

Research Article

## Quantitative analysis and expression of salivary opiorphin in painful oral soft-tissue conditions: A descriptive study

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### ABSTRACT

**Objectives:** Opiorphin is an enkephalinase inhibitor which suppresses pain by acting on the opioid system. The levels of opiorphin in plasma and saliva have shown to vary in patients with burning mouth syndrome. This descriptive study was designed to estimate the salivary levels of opiorphin among individuals with painful oral soft-tissue conditions.

**Materials and Methods:** Unstimulated whole saliva was collected from 60 individuals (20 controls, 20 traumatic and inflammatory conditions, and 20 patients with oral potentially malignant disorders [OPMDs] and oral cancer). The salivary levels of opiorphin were assessed through competitive enzyme-linked immunosorbent assay.

**Results:** The mean level of opiorphin among controls was  $7.108 \pm 2.535$  ng/ml, among individuals with traumatic and inflammatory conditions was  $9.409 \pm 2.369$  ng/ml, and in individuals with OPMDs and oral cancer was  $8.268 \pm 2.414$  ng/ml. A positive correlation was observed between salivary opiorphin levels and age of the patient ( $r = 0.028$ ).

**Conclusion:** The varying levels of opiorphin in painful oral mucosal conditions and with age indicate its role in local pain modulating mechanisms.

**Keywords:** Enzyme-Linked Immunosorbent Assay, Endorphins, Opiorphin, Orofacial pain, Saliva

### INTRODUCTION

Pain diagnosis and management would benefit from the development of objective markers of nociception and pain. Oral mucosa is affected by various inflammatory, infectious, autoimmune, and premalignant and malignant conditions. These conditions manifest as painful ulcers and erosions. The salivary glands form a part of the neuroendocrine system and hence the composition of saliva varies with many local and systemic conditions.<sup>[1]</sup> Some of the components secreted in saliva have served as biomarkers for various physiological and pathological conditions, including pain.<sup>[2]</sup> Opiorphin is a pentapeptide isolated from human saliva that suppresses pain from chemically induced inflammation and acute physical pain.<sup>[3]</sup> It is a natural inhibitor of endorphin and enkephalin degrading enzymes (neprilysin and neutral endopeptidase N) that are devoid of the adverse effects of morphine and its derivatives. Apart from being an effective analgesic, opiorphin also has potent anti-inflammatory, anti-depressant, and anti-tumor activity.<sup>[3,4]</sup>

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The concentration of opiorphin in human saliva was quantified as 2.8–25.9 ng/ml.<sup>[5]</sup> Being involved in the endogenous analgesic system, the salivary levels of opiorphin have been reported to increase in painful oral conditions such as burning mouth syndrome. A regional pain modulation activity of opiorphin has been suggested in patients with burning mouth syndrome.<sup>[6]</sup> Thus, with the involvement of opiorphin in pain modulation, the levels are subjected to change in any painful condition affecting the oral mucosa. The previous studies have estimated the levels of opiorphin in various human biological fluids in individuals with psychosomatic disorders as well as post local anesthetic administration in saliva.<sup>[6-9]</sup> The results of these studies have been inconclusive. We hypothesized that opiorphin being an enkephalin protecting peptide, the salivary levels of this peptide will vary in painful oral soft-tissue conditions. Accordingly, the study was designed to estimate the salivary levels of opiorphin among individuals with painful oral soft-tissue conditions and normal healthy controls and correlate the change with the severity of pain as well as with the emotional status of the individual.

## MATERIALS AND METHODS

### Study population

Patients who reported to the outpatient department of the institution in the age group of 20–75 years with painful oral soft-tissue conditions such as traumatic ulcer, recurrent aphthous ulcer, oral candidiasis, and oral potentially malignant disorders (OPMDs) such as lichen planus, oral submucous fibrosis, carcinoma of oral cavity, and burning mouth syndrome (BMS) were included in the study.

Patients with chronic systemic illness such as diabetes mellitus and hypertension who were under medication, patients undergoing treatment for the painful oral soft-tissue conditions and psychosomatic disorders and who have undergone treatment within the past 4 weeks, those with subjective perception of dry mouth and with salivary glands disorders, conditions affecting the quantity and quality of saliva, as well as those patients with conditions associated with active purulent discharge such as pericoronal abscess, chronic periapical abscess with sinus tract formation, chronic periodontitis, and periodontal abscess were excluded from the study.

### Study design and sample collection

Based on literature survey done by Boucher *et al.*<sup>[8]</sup> on comparative evaluation of salivary opiorphin levels in individuals with idiopathic burning mouth syndrome, expecting a power of 80% and type 1 error of 5% with a confidence interval of 95%, a sample size of 60 (20 in each group of control, traumatic and inflammatory conditions,

and OPMDs and oral cancer) was determined for a three group study.

The study was conducted between April 2018 and December 2018. The study was approved by the Ethical Board of the institution and informed consent was obtained from all the participants before sample collection. The subjects were asked to rinse their mouth with alcohol free mouthwash and unstimulated whole saliva (UWS) was collected using spit method. The samples were then centrifuged and the supernatant was stored at  $-80^{\circ}\text{C}$  till analysis.

A brief medical and personal history of the participants pertaining to their systemic condition and presence of deleterious habits such as alcohol, tobacco use, and pain history of the oral lesions was recorded, followed by clinical examination of the oral lesions. Hospital anxiety and depression scale (HADS) questionnaire was used with an aim to facilitate detection and grading of anxiety and depression levels if any among the participants<sup>[10]</sup> and visual analog scale (VAS pain score) was used to assess pain intensity. The levels of opiorphin in saliva were analyzed using Human Opi ELISA kit (Wuhan Fine Biotech Co., Ltd.), a competitive ELISA (enzyme-linked immunosorbent assay) kit, with a detection range of 0.156–10 ng/mL and sensitivity  $<0.094$  ng/mL.

### Statistical analysis

SPSS version 20.0 for Windows version (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The numeric data were described using arithmetic mean and standard deviation. Correlation of opiorphin levels was analyzed with VAS score, HADS score, and age of the subject. Statistical correlation of opiorphin levels between the three groups was also assessed. The correlation of level of opiorphin with age was assessed using Pearson's correlation coefficient. Statistical significance of differences in mean values was tested using a non-parametric test, Mann-Whitney U-test. The level of significance was considered at  $P \leq 0.05$ .

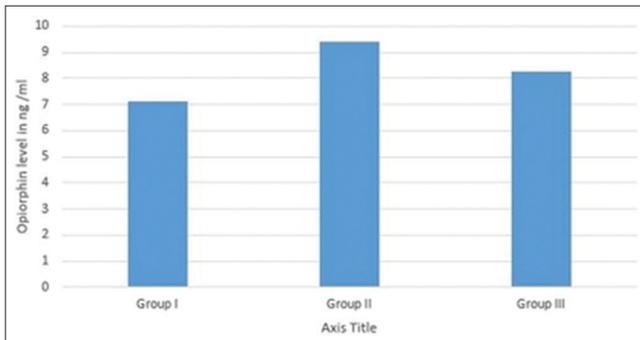
## RESULTS

A total of 60 age- and sex-matched individuals were categorized into three groups, Group I – controls ( $n = 20$ ), Group II – traumatic and inflammatory conditions of oral mucosa ( $n = 20$ ), and Group III – OPMDs and oral cancer patients ( $n = 20$ ). The study consisted of subjects within the age group of 20–75 years (mean:  $36 \pm 2.7$  years). The mean level of opiorphin in control subjects without pain was  $7.108 \pm 2.535$  ng/ml, among individuals with traumatic and inflammatory conditions, it was  $9.409 \pm 2.369$  ng/ml and  $8.268 \pm 2.414$  ng/ml, in OPMDs and oral cancer [Table 1 and Figure 1]. No significant correlation was found between opiorphin levels, VAS, and HADS score. However, a positive correlation was observed between salivary opiorphin levels and age of the patient ( $r = 0.028$ ).

**Table 1: Salivary levels of opiorphin among different study groups.**

Study group	Opiorphin level (ng/ml) (mean±SD)
Controls ( <i>n</i> =20)	7.108±2.535
Traumatic and inflammatory conditions ( <i>n</i> =20)	9.409±2.369
OPMDs and oral cancer ( <i>n</i> =20)	8.268±2.414
<i>P</i> -value	NS*

NS\*: Not significant, *P*>0.05

**Figure 1:** Salivary levels of opiorphin among the three groups (Group I: Controls; Group II: Traumatic and inflammatory conditions; and Group III: oral potentially malignant disorders and oral cancer).

## DISCUSSION

Opiorphin is a peptide of five amino acids present under two forms of amino acid sequence Gln-Arg-Phe-Ser-Arg and pGlu-Arg-Phe-Ser-Arg, was first extracted, purified, and characterized by a functional biochemical approach from human saliva.<sup>[3,11,12]</sup> The precursor of opiorphin peptide, PROL1 gene is expressed by lacrimal glands and salivary glands. The major pool of saliva in oral cavity is contributed by the submandibular salivary gland and an association of opiorphin has been established with the human submandibular salivary gland forming the cervical sympathetic trunk submandibular gland axis with the cervical superior ganglia.<sup>[6]</sup> This suggests a neuronal modulation of opiorphin secretion.

Boucher *et al.*, in 2016, assessed opiorphin levels in saliva, blood, and urine samples from patients with BMS and compared it with normal healthy individuals. They reported a lack of significant difference in salivary opiorphin levels between BMS and controls, but a significant increase in blood levels of opiorphin in patients with BMS. Although the opiorphin level in urine of BMS patients was higher than that of controls, there was no significant difference in opiorphin levels between the groups.<sup>[8]</sup>

Salarić *et al.*, in their study assessing opiorphin level in saliva of individuals with and without BMS, showed a significantly

higher level of opiorphin in UWS among BMS subjects ( $8.129 \pm 6.445$  ng/mL), compared to the control group ( $5.017$  ng/mL  $\pm 2.585$ ). Although the opiorphin levels in stimulated whole saliva (SWS) were higher in BMS group as compared to controls, it was not statistically significant. Similarly, the difference in opiorphin level between UWS and SWS was not statistically significant, although opiorphin level in UWS was higher than in SWS. The increase in opiorphin levels in BMS patients was elucidated as an adaptive reaction ensuing chronic pain.<sup>[6]</sup>

A similar observation is noted in our study with reference to the mean level of unstimulated salivary opiorphin level between the control and study groups denoting an increase in the opiorphin levels among the subjects with pain. In the present study, the salivary opiorphin levels among both controls and the subjects were higher as compared to the results obtained by Salarić *et al.* Moreover, BMS being a chronic condition and opiorphin being a peptide, long-term continuous release of opiorphin is easily degraded by the peptidases in saliva, resulting in lower opiorphin levels. Whereas, in the present study, acute inflammatory and traumatic oral conditions are also considered, wherein the release of opiorphin is rather for a shorter period as compared to BMS which is a chronic condition which could be attributed to the method of opiorphin assessment. The higher values obtained in our study can be due to the binding of immature translational products derived from the PROL 1 protein to the antibody in our ELISA based study in contrast to the liquid chromatography mass spectroscopy assessment by Salarić *et al.*<sup>[6]</sup> The salivary opiorphin levels were also found to increase as the chronicity of the pain increased though it was not statistically significant.

BMS being a psychosomatic disorder, the psychological status of the individuals can also contribute to the altered pain threshold in these individuals. This can be due to altered opiorphin levels locally or centrally. Thus, evaluation of psychological and emotional status of the individual can also explain the altered levels of opiorphin as it is also considered to possess anti-depressive effects.<sup>[13]</sup> Hence, in the present study, we assessed the psychological and emotional status of the subjects through HADS and compared it to the opiorphin levels. However, there was no significant correlation between the two.

Al Saffar *et al.*, in 2013, showed a significant difference of salivary opiorphin before ( $5.96 \pm 5.38$  ng/ml) and after administration of local anesthesia ( $14.49 \pm 3.66$  ng/ml) and also an inverse relationship of salivary opiorphin levels with VAS.<sup>[7]</sup> The increase in level of opiorphin found was more than that estimated by Salarić *et al.* in patients with BMS.<sup>[6]</sup> The nature of pain can also determine the concentration of opiorphin. The momentary pain induced in their study by needle penetration has led to two-fold increase in opiorphin

which is higher than the opiorphin levels quantified in chronic painful condition like BMS.<sup>[6]</sup> While the pain due to local anesthetic injection is acute, burning sensation and pain observed in BMS and other mucosal conditions are chronic. This explains that the nature and intensity of pain (acute and chronic) can thus influence the opiorphin levels in saliva. Thus, a natural pain modulation mechanism may result in altered opiorphin levels that pave to adaptation and increased pain threshold. The chronicity of pain observed in these conditions could have triggered the physiological pain modulation system involving the enkephalins and opiorphin.

In the present study, no significant difference in the opiorphin levels between the genders was observed in contrast to the study by Dufour *et al.* due to the unequal distribution of sample size.<sup>[14]</sup> Increased level of opiorphin with advancing age in the present study indicates a possible natural adaptation compensating for decreased pain threshold with increase in age, although there is insufficient literature to support this. In the present study, highest salivary opiorphin levels were found in Group II patients (traumatic and inflammatory oral conditions). The level of opiorphin found in our study in the mucosal lesion groups is more than that demonstrated in individuals with BMS as described in the previous studies.<sup>[6,8]</sup> The increased levels of salivary opiorphin found in the mucosal lesion groups (II and III) as compared to the controls can be attributed to the protective release of opiorphin at the site of injury where the opioid peptides are released from the immune cells.<sup>[15]</sup> The decreased level of opiorphin in BMS patients can also be attributed to the decreased salivation found in these patients.<sup>[16,17]</sup> Similarly, the increased opiorphin levels in OPMDs and oral cancer patients can also support the anti-tumor activity of opiorphin, the levels being increased due to natural protective mechanism.<sup>[18]</sup>

Pain results from an initial noxious stimulation of nociceptors on primary afferent nerve endings that are present in skin, joints, muscles, and viscera. Noxious stimuli can be blocked or largely reduced at their source by enhancing the extracellular concentrations of enkephalins. The local availability of enkephalins to block the transmission of pain and produce analgesia is regulated by neprilysin and aminopeptidase N, which break down enkephalins. Along with enkephalins, these enzymes are released at the site of nerve injury from noxiously stimulated nerve cells, fibroblast, keratinocytes, lymphocytes, and neurons.<sup>[15,19]</sup> The analgesic effect of enkephalins is thus annulled by the enzymes degrading it. Opiorphin thus forms a protective role for enkephalin by blocking the enkephalinase enzyme. It can be proposed that with the natural increase in the enkephalins at the time of noxious stimuli, there is concurrent increase in the concentration of opiorphin. The cervical sympathetic plexus in the submandibular ganglia being the source of

opiorphin can possibly explain the local increase of opiorphin in painful oral mucosal lesions. This can be interpreted as a natural adaptive and protective mechanism of the body to tissue injury. This implies that with the increase in opiorphin level in saliva, there should be a decrease in pain perception, but which is not so. The peptide nature of the opiorphin accounting for its rapid degradation and short duration of action, ultimately results in loss of its enkephalin protective role. Thus, resulting in termination of enkephalin action, which decreases pain.

### Limitations

There is a wide range in the salivary opiorphin levels in our study as well as in the previous studies. This makes it challenging to establish and standardize the opiorphin level as a marker for objective pain assessment. A larger sample size with various oral painful conditions and psychological disorders is suggested to understand its role as an antidepressant. Furthermore, the pain experience can be better analyzed by comparing the level of opiorphin with neurogenic pain markers like nerve growth factor in saliva.

### CONCLUSION

The varying levels of opiorphin in painful oral mucosal conditions can thus indicate its role in pain modulating mechanisms. At present, there are no validated objective markers for quantitative measurement of pain that can be endorsed for clinical use. In this scenario, opiorphin levels in saliva can prove to be a useful biomarker in objective assessment of intensity of pain, which is otherwise subjective. It can also be used as a diagnostic tool in study of pain. Extending the clinical significance, the knowledge about the role of opiorphin in modulating pain can herald newer analgesics that can potentiate the endogenous enkephalins.

### Declaration of patient consent

The study was approved by the University Ethics Committee for human trials and written informed consent was obtained from all the participants before sample collection.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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