

Original Paper

Is Salivary A-Amylase a Reliable Indicator of Psychological Status and Quality of Life in Patients with Oral Lichen Planus: a Case-Control Study

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Key Words

Alpha-amylase • Biomarkers • Oral lichen planus • Quality of life • Saliva

Abstract

Background/Aims: The objectives of our study were to determine salivary α -amylase activity (stress biomarker) and its association with psychological status and quality of life (QoL), disease duration and intensity of symptoms (pain/burning) in patients with OLP. **Methods:** A total of 50 subjects participated in this case-control study: 30 patients with oral lichen planus (OLP); 20 control subjects. Unstimulated whole saliva (UWS) was collected between 9 and 10 am to avoid diurnal fluctuations. Psychological status was assessed using the Croatian validated version of the original Depression, Anxiety and Stress Scale (DASS-21). The impact of oral health on QoL was assessed using the Croatian version of the Oral Health Impact Profile Questionnaire (OHIP-CRO14). **Results:** There was no statistically significant difference in salivary α -amylase activity between patients with OLP (N=30) and control subjects (N=20) (133813.3 vs. 166815.5 U/L, $p=0.314$; t-test). Depression, anxiety and stress showed no statistically significant difference between patients with OLP and control subjects ($p=0.076$, $p=0.111$, $p=0.209$; t-test). The patients with OLP had statistically significantly poorer QoL (total) compared to control subjects ($p=0.004$, t-test). There was a moderate positive correlation between symptom intensity (pain/burning) and poor QoL (total) ($r=0.584$, $p<0.001$), the OHIP-CRO14 dimension "physical pain" ($r=0.661$, $p<0.001$), "psychological impossibility" ($r=0.555$, $p<0.01$), "handicap" ($r=0.546$, $p<0.01$). **Conclusion:** Although salivary α -amylase showed no statistically significant

difference between patients with OLP and control subjects, the patients with OLP had poorer psychological status (three times higher scores for depression and two times higher scores for anxiety) and poorer QoL compared to the control subjects. Recognising and treating mental disorders in patients with OLP is important in order to break the „vicious circle“ and achieve a better QoL in these patients.

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Introduction

Oral lichen planus (OLP) is a chronic autoimmune mucocutaneous disease. It is a disease of unknown aetiology in which genetic and environmental factors are involved. It is assumed that cellular immunity plays a key role in the development of OLP. The role of psychological stress is not clear [1]. Numerous studies show a dysregulated immune response in OLP, suggesting the possibility of autoimmunity [2]. Hepatitis C virus (HCV) is the only microorganism convincingly associated with OLP, and only in some geographical areas [3]. In patients with OLP, particularly with the reticular or erosive form of the disease, a type 1 immune response has been observed, resulting in damage to the epithelium of the oral mucosa [4]. This response involves plasmacytoid dendritic (pDC) and myeloid dendritic cells, CD4+ and CD8+ T-cells, natural killer (NK) cells and mast cells, and is dominated by soluble factors characteristic of the type 1 immune response [interferons, interleukin 12 (IL-12), tumour necrosis factor alpha (TNF- α)] [5]. Important studies investigating the oral microflora and the receptors that control the antimicrobial response are currently at an early stage [6, 7]. To date, it is not clear whether the destruction of keratinocytes in OLP is due to autoreactivity or a dysregulated response to an exogenous antigen.

The incidence of OLP is up to 2.2% worldwide [8]. The clinical picture of OLP is diverse and several clinical forms can occur in one patient, making an accurate diagnosis difficult. Despite the diverse clinical picture, the histopathological diagnosis of OLP is unique. Microscopic features of OLP include hyperparakeratosis, hyperorthokeratosis and their combination; cytoid (*Civatte*) bodies; hydropic degeneration of the basal layer of the epithelium and, most importantly, a dense band-like inflammatory lymphocytic infiltrate in the lamina propria [9]. In case of uncertainty or doubt, an active discussion between an oral medicine specialist and a pathology specialist is always recommended. Reported rates of malignant transformation of OLP vary from 0.4% to 12.5%, with an average rate of 1.1% reported in a recent meta-analysis and systematic review of 7,806 patients in 16 studies [10, 11]. OLP has a significant negative impact on quality of life (QoL) [8, 12].

Several studies have provided direct evidence that salivary α -amylase activity correlates with changes in blood catecholamine concentrations and thus serves as a surrogate marker of stress, indicating changes in acute psychosocial stress [13, 14]. Studies have shown that salivary α -amylase activity is associated with stressful conditions and that its activity increases during physical (treadmill training, running, cycling) and psychological stress (academic stress in students) [15, 16]. The effects of psychological and physical stress on the functions of the immune system are well known. By understanding these interactions, psychoneuroendocrinology (PNEI) contributes to a better understanding of the association between mental and physical health. Psychological factors can alter the course of various diseases by affecting different levels of the endocrine and immune systems [17]. Patients with OLP reported experiencing more stressful life events and scoring higher on anxiety and depression tests than control subjects [18]. Salivary biomarkers (such as α -amylase) represent an attempt to quantify and objectify stress mechanisms using simple and non-invasive diagnostic methods.

The objectives of our study were to determine salivary α -amylase activity, psychological status and QoL, disease duration and intensity of symptoms (pain/burning) in patients with OLP. The hypothesis of our study was that higher salivary α -amylase activity correlates with poorer mental health, poorer QoL, longer disease duration and higher intensity of symptoms (pain/burning) in patients with OLP.

Materials and Methods

Study Design and Subjects

A total of 50 subjects took part in this case-control study: 30 patients with a clinically and histopathologically confirmed diagnosis of OLP; 20 control subjects. The group of patients with OLP consisted of 15 patients with an erosive form and 15 patients with a non-erosive form of the disease. The control group consisted of randomly selected healthy patients who came to the Department of Diagnostic Radiology, Dental Clinic Split, Split, Croatia. Patients with diseases of the oral mucosa and/or systemic diseases, smokers and underage patients (<18 years) were excluded from the study.

Anamnestic data, a list of medications, the disease duration (months) and the topography of the lesions according to the WHO classification were collected from all subjects [19]. A visual analogue scale (VAS, from 0 to 100 mm) was used to assess the intensity of pain and/or burning (0 = no pain/burning, 100 = worst possible pain/burning) in patients with OLP. The same oral medicine specialist (A.G.) performed a clinical oral examination and an incisional/punch biopsy of the oral mucosa. The study protocol was explained to each subject, and after signing the informed consent form, they were enrolled in the study. The study lasted three months (from April 2023 to June 2023) and was conducted with the approval of the Ethics Committee of the School of Medicine, University of Split, Split, Croatia (study of Dental Medicine) (approved on 28 April 2023) (class: 003-08/23-03/0015; registration number: 2181-198-03-04-23-0025). The study was conducted in accordance with the principles of the Declaration of Helsinki (1964) and its subsequent amendments.

The inclusion criterion for OLP was:

1. Patients with a clinically and histopathologically confirmed diagnosis of OLP according to the modified WHO criteria [20].

The exclusion criteria for OLP were:

1. Patients with a history of systemic and/or autoimmune diseases and/or cancer;
2. Pregnant women;
3. Medication (corticosteroid, immunosuppressants, psychoactive therapy within the last three months), hormone therapy;
4. Harmful habits such as chewing betel nuts or tobacco or smoking;
5. Cutaneous lichen planus (LP);
6. High blood pressure;
7. Patients with inflammatory diseases of the oral cavity (gingivitis, parodontitis);
8. Patients who have not understood the purpose of the study and the content of the informed consent form.

Saliva Sampling

Unstimulated whole saliva (UWS) was collected between 9 and 10 am according to the following protocol:

1. Patients were asked to refrain from intense physical activity and mental exhaustion for three days before sampling;
2. Patients were asked to stop eating, drinking, smoking and brushing their teeth 90 minutes before sampling;
3. In women of reproductive age, the UWS was taken during the follicular phase of the menstrual cycle;
4. Approximately 2.00 to 2.50 mL of saliva was collected from each subject in graduated tubes (*Salivette*) (ref. 51. 1534.500, SARSTEDT AG & Co. KG, Nümbrecht, Germany) using the "spit method" (subjects collected saliva in the mouth for 60 seconds and then spit it out into a graduated tube; the process was repeated for a further ten minutes).

The samples were sent to the Department of Medical Laboratory Diagnostics, Clinical Hospital Centre Split, Split, Croatia, and then centrifuged at 1500 x g for five minutes. The saliva samples were stored at -20°C until they were used for study purposes. For the determination of salivary α -amylase activity, the frozen samples were first stored at room temperature for 30 minutes and then centrifuged at 1500 x g for five minutes. The salivary α -amylase activity was measured using the kinetic colourimetric method on the Roche/Hitachi cobas c 701/702 Systems instrument with reagents from the same manufacturer. Haemolysis in saliva was tested by visual inspection and saliva samples containing blood were excluded from the study [21].

Instruments

Depression, Anxiety and Stress Scale (DASS-21)

Psychological status was assessed using the Croatian validated version of the original Depression, Anxiety and Stress Scale, the DASS-21 scale (Lovibond & Lovibond, 1995a; Croatian adaptation Jokić-Begić, Jakšić, Ivezić and Surányi, 2012) [22]. The same researcher (A.G.) collected data through interviews. The three-factor structure scale (depression, anxiety, stress) consists of 21 statements, each of which contains seven statements. The depression subscale refers to symptoms such as dysphoria, hopelessness, self-deprecation, apathy and disinterest. The anxiety subscale refers to arousal of the autonomic system and situational anxiety. The stress scale includes indicators for chronic, non-specific arousal, relaxation difficulties, restlessness, impatience, etc. They are used to assess the level of depression, anxiety and stress in the last seven days. The DASS-21 scale proved to be an instrument with high internal reliability (reliability coefficients 0.90 vs. 0.89 vs. 0.91). The items are rated on a four-point Likert scale (0 - does not apply to me at all, to 3 - applies to me almost completely or mostly). The results obtained for the subscales must be multiplied by two. The maximum score for each subscale is 42. A higher score on each of these scales means poorer mental health [23].

Croatian version of the Oral Health Impact Profile Questionnaire (OHIP-CRO14)

The impact of oral health on QoL was assessed using the OHIP-CRO14. The OHIP-14 questionnaire consists of 14 questions, which can be divided into seven dimensions according to Locker's theoretical model of oral health: Functional impairment, Physical pain, Psychological discomfort, Physical impossibility, Psychological impossibility, Social impossibility and Handicap. These seven dimensions can be divided into three categories, with functional impairment, physical pain and physical impossibility belonging to the physical factor, psychological discomfort and psychological impossibility to the psychological factor and social impossibility and handicap to the social factor. Subjects were asked how often they had experienced a particular problem in the last month. The answers were categorised using a Likert scale: 0 - never, 1 - almost never (rarely), 2 - occasionally, 3 - relatively often, 4 - very often. Lower scores on the scale indicate good oral health, while higher scores indicate poorer oral health, i.e. a poorer QoL [24, 25].

Statistical Analysis

Data were collected, coded and entered into Microsoft Excel, version 16.0 (Microsoft Corporation, Washington, USA). Data were analysed using IBM SPSS Statistics software, version 23.0 (IBM Corporation, New York, USA). We used an absolute number with a percentage to represent categorical variables and an arithmetic mean with standard deviation (AM±SD) to represent ordinal variables. The age of the subjects was expressed as median (M) with interquartile range (IQR). The chi-square (χ^2) test was used to compare between groups for categorical variables, while the t-test was used to compare the AM of independent variables. We used the Pearson correlation test to examine a statistically significant linear relationship between two numerical variables. A three-way ANOVA with Wald correction was used to determine the influence of the different variables on the DASS-21 subscales (depression, anxiety, stress). The images (Figures 1 and 2) were processed using the Paint programme (Microsoft Corporation, Redmond, USA). The significance level was set at 0.05.

Results

Study Subjects

The sample of OLP patients consisted of 30 subjects aged 36 to 86 years, median 60.0 (IQR 50.8 to 75.0). The control group consisted of 20 subjects aged 41 to 80 years, median 61.0 (IQR 51.3 to 72.0). In both groups, the majority of subjects were women (66.7% vs. 65.0%). There was no statistically significant difference between the groups in terms of age and gender ($p=0.945$ and $p=0.903$, respectively) (Table 1).

The mean disease duration in patients with OLP was 38.0 ± 59.2 and the mean VAS score was 1.9 ± 2.6 . The most common topographic localisations of OLP according to the WHO scheme were 19 and 20, i.e. the mucosa of the right cheek (86.7%) and the mucosa of the

left cheek (83.3%). In a quarter of the patients with OLP, the attached gingiva of the maxilla and mandible on the right and left, the premolar and molar region (WHO 27, 28, 29, 30) was affected (Figures 1 and 2).

Salivary Stress Biomarker

There was no statistically significant difference in salivary α -amylase activity between patients with OLP (N=30) and control subjects (N=20) (133813.3 vs. 166815.5 U/L, $p=0.314$; t-test). In both subject groups (OLP, control subjects), there was no statistically significant difference in salivary α -amylase activity between men and women ($p=0.779$ and $p=0.794$, respectively). There was also no statistically significant difference in salivary α -amylase activity between patients with erosive and non-erosive forms of OLP (142682.7 vs. 124944.0 U/L, $p=0.560$).

Psychological status and QoL

Depression, anxiety and stress showed no statistically significant difference between patients with OLP and control subjects ($p=0.076$, $p=0.111$, $p=0.209$; t-test). Stress was the leading mental disorder, i.e. it showed the highest scores in patients with OLP and control subjects. The patients with OLP had three times higher scores for depression and two times higher scores for anxiety than control subjects (Table 2). Women had statistically significantly higher scores for depression, anxiety and stress compared to men in patients with OLP ($p=0.008$, $p=0.002$, $p=0.010$) and the control group ($p=0.015$, $p=0.033$, $p=0.034$).

The patients with OLP had statistically significantly poorer QoL (total) compared to control subjects, as measured by the OHIP-CRO14 ($p=0.004$, t-test). The patients with OLP had statistically significantly higher scores on the dimensions (OHIP-CRO14) "physical pain", "psychological discomfort", "physical impossibility" and "handicap" compared to control subjects ($p=0.002$, $p=0.043$, $p=0.040$, $p=0.011$, t-test) (Table 3). Compared to men, women had statistically significantly poorer QoL (total) in patients with OLP (11.4 vs. 4.2, $p=0.018$; t-test).

Table 1. Demographic data. * t-test for independent samples, † Chi-square test. Abbreviations: IQR, interquartile range; M, median; OLP, oral lichen planus

	OLP Group N=30	Control Group N=20	P
Age [years; M (IQR)]	60.0 (50.8-75.0)	61.0 (51.3-72.0)	0.945*
Gender [female; n (%)]	20 (66.7%)	7 (65.0%)	0.903†

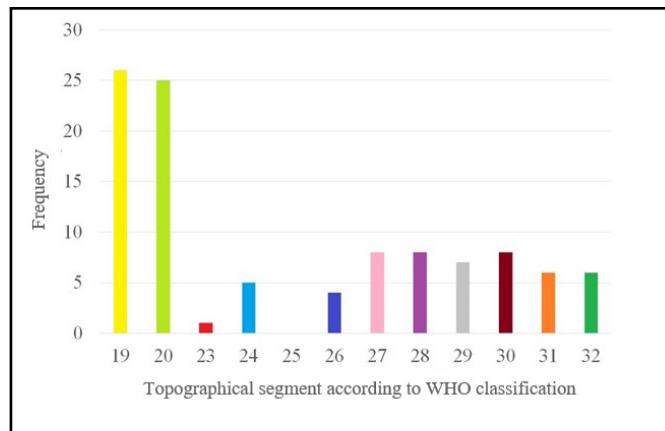


Fig. 1. Frequency of involvement of parts of the oral mucosa according to the WHO classification.

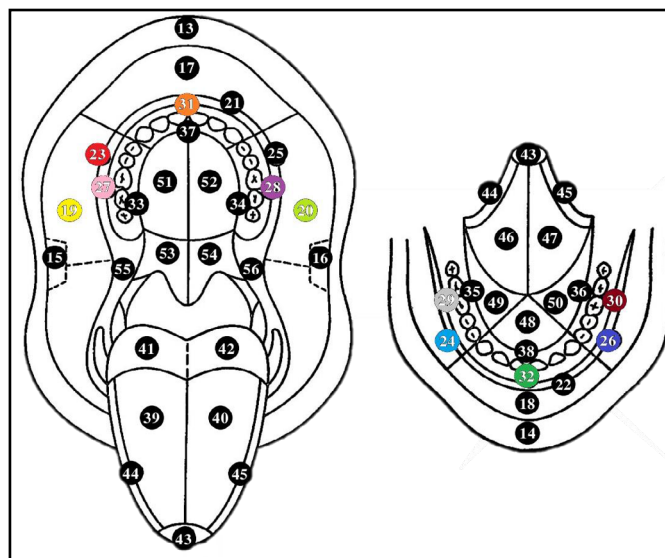


Fig. 2. The most common topographical localisations of OLP according to the WHO classification.

There was a weak/moderate positive correlation between the disease duration and the intensity of symptoms (pain/burning) ($r=0.409$, $p<0.05$) and the OHIP-CRO14 dimension “psychological impossibility” ($r=0.455$, $p<0.05$). There was a moderate positive correlation between symptom intensity (pain/burning) and poor QoL (total) as measured by the OHIP-CRO14 ($r=0.584$, $p<0.001$), the OHIP-CRO14 dimension “physical pain” ($r=0.661$, $p<0.001$), “psychological impossibility” ($r=0.555$, $p<0.01$), “handicap” ($r=0.546$, $p<0.01$). There was a moderate positive correlation between poor QoL (total) and depression ($r=0.651$, $p<0.001$), anxiety ($r=0.653$, $p<0.001$), stress ($r=0.587$, $p<0.001$) (Table 4).

Multiple linear regression was performed for depression, anxiety and stress as dependent variables, while the form of OLP (erosive/non-erosive), age, gender, salivary α -amylase, disease duration and VAS were covariate factors in patients with OLP. There was a statistically significant effect of form of OLP ($p=0.041$) and VAS ($p=0.015$) on the level of depression and a statistically significant effect of gender ($p=0.024$) on the level of anxiety in patients with OLP (Table 5).

Erosive and Non-Erosive forms of OLP

There was a statistically significant difference in VAS between patients with erosive and non-erosive forms of OLP (3.5 vs. 0.3; $p<0.001$). Patients with an erosive form of OLP had statistically significantly higher scores on the OHIP-CRO14 dimension “physical pain” compared to patients with a non-erosive form of OLP (2.7 vs. 0.8; $p=0.012$) (Table 6).

Table 2. Psychological status of patients with OLP and control subjects. * t-test for independent samples. Abbreviations: AM, arithmetic mean; OLP, oral lichen planus; SD, standard deviation

	OLP Group N=30 (AM±SD)	Control Group N=20 (AM±SD)	p
Depression	6.2±9.2	2.7±4.2	0.076*
Anxiety	6.6±9.8	3.3±4.2	0.111*
Stress	11.7±11.1	8.1±9.0	0.209*

Table 3. QoL of patients with OLP and control subjects. * t-test for independent samples. Abbreviations: AM, arithmetic mean; OHIP-CRO14, oral health impact profile; OLP, oral lichen planus; QoL, quality of life; SD, standard deviation

	OLP Group N=30 (AM±SD)	Control Group N=20 (AM±SD)	p
OHIP-CRO14	9.0±8.9	3.1±4.8	0.004*
Functional limitation	1.2±1.4	0.6±1.2	0.087*
Physical pain	1.7±2.1	0.4±0.8	0.002*
Psychological discomfort	2.5±2.4	1.4±1.6	0.043*
Physical impossibility	1.1±2.0	0.3±0.8	0.040*
Psychological impossibility	1.1±1.8	0.5±1.1	0.128*
Social impossibility	0.5±1.4	0.0±0.0	0.062*
Handicap	0.8±1.4	0.1±0.2	0.011*

Table 4. Presentation of r values of Pearson’s correlation of characteristics in patients with OLP. * Pearson correlation test † $p < 0.05$, ‡ $p < 0.01$, § $p < 0.001$. Abbreviations: OHIP-CRO14, oral health impact profile; OLP, oral lichen planus; VAS, visual analogue scale

	Age (years)	α -Amylase (U/L)	Disease duration (months)	VAS	Depression	Anxiety	Stress
α -Amylase	0.270						
Disease duration	0.217	0.094					
VAS	0.194	0.129	0.409†				
Depression	0.216	-0.118	-0.052	0.293			
Anxiety	0.204	-0.025	-0.090	0.080	0.746§		
Stress	0.167	0.018	-0.057	0.214	0.825§	0.873§	
OHIP-CRO14	0.155	-0.097	0.155	0.584§	0.651§	0.653§	0.587§
Functional limitation	0.272	-0.060	-0.087	0.135	0.528§	0.670§	0.564†
Physical pain	0.113	-0.048	0.220	0.661§	0.282	0.174	0.217
Psychological discomfort	0.715	0.049	0.223	0.344	0.565§	0.598§	0.538†
Physical impossibility	-0.149	-0.268	-0.140	0.285	0.276	0.281	0.210
Psychological impossibility	0.201	-0.001	0.455†	0.555†	0.362†	0.251	0.250
Social impossibility	0.122	-0.049	-0.123	0.322	0.699§	0.843§	0.736§
Handicap	0.246	-0.138	0.092	0.546†	0.637§	0.603§	0.541†

Table 5. Results of three-way ANOVA for depression, anxiety, stress as dependent variables. Abbreviations: ANOVA, analysis of variance; OLP, oral lichen planus; VAS, visual analogue scale

Covariates	Depression	Anxiety	Stress
	p	p	p
Form of OLP	0.041	0.080	0.103
Age	0.145	0.207	0.381
Gender	0.054	0.024	0.059
α -Amylase	0.294	0.780	0.954
Disease duration (months)	0.526	0.824	0.701
VAS	0.015	0.241	0.084

Table 6. Comparison of salivary α -amylase activity, psychological profile and QoL between patients with erosive and non-erosive forms of OLP. * t-test for independent samples, † Chi-square test. Abbreviations: AM, arithmetic mean; IQR, interquartile range; M, median; OHIP-CRO14, oral health impact profile; OLP, oral lichen planus; QoL, quality of life; SD, standard deviation; VAS, visual analogue scale

	Erosive OLP N=15 (AM±SD)	Non-Erosive OLP N=15 (AM±SD)	p
Age [years; M(IQR)]	63.0(53.0-76.0)	55.0(47.0-73.0)	0.244*
Gender [female; n(%)]	11(73.3%)	9(60.0%)	0.439†
α -Amylase (U/L)	142682.7±80250.5	124944.0±84400.3	0.560*
Disease duration (months)	57.1±77.6	18.9±20.9	0.084*
VAS	3.5±2.6	0.3±1.3	<0.001
Depression	5.7±10.7	6.7±7.8	0.787*
Anxiety	5.2±9.6	8.0±10.3	0.446*
Stress	11.1±10.4	12.4±12.0	0.747*
OHIP-CRO14	11.1±10.1	6.8±7.2	0.189*
Functional limitation	1.1±1.5	1.3±1.4	0.803*
Physical pain	2.7±2.3	0.8±1.3	0.012*
Psychological discomfort	3.1±2.2	2.0±2.6	0.231*
Physical impossibility	1.5±2.5	0.7±1.3	0.320*
Psychological impossibility	1.4±2.3	0.9±1.2	0.437*
Social impossibility	0.5±1.6	0.5±1.2	0.900*
Handicap	0.9±1.7	0.7±1.2	0.709*

Discussion

OLP is a disease of unknown aetiology in which (among others) psychological difficulties play a certain role (cause or consequence). It is a disease of PNEI origin. Therefore, in this study we analysed salivary α -amylase activity as a stress biomarker and its association with disease duration, intensity of symptoms (pain/burning), psychological status and QoL.

In our study, there was no statistically significant difference in salivary α -amylase activity between patients with OLP and control subjects ($p=0.314$). There are few studies on their association, and most do not support the association between salivary α -amylase activity and OLP. The results of the study by Silva Simoura JA et al. showed increased salivary α -amylase activity [26]. Pippi R et al. and Glavina A et al. showed no statistically significant difference in salivary α -amylase activity between patients with OLP and control subjects [27, 28]. Their results are consistent with the results of our study.

The discrepancies in the study results on salivary α -amylase activity can be explained by the small number of studies performed, the heterogeneity of the diagnostic criteria for OLP, the lack of a comprehensive tool to distinguish between the different clinical forms of OLP, the different wake-up times of the subjects and the different times of sample collection. The inclusion and exclusion criteria for the control group were also not clearly defined in the studies. The studies by Pippi R et al. and Glavina A et al. define the inclusion criteria for the control group as „healthy“ so that the results can be compared with ours [27, 28]. The objective association between acute stress and salivary α -amylase activity has been confirmed in studies [13, 14]. The role of salivary α -amylase as a mediator of chronic stress needs to be clarified in future studies. Since OLP is a chronic disease, we did not demonstrate a relationship between α -amylase and chronic stress in our study.

When interpreting the results of different studies, it is necessary to know the daily fluctuations of salivary biomarkers. Salivary α -amylase has a distinct daily excretion profile. Salivary α -amylase activity decreases sharply in the first 30 minutes after waking and then increases steadily towards the afternoon and evening [29]. Studies on salivary α -amylase activity have used different units of measurement (i.e. U/ml, μ U/L, ng/ml, IU/L) that could not be converted into a single unit of measurement. Therefore, no meta-analysis was performed [30]. Most studies that analysed salivary biomarkers used UWS, a smaller number of studies used stimulated whole saliva (SWS), while individual studies used both types of whole saliva. It is important to note that studies that collected both types of whole saliva obtained similar concentrations/activities of salivary biomarkers. Therefore, we can conclude that UWS is a reference for conducting future controlled longitudinal studies with standardised diagnostic criteria [30].

The patients with OLP did not have a poorer psychological status compared to the control subjects ($p=0.076$, $p=0.111$ and $p=0.209$ respectively). The results of the studies on the relationship between psychological status (depression, anxiety, stress) in patients with OLP are inconsistent. However, a large number of studies support the association between OLP and psychological disorders. The medical history of patients with OLP often indicates that the exacerbation of OLP lesions was preceded by a stressful event [18]. Shah B et al., Chaudhary S et al., Araya MS et al., Shetty S et al., Pires ALPV et al. showed higher scores for depression, anxiety and stress in patients with OLP compared to control subjects [31-35]. Their results are not consistent with the results of our study. Patients with OLP show discrepancies in depression, anxiety and stress scores. This could be due to the fact that no standardised diagnostic tool was used to identify mental disorders and that the inclusion and exclusion criteria of the control group were not defined. Most studies specified “healthy” as the inclusion criterion for the control group, while only one study by Araya MS et al. used a double control (positive and negative) [31-35]. The results of our study showed that patients with OLP had three times higher scores for depression and two times higher scores for anxiety compared to control subjects. Patients with OLP who have psychological problems and need medical help very often deny this condition. There are several reasons for this: shame, stigmatisation, fear of misunderstandings in the family, side effects of psychotropic drugs. Mental disorders have a significant impact on the poor QoL of these patients, which was initially affected by the diagnosis [33]. Psychological assessment and treatment of patients with OLP is as important as oral therapy to control the disease. It is necessary to involve psychiatric specialists and psychologists in the diagnosis of these comorbidities (depression, anxiety, stress).

The QoL (total), measured with the OHIP-CRO14, was statistically significantly different between patients with OLP and control subjects ($p=0.004$). Patients with OLP had statistically significantly poorer QoL compared to control subjects (9.0 vs. 3.1). The patients with OLP had statistically significantly higher scores in the OHIP-CRO14 dimensions “physical pain”, “psychological discomfort”, “physical impossibility” and “handicap” compared to the control subjects. The physical factor category was most strongly affected in patients with OLP. This could therefore indicate a secondary effect of depression, anxiety and stress rather than a causal effect (although this cannot be established in a case-control study). There was a moderate positive correlation between the disease duration and the psychological factor category (psychological impossibility, $r=0.455$, $p<0.05$). There was a moderate positive correlation between symptom intensity (pain/burning) and poor QoL (total) in patients with OLP ($r=0.584$, $p<0.001$). The symptom intensity (pain/burning) showed a moderate positive correlation with all three categories (physical, psychological, social) (physical pain, $r=0.661$, $p<0.001$; psychological impossibility, $r=0.555$, $p<0.01$; handicap, $r=0.546$, $p<0.01$). This indicates that all three factors (physical, psychological, social) are involved, i.e. that the QoL of patients with OLP who have symptoms (erosive form) is severely restricted. This leads to a “vicious circle” in which poor QoL and social isolation become perpetuating factors that further and repeatedly exacerbate the clinical picture and the worsening of OLP symptoms. The OHIP-CRO14 dimension “physical pain” showed statistically significantly higher values in patients with erosive OLP compared to non-erosive OLP ($p=0.012$).

Our study has several limitations: small sample size; determination of salivary α -amylase activity at a single time point; different sensitivity of the kits in the different studies performed. The determination of haemoglobin or transferrin are more reliable methods for the detection of blood in saliva. Although they are much more sensitive than visual inspection, the accuracy of these methods can be affected by various factors such as age, hormones and salivary flow [29]. In our study, the exclusion criterion was drug therapy (corticosteroids, immunosuppressants, psychoactive therapy) three months before inclusion in the study. However, it is known that some medications can have a long-term effect [36].

Future longitudinal studies should use a standardised methodology: UWS and standardisation of the method for determining salivary α -amylase activity (international measure for assessing salivary α -amylase activity), use of agreed international diagnostic

criteria for OLP, consideration of ethnicity and prevalence of clinical forms of OLP, consideration of sensitivity of kits and inclusion of a double control group (positive and negative).

Conclusion

The patients with OLP did not show higher salivary α -amylase activity or a correlation with psychological status and QoL. However, this does not mean that salivary α -amylase is not an indicator of chronic psychosocial stress when the characteristics of the stressed control group are taken into account. The patients with OLP had three times higher scores for depression and two times higher scores for anxiety, i.e. poorer psychological status. The patients with OLP had a poorer QoL (in terms of physical, psychological and social factors) compared to the control subjects. The DASS-21 can be a useful tool for the early detection of mental disorders, highlighting the need to involve psychiatric specialists (psychologists) in the therapeutic approach. In this way, the “vicious circle” can be broken and a more successful treatment of these patients can be ensured.

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Author Contributions

Conceptualization, Supervision, Writing – original draft, Writing – review & editing, A.G.; Conceptualization, Funding acquisition, Supervision, L.L.M.; Data curation, Funding acquisition, Methodology, L.C.; Data curation, Formal analysis, Methodology, D.Š.D.; Investigation, Writing – original draft, A.Z.; Conceptualization, Supervision, Visualization, A.T. All authors approved the final content for journal submission and publication.

Statement of Ethics

The study protocol has been approved by the research institute’s committee on human research. The study was conducted with the approval of the Ethics Committee of the School of Medicine, University of Split, Split, Croatia (study of Dental Medicine) (approved on 28 April 2023) (class: 003-08/23- 03/0015; no: 2181-198-03-04-23-0025). The study was conducted in accordance with the principles of the Declaration of Helsinki (1964) and its subsequent amendments.

Disclosure Statement

The authors have no conflicts of interest to declare.

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