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Assessment of salivary opiorphin in oral lichen planus

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Opiorphin is a pentapeptide, which could be isolated from human fluids and has a decreasing effect on pain. **Aim:** Since lichen planus is a chronic mucocutaneous disease, which causes pain or burning feeling in the oral mucosa, this study aimed to compare salivary opiorphin levels of oral lichen planus (OLP) patients with healthy subjects. **Methods:** This case-control study, was performed on 24 patients with OLP lesions and 21 healthy subjects. After collecting un-stimulated saliva, opiorphin levels were compared between two groups through statistical analyses. **Results:** There was not any significant difference between OLP patients and healthy subjects according to salivary opiorphin concentration (p=0.378). Also, in the OLP group, opiorphin concentration was not significantly different between males and females (p=0.601). Analytical analysis could not show any remarkable difference between various severity of OLP lesions regarding to salivary opiorphin levels (p=0.653). **Conclusion:** In this study, salivary opiorphin levels was not significantly different between patients with OLP and healthy subjects; however, more studies are suggested for better assessment of salivary opiorphin levels in various types of OLP lesions and its correlation with pain severity.

Keywords: Oligopeptides. Lichen planus, oral.

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Introduction

Opiorphin is a pentapeptide, which could be isolated from human fluids including saliva[1].

It seems that opiorphin inhibits encephalin- inactivating enzymes and increases half-life of encephalin[2]. Moreover, opiorphin could inhibit nociception due to chronic and acute stimulation, and perception of pain, this effect is compatible with morphine[1].

Since, this peptide has a preventive effect on pain, there is a hypothesis, which depict that every chronic disease imposes chronic pain or discomfort, might influence on opiorphin levels.

Lichen planus is a chronic mucocutaneous disease, in which, the patients mostly tolerate a long time burning and pain in their oral mucosa. Despite huge progression about etiology of OLP, the exact mechanism and definite treatment of this condition is unclear.

The researchers have been trying to find some acceptable and noninvasive methods for diagnosis and assessment of efficacy of the treatments. Up to now, several studies have been performed on some salivary biomarkers in OLP patients; however, none of them evaluate salivary opiorphin, as an index of chronic pain.

This study was conducted to investigate salivary opiorphin concentrations of OLP patients and compare this marker with healthy subjects.

Methods and Materials

This case-control study was performed in the oral and maxillofacial medicine department of Mashhad Faculty of Dentistry from September 2018 until June 2019.

Twenty-four patients with OLP (both keratotic and non-keratotic), and 21 healthy subjects, after signing a written informed consent form, enrolled in this study.

Diagnosis of OLP was confirmed through clinical and histopathological examinations, according to WHO modified criteria[3].

The healthy individuals were selected from the patients, who were referred to Mashhad Faculty of Dentistry, who had no oral lesions or systemic diseases, or any pain; they had only been referred for common dentistry procedures.

The inclusion criteria for the case group were: newly diagnosed oral lichen planus patients, as confirmed by clinical and histopathological examination; patients who had signed the informed consent form. The exclusion criteria for the case group were as bellow: the subjects, who had used any drugs interacting with opioids or analgesics; the patients, whose histopathological examination was reported as lichenoid reactions. The exclusion criteria for the control group was the usage any medicine for relief of the pain, in recent days.

Prepared checklists were completed for all subjects including demographic characteristics consisting of age, gender, and medical history; lesion properties including the site of lesion, clinical features (keratotic and non-keratotic), type and severity of

OLP lesion, based on Thongprasom criteria[3]. The patients were categorized into five categories 20-30, 30-40, 40-50, 50-60, and ≥60 years according to their age. The correlation of opiorphin levels, and sex and age groups was statistically analyzed.

Assessment of opiorphin

All subjects were asked not to drink, eat, or smoke for 90 minutes before collecting unstimulated saliva. Saliva samples were collected through spitting method between 9 to 12 a.m. The patients were asked to sit in a convenient position and after gathering the saliva in their mouth, spit into 50 ml falcon sterilized tube every 1 minute for 5-10 minutes[4]. The salivary samples were stored with ice and sent to the laboratory and kept at -20°C for 24 hours for mucolysis. In order to separate debris and mucous, the saliva samples were centrifuged for 15 minutes at 3000 rpm. Until collecting all samples and analysis, the samples were kept at -20°C.

After that, the watery parts of the salivary samples were transferred to microtubes with a volume of 1.5 cc. Then opiorphin levels were measured using the kits of "Human Opiorphin ELISA Kit (Catalog # MBS760008)" according to the protocol of the company.

The bioten double antibody sandwich was used for the assessment of opiorphin. First, 40 µL of the salivary sample and 10 µL of opiorphin antibody were added to 50 µL of standard solution and 50 µL of streptavidin-HPR; after that, the mixed solution was incubated at 37ºC for 60 min.

Next, this solution was washed with 300 µL diluted buffer five times. Then, 50 µL of chromogen A and 50 µL of chromogen B solutions were added to prepare the samples. After mixing them, the samples were incubated at 37ºC for 10 minutes.

Finally, 50 µL of stop solution was added to them, which change the yellow color to blue color. After 10 minutes, the optical density was measured at the wavelength of 450 nm to assess the opiorphin concentration.

The opiorphin levels were compared between healthy subjects and OLP patients through statistical analyses.

Statistical analysis

The data were entered into SPSS software (version 24) and statistical analysis was performed. Kolmogorov-Smirnov test was employed for quantitative data with normal distribution. In order to assess the qualitative variables between the groups, Chi-do and Fisher's exact test were used. For quantitative variables with normal distribution, parametric statistical methods including independent t-test and ANOVA, and for nonparametric methods in case of abnormal distribution, Mann-Whitney test and Kruskal-Wallis were employed. The significance level for the statistical tests was considered as 0.05.

The protocol of this study was approved by ethical committee of Mashhad University of Medical Sciences (the ethical code: IR.MUMS.DENTISTRY.REC.1397.045).

Results

In this study, salivary opiorphin levels of 24 OLP patients and 21 healthy subjects were assessed.

Kolmogorov-Smirnov test showed that the variables of age and opiorphin have normal distribution. Therefore, independent t- test was used for assessment of these variables.

The mean ages of OLP patients and healthy persons were 46.41±11.89 and 46.09±9.12 years, respectively. Statistical analysis revealed that there was no any significant variations between the two groups regarding the mean age (p=0.920).

Also, more number of OLP patients belonged to 50-60-year group; however, chi square test showed that there was no remarkable correlation between development of OLP and subjects' age (p=0.389) (Table 1).

Table 1. demographic data and opiorphin levels in the case and control groups.

Variables Number (%)		Case group	Control group	p-value
		Number (%)		
Sex	Male	6(62.1)	10(37.5)	p=0.114*
	Female	18(37.9)	11(62.5)	
	Total	24(100)	21(100)	
Age (years)	20-30	3(12.5)	0(0)	p=0.389*
	30-40	5(20.8)	8(38.1)	
	40-50	6(25)	6(28.6)	
	50-60	8(33.3)	6(28.6)	
	≥60	2(8.3)	1(4.8)	
	Total	24(100)	21(100)	
Opiorphin		2.76±0.66	2.93±0.55	0.378**

*Chi square test
**Independent samples t-test

The mean of salivary opiorphin levels were 2.76±0.66 and 2.93±0.55 for the case and control groups, respectively (Table 1 and Figure 1). Independent t- test did not show any significant difference between OLP patients and healthy subjects according to salivary opiorphin levels (p=0.378) (Table 1).

Pearson's Chi-Square test did not reveal any significant correlation between OLP patients' age and salivary opiorphin levels (p=0.187). Furthermore, this statistical analysis did not show any remarkable relationship between healthy subjects' age and salivary opiorphin concentration (p=0.597).

In the case group, the mean of salivary opiorphin levels were 2.72±0.69 and 2.89±0.58 for the females and males, respectively. Statistical analysis did not show any remarkable difference between two genders in the OLP patients according to salivary opiorphin levels (p=0.601) (Table 2). Also, in the control group, the mean of salivary opiorphin levels were 2.79±0.40 and 3.07±0.67 for the females and males, respectively, which were not significantly different (p=0.257) (Table 2). Thus, we concluded that subjects' gender has no effect on salivary opiorphin level.

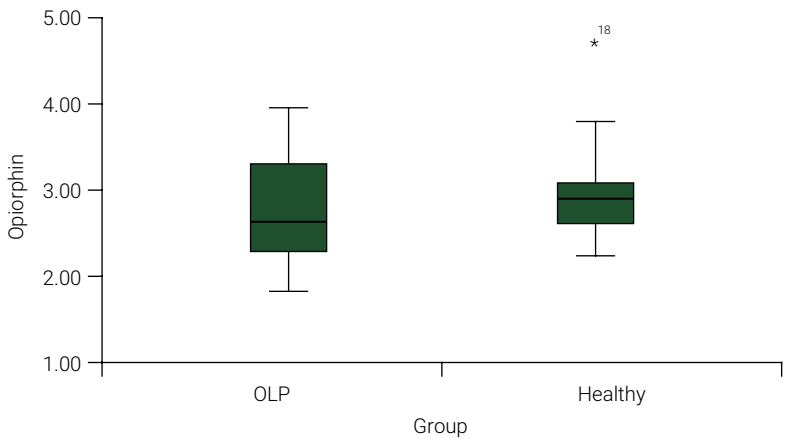

Figure 1. box plot of opiorphin levels in the case and control groups.

Table 2. opiorphin levels based on the sex in the case and control groups.

Variables	Sex	Case group	Control group	p-value*
Opiorphin	Female	2.72±0.69	2.79±0.4	0.758
	Male	2.89±0.58	3.07±0.67	0.587
p-value*		0.601	0.257	

*Independent samples t-test

Moreover, according to opiorphin levels, there was no remarkable difference between OLP females and healthy females (p=0.758), as well as between OLP males and healthy males (p=0.587).

Analytical analysis showed that opiorphin levels were not statistically different between various severity of OLP lesions, based on Tongprasom criteria (p=0.653); and there was no significant difference between keratotic, and non-keratotic types of OLP lesions (p=0.453). We concluded that there is no association between salivary opiorphin levels, and severity of OLP lesions.

Discussion

Some biomarkers, which are secreted into saliva, could indicate some physiologic and pathologic conditions. Opiorphin is a pentapeptide, which might be isolated from human saliva[1] and could alleviate pain due to inflammation and physical damage. Also, this biomarker is a natural suppressor of enzymes, which protects enkephalins and endorphin from degradation. Since enkephalin and endorphin are the native painkillers; this process could reduce the pain sensation. Moreover, opiorphin has anti-depressant[5], anti-inflammatory and anti-tumoral effects[1,6].

Sobocińska et al.[7] (2020) studied the effects of sialorphin and spinorphin on a mouse model of colitis and suggested these peptides as anti-inflammatory elements.

Nejad et al.[6] (2020) study revealed that salivary opiorphin levels in the patients, who had painful oral soft tissue lesions including traumatic and inflammatory

conditions, as well as, in patients with oral pre-malignant or malignant lesions, were higher than in healthy subjects; their study confirmed that the severity and type of pain could effect on opiorphin levels. Therefore, assessment of patients' salivary opiorphin could be an indicator of the severity of pain. Furthermore, these rising opiorphin levels in oral cancer patients could suggest an anti-tumoral role of this marker.

An in-vivo study on a mouse model of melanoma showed that opiorphin and sialorphin conjugate to a proapoptotic and antimicrobial peptide (called klak) leading to the formation of compounds, sialo-klak and opio-klak. These compounds have positive cytotoxic effects on cancer cells[8].

In the present study, the opiorphin levels were assessed in the patients involved with OLP, as potentially malignant lesions. Although opiorphin levels of OLP patients were higher than in healthy persons, the difference was not significant.

Concurrently, similar study on painful oral lesions, showed that the opiorphin levels in these patients were higher than the controls[6]. Furthermore, Ozdogan et al.[9] (2019) study showed that rate of salivary opiorphin concentration has a positive correlation with the severity of tooth pain. They concluded that inflammation due to the pulp or periodontal diseases could raise opiorphin levels.

In the present study, which was performed on 24 OLP patients including 7 keratotic and 17 non-keratotic OLP; there was not statistically significant difference between salivary opiorphin levels of patients with keratotic and non-keratotic lesions. Also, this biomarker level did not noticeably rise in severe lesions based on Tongprasom criteria. Therefore, this study could not prove that inflammation due to an erosive form of OLP increase opiorphin levels in these patients.

The results of this study were contradictory with Ozdogan et al.[9] (2019) study, which observed that there was a direct correlation between opiorphin concentration and severity of inflammation.

With respect to the fact that another study did not perform on various types of OLP lesions and opiorphin levels, a comprehensive study with a greater sample size on different severity of OLP is suggested for better judgment about the relationship of salivary opiorphin levels and severity of OLP lesions.

In this study, there was no significant difference between the salivary opiorphin concentration of OLP patients and healthy subjects; as well as, various types of OLP lesions. Further studies are recommended to evaluate salivary opiorphin levels in different scores of OLP lesions and its correlation with pain severity.

Author Contribution

Zohreh Dalirsani, Maryam Amirchaghmaghi, Ala Ghazi and Seyed Isaac Hashemy contributed in designing and performing the project. Mahboobeh Taherizadeh contributed in analyzing the data.

All authors actively participated in the manuscript's findings and have revised and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest in this study.

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