compare the means of CAR, diurnal decline, and salivary flow rate between groups.

The relationship between cortisol and scores for anxiety, depression, stress, and salivary flow were examined using the Spearman correlation coefficient. The level of significance used was 5%, where $P \leq .05$ was considered significant. Analyses were carried out using the Statistical Package for the Social Sciences software, version 17.0.

RESULTS

Study Population

The mean age of the OLP group was 49.19 (±13.97) years and the mean age of the control group was 48.10 (±13.82). In both groups, 71.4% of patients were female and 28.6% were male. Further, 85.7% of the cases showed a combination of reticular and plaque forms of the disease (n = 18), and 14.3% presented with atrophic-erosive lesions (n = 3). The lesions varied in several sites of the oral cavity, affecting the buccal mucosa in most cases (52.38%).

Symptoms of Depression, Anxiety, and Stress

Statistically significant differences were observed in anxiety, depression, and perceived stress scores (Mann-Whitney *U*, P = 0.001, P = .005, P = .026, respectively; Table I). A statistically significant association was also seen for the anxiety-depression component (McNemar's χ^2, odds ratio [OR] = 5.84; 95% confidence interval [CI], 1.06-32.08), as well as the presence of perceived stress (McNemar's χ^2, OR = 4.0; 95% CI, 1.11-14.80; Table II).

Table I. Median (interquartile range) of anxiety, depression, and perceived stress scores in individuals with OLP and controls

Scores	*OLP group*	*Control group*	*P value**
Anxiety	11.0 (1-3)	3.0 (1-3)	.001
Depression	12.0 (6-11)	3.0 (6-12)	.005
Perceived stress	26.0 (15-26)	17.0 (12-17)	.026

OLP, oral lichen planus.
*Mann-Whitney *U* test.

Salivary Flow Rate and Cortisol Levels

Individuals with OLP had a mean salivary flow of 0.27 (±0.24) mL/min and the control group had a mean salivary flow rate of 0.21 (±0.14) mL/min (Student's *t* test, P = .29).

A significant difference was observed in the variation in salivary cortisol secretion pattern throughout the day (Friedman, P = .00). Figure 1 shows the mean levels of salivary cortisol at different collection times.

There was no statistically significant difference in the means of salivary cortisol secretion pattern at awakening, 30 min post-awakening, at bedtime or in CAR or diurnal decline (Table III).

A positive correlation was shown between CAR and perceived stress scores (ρ = 0.32; P = .04; Table IV).

DISCUSSION

The present study indicates that individuals with OLP have significantly higher levels of depression, anxiety, and stress than controls. However, there was no difference in salivary parameters.

To evaluate the psychological factors, the Beck and PSS inventories were used, considering the reliability,

Table II. Anxiety-depression and perceived stress in individuals with OLP and controls

Variables	*OLP group*		*Control group*		*OR*	*CI (95%)*
	n	%	*n*	%		
Anxiety-depression						
With anxiety-depression	08	38.10	02	8.52	5.8	1.06-32.08*
Without anxiety-depression	13	61.90	19	90.48	*	
Perceived stress						
With perceived stress	15	71.42	06	39.13	4.0	1.11-14.80*
Without perceived stress	08	38.08	13	60.87		

OLP, oral lichen planus; *OR*, odds ratio; *CI*, confidence interval.
*McNemar's χ^2 test.

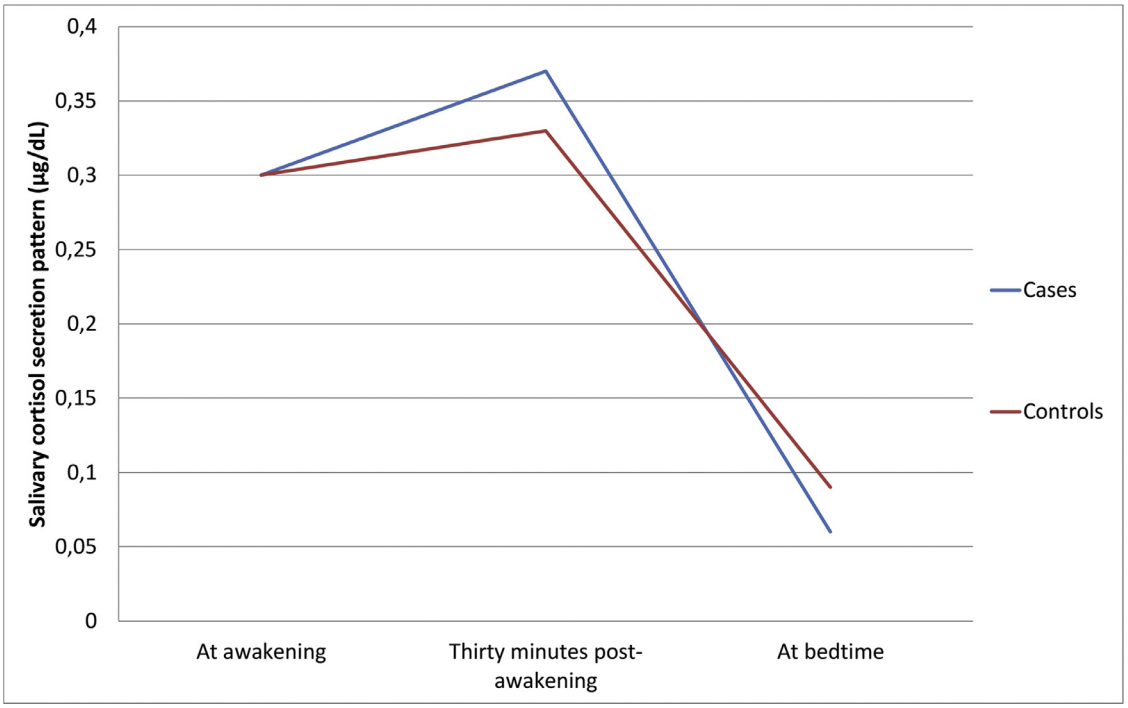

Fig. 1. Mean levels of salivary cortisol secretion pattern at awakening, 30 min post-awakening, and at bedtime.

Table III. Salivary cortisol secretion pattern (μg/dL) in individuals with OLP and controls

Concentration	*OLP group Mean (±SD)*	*Control group Mean (±SD)*	*P value*
At awakening	0.30 (±0.12)	0.30 (±0.15)	.98*
Thirty minutes post-awakening	0.37 (±0.21)	0.33 (±0.14)	.95*
At bedtime	0.06 (±0.06)	0.09 (±0.09)	.97†
Cortisol awakening response	0.07 (±0.22)	0.03 (±0.17)	.25†
Diurnal decline	0.30 (±0.23)	0.24 (±0.21)	.38†

OLP, oral lichen planus.
*Mann-Whitney *U* test.
†Student's *t* test.

internal consistency, and validity of these instruments, and because they are self-evaluative and quick to apply.[16,17] Our findings show a nearly 6-fold greater chance (OR = 5.8) of individuals with OLP being anxious and depressed compared to the control group. In a study by Pippi et al.,[18] depression was highly associated (OR = 4.7, $P < .0001$) with OLP, especially in the reticular and papular clinical forms. However, Girardi et al.[6] diverge in their results.

In addition, there was a 4-fold greater chance (OR = 4.0) of stress among individuals with OLP in our study. Lundqvist et al.[19] found, in addition to depression and anxiety, a strong correlation between OLP and stress using the PSS. In a study by Čanković et al.,[20] it was revealed that with each new stressful experience, the odds of developing OLP (OR = 1.97, $P < .001$) increased almost twice. The controversies and difficulty of establishing causality between OLP and psychological factors may be related to the use of different measurement instruments, as well as subjectivity, lack of a methodological standard, and the low statistical power of the studies because of small sample sizes.[6,18] Salivary biomarkers have been used to obtain more objective patterns of psychological factors measurement.[6]

Cortisol is produced by the adrenal glands, and its action on the body influences metabolism, immunoregulation, blood pressure, cognition, and behavior and has an effect on some pathologic conditions, including inflammatory autoimmune diseases.[8,21] Cortisol in plasma can

Table IV. Spearman's correlation coefficient between salivary cortisol secretion pattern and depression, anxiety, and stress scores and salivary flow

Concentration	*Depression*		*Anxiety*		*Stress*		*Salivary flow*	
	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*
Cortisol awakening	0.12	.47	0.16	.31	0.32	.04	0.24	.12
At bedtime	−0.14	.37	−0.12	.42	−0.23	.14	0.17	.28

be free (5%-10%) or bound to plasmatic proteins. Saliva, like urine, contains free cortisol. The correlation between free cortisol in plasma and saliva is positive and significant ($R = 0.54$ and $P < .0001$).[22] According to Laudat et al.,[23] salivary cortisol measurements are an excellent index of plasma free cortisol concentrations. They reported that normal patients had a mean (range) salivary cortisol concentration of 0.558 μg/dL (0.367-0.982) at 8 AM and 0.140 μg/dL (0.079-0.147) at 8 PM.

The concentration of cortisol at awakening is higher and decreases throughout the day, reaching lower concentrations before bedtime. A normal daytime cortisol rhythm consists of an acute increase for 30 to 40 min after awakening, referred to as the CAR. This peak is followed by a gradual decline throughout the day, reaching the lowest levels before bedtime. It has been suggested that CAR is an important preparation of the body to meet the demands of the day.[24] For example, CAR has been shown to be higher on working days compared to on rest days.[25]

In this study, the cortisol secretion pattern showed an average increase of 23.3% in cases and 10% in controls between awakening and 30 min post-awakening, followed by a bedtime decline, in agreement with the pattern previously described by Hellhammer et al.[24]

A comparison of the salivary cortisol secretion pattern between collection times showed no statistically significant difference in the concentration of this hormone, as evidenced by Girard et al.[6] and Pippi et al.[26] Contrary to these findings, Koray et al.[7] found significantly higher levels of salivary cortisol in patients with OLP. However, in their study, saliva collection was only performed before the biopsy procedure, which may have induced increased stress and consequent elevation of cortisol secretion. Shah et al.[8] reported higher levels of stress, anxiety, depression, and cortisol in patients with OLP.

In our study, cortisol levels did not differ between individuals with OLP and controls, though there was a significant difference in the levels of perceived stress after the psychological test. A possible explanation for these findings is that in some autoimmune diseases, such as lupus erythematous and rheumatoid arthritis, elevated levels of stress would increase activation of the sympathetic nervous system and reduce the response of the HPA axis, with a decrease in its final product, cortisol,[27] which could contribute to maintenance of the inflammatory process.[28] This would explain, at least in part, why patients with stress and OLP, an immune-mediated disease, did not present elevated levels of cortisol compared to the control group in our study.

Another possibility is that chronic stress may result in hypocortisolism.[29] In other words, initially stressful events would increase the response of the HPA axis. However, as time went on, the maintenance of these events would result in adaptation of the HPA axis and decrease of cortisol secretion. Because individuals with OLP in the present study presented with the disease for more than 6 months, we could therefore suggest that, at the time of saliva collection, they were already in the process of adapting the HPA axis and, thus, the levels of cortisol would not be higher.

In addition, our results show that the unstimulated salivary flow rate of patients with OLP (0.27 ± 0.24) is within normal range (0.25 to 0.35 mL/min)[15] and is slightly below normal in controls (0.21 ± 0.14), with no statistically significant difference between the groups. Therefore, increased perceived stress was not able to reduce salivary flow in individuals with OLP. The salivary glands are innervated by the autonomic nervous system, and sympathetic stimulation is responsible for protein secretion in saliva, whereas parasympathetic stimulation is responsible for increased salivary flow. For some authors, the subdivisions of the autonomic nervous system present antagonistic and mutually inhibitory actions on the target organs. Thus, stress would result in greater sympathetic tonus on the salivary glands and, consequently, inhibition of parasympathetic stimulation, resulting in decreased salivary flow.[10] However, some studies have shown that stress may decrease,[30] not alter[31] or even increase, salivary flow.[10] Furthermore, it has been suggested that dehydration that occurs during prolonged physical exercise has a greater influence on the decrease in salivary flow rate than neuroendocrine regulation.[32] It is noteworthy that in the present study, saliva collection took place at home, at rest, which minimizes the chances of volunteers becoming dehydrated.

Differences in the results found regarding the salivary cortisol secretion pattern in OLP studies could relate to the size of the samples, the use of different methods with variations in the cortisol collection and dosing protocol, as well the inclusion criteria, thus limiting generalization and data comparison.

This study should be considered in the light of some limitations, taking it as a preliminary exploratory study that had a small convenience sample, in which generalizations or inferences are limited to the researched population, in addition to memory bias that is inherent to the design of the case-control study model. In addition, patients with OLP were not differentiated based on disease type (reticular versus erosive). Such differentiation of patients may be important because of the severity of symptoms in erosive OLP, which may potentiate emotional tension in patients.

The use of cortisol as a salivary biomarker in individuals with immune-mediated diseases should be analyzed with caution. These findings indicate the need to investigate new biomarkers, involving other axes of the autonomic nervous system that may better reflect the response to stress in individuals with OLP. Hence, future studies with larger samples, which may also include other biomarkers, are needed to investigate the

possible ways in which psychological factors could be related to the etiopathogenesis of OLP.

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PRESENTATION

This study was presented at the 95th General Session & Exhibition of the IADR (International Association for Dental Research), San Francisco, CA, in 2017 (ID 1052).

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