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Opiorphin in burning mouth syndrome patients: a case-control study

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Abstract

Objectives: Opiorphin is a pentapeptide isolated from human saliva that suppresses pain from chemically induced inflammation and acute physical pain. Burning mouth syndrome (BMS) is a chronic condition of a burning sensation in the mouth, where no underlying dental or medical cause can be identified. We aimed to measure the level of opiorphin in whole unstimulated (UWS) and stimulated (SWS) saliva of patients with BMS.

Materials and methods: Originally developed and validated LC-MS/MS method was used for opiorphin quantification.

Samples were obtained from 29 BMS patients and 29 age and sex-matched controls.

Results: The average concentration of opiorphin in UWS and SWS in the BMS group was 8.13 ± 6.45 and 5.82 ± 3.59 ng/ml, respectively. Opiorphin in BMS patients' UWS was significantly higher, compared to the control group (t = 2.5898; p = 0.0122). SWS opiorphin levels were higher, but not significantly, in BMS patients than in controls.

Conclusions: Our results indicate that higher quantities of salivary opiorphin in BMS may be a consequence of chronic pain, but we cannot exclude that they occur as a result of emotional and behavioral imbalances possibly associated with BMS. To our knowledge, this is the first original article measuring opiorphin in a pain disorder.

Clinical relevance: Opiorphin may be a measurable biomarker for chronic pain, which could help in objectifying otherwise exclusively a subjective experience. Increased opiorphin could serve as a universal objective indicator of painful conditions.

Since opiorphin may also reflect emotional and socio-relational imbalances occurring with BMS, it could as well represent a biomarker for BMS. Knowledge on opiorphin's involvement in pain pathways could contribute to developing new clinical diagnostic methods for BMS.

Keywords: Opiorphin, Burning mouth syndrome, Saliva, Chromatography, Liquid, Spectrometry, Mass

Introduction

Opiorphin is an endogenous pentapeptide isolated in 2006 from human saliva [1]. It was described as a mature product of the proline-rich, lacrimal 1 (PROL1) protein and shown that it inhibits enkephalin-inactivating ectopeptidases, human neutral ecto-endopeptidase (hNEP), and human ectoaminopeptidase (hAP-N), thus resulting in extended analgesic activity of enkephalins. Initial research on rats showed that it suppresses pain sensation for both chemical-induced and acute mechanical pain as efficiently as morphine but without causing drug tolerance and with fewer morphine-associated side effects [1, 2]. It was previously observed that central administration of opiorphin induced an antidepressant-like effect by activation of μ and δ opioid receptors [3–5], as well as showed to affect colonic motility [6, 7]. Apart from saliva, opiorphin has been detected in plasma, cerebrospinal fluid, urine, tears, semen, and breast milk [8, 9]. To our knowledge, limited data are available on its levels, as wells as age and gender distribution. Only few papers have been published on opiorphin quantification [9, 10].

Being involved in pain pathways, opiorphin could potentially represent a valuable biomarker for different types of physiological disorders. Because of its abundance in the oral milieu, we hypothesize that it might affect pain regionally, especially in common idiopathic orofacial pain conditions, such as “burning mouth syndrome” (BMS).

“BMS is a debilitating chronic condition of a burning sensation in the mouth, where no underlying dental or medical cause can be identified and no oral signs can be found” [11, 12]. The discomfort may affect the tongue, gums, lips, inside of the cheeks, roof of the mouth, or the whole mouth and is often accompanied by dry mouth and altered taste sensation [11–13]. Although BMS can affect anyone, it occurs most commonly in middle-aged or older women. It is estimated that it affects up to 14%postmenopausal women and about 1% of general population with a male-to-female ratio being 1:7 [12, 14]. Till this day, the etiology of BMS is poorly understood.

Various published treatment attempts generally lack evidence of real long-term efficiency [12]. For the past several decades, literature has been pointing towards the involvement of neural mechanisms in the etiology of BMS [15–18] and the current research suggests the same [19]. Therefore, we believe that levels of salivary opiorphin may present a biomarker for this condition.

Differences in saliva composition have previously been observed in BMS patients, compared to healthy individuals. Nagler and Hershkovich have suggested oral neuropathy or neurologic transduction interruption background induced by compositional saliva alterations in patients with oral sensory complaints, such as BMS [17]. Additionally, Granot and Nagler have corroborated the altered salivary composition, i.e., elevated Na, K, Cl, Ca, IgA, and amylase concentrations in patients with oral sensory complaints, including BMS patients [18]. Both studies suggested a local neuropathic process underlying the patients’ complaints; however, central involvement could also partake in the onset and perpetuation of burning symptoms [15, 19].

Opiorphin, because of its involvement in pain pathways, could present a salivary marker for the local neuropathic condition, such as BMS.

Aim of the study was to investigate levels of salivary opiorphin in patients with BMS in unstimulated whole saliva (UWS) and stimulated whole saliva (SWS) and to compare the results with the healthy control group. Different opiorphin levels than in control subjects would confirm the neuropathic background in the development of the BMS. We had hypothesized that we may encounter lower opiorphin levels in BMS patients, which would implicate that a relative scarcity of this potent pain suppressor could, in certain individuals, lead to development of BMS.

Materials and methods

Patients and controls

Saliva samples were obtained from 29 patients with BMS (24 females and 5 males, mean age 67.45 ± 9.44 years, range 43–84) treated at the Department of Oral Medicine, Zagreb School of Dental Medicine, and 29 age- and sex-matched control subjects (20 females and 9 males, mean age 67.31 ± 12.66 years, range 38–89) recruited from patients of the Department of Endodontics and Restorative Dentistry, Zagreb School of Dental Medicine from February to May 2014. A statistical power analysis was performed for sample size estimation, based on data from our pilot study [20, 21]. Based on the results of that study, the average concentration of opiorphin in saliva of healthy volunteers was 8.5 ± 5.0 ng/ml.

There was no significant difference in opiorphin level in female vs. male subjects (9.4 ± 5.4 vs. 7.2 ± 4.6 ng/ml). Since the difference between female and male was 2.2 ng/ml, it would be reasonable to assume that differences between study and control group should be at least twice as large in order to verify differences between the study groups. With an alpha = 0.05 and power = 0.90, the projected sample size needed with this variance (GPower Win 3.1.9.2. (Heinrich-Heine-Universität, Düsseldorf, Germany)) was approximately N = 26 for the between group comparison. Thus, our proposed sample size of 29 should be adequate for the main objective of this study especially since there is no attrition possible in this type of study.

Diagnosis of BMS was established by experienced oral medicine specialists, in accordance with the presence of typical symptoms: daily and symmetrical burning pain sensation of the oral mucosa, lasting for at least 3 months, with constant or increasing intensity, that endures more than 2 h during the day and improves during eating or drinking, having no interference with sleep, and unaccompanied by any dental, oral, or medical clinical signs [11, 12]. Subjects abstained from food and beverages at least for 8 h and did not brush their teeth prior to sample collection. Saliva sampling took place in the morning, between 8 and 10 a.m. Periodontal health was assessed by papillary bleeding index (PBI) on Ramfjord index teeth (nos. 3, 9, 12, 19, 25, and 28). When a subject was missing a Ramfjord index tooth, a tooth closest to it was assessed.

BMS group inclusion criteria are as follows: no systemic pain, no oral or dental clinical signs, PBI 0 or 1, regular medication use if under therapy, and well-controlled systemic diseases or conditions. Control group inclusion criteria are no oral/dental or systemic pain, seen by dentist for regular checkups (at least once a year), saliva samples taken only if dental procedures were not required, PBI 0 or 1, regular medication use if under therapy, and well-controlled systemic diseases or conditions. Prior to sample collection, the subjects' medical history was recorded. In order to eliminate other possible influences on opiorphin levels and ensure that groups are well matched, BMS and control subjects were compared according to their medical history, drug consumption, and oral clinical findings. Subjects' medications were indexed by the

Anatomical Therapeutical Chemical Classification System (ATC) [22], while the subjects' systemic conditions were indexed by the International Statistical Classification of Diseases and Related Health Problems (ICD-10) [23].

The experimental procedures were conducted in accordance with ethical standards of the Declaration of Helsinki and approved by the Ethics Committee of the School of Dental Medicine on 9 December 2010.Written informed consent was obtained from all participants.

The method, sample collection, and manipulation were previously described in detail and validated by our group [21]. Briefly, an original sensitive, specific, and reliable liquid chromatography-mass spectrometry method was utilized for quantification of salivary opiorphin.

One saliva sample contained approximately 2 ml of saliva (2 ml UWS + 2 ml SWS per patient). Saliva was obtained from mouth floor with a vacuum saliva collector that delivered it into a pre-weighed graduated tube containing 300 μl of trifluoroacetic acid (TFA) kept on ice. Salivation was induced by 1 % ascorbic acid solution by applying it onto the antero-dorso-lateral surfaces of the tongue and vestibular mucosa using a cotton swab for 60 s, followed by a mouth rinse using the rest of the ascorbic acid solution for 30 s. Afterwards, the samples were vortexed and left on ice for 20 min and afterwards centrifuged (20,000×g, 30 min, at 4 °C). Supernatant (800 μl) was placed into a separate tube and then freeze-dried. The residue was dissolved in 200 μl of 0.1%formic acid (FA) in water, and an aliquot of 30 μl was analyzed by LC-MS/MS (Agilent Technologies 1200 series HPLC system, Agilent Technologies Inc., Palo Alto, CA, USA).

Electrospray positive ionization-mass spectrometric multiple reaction monitoring (ESI+/MRM) experiments were used for quantifying opiorphin. Agilent MassHunter software was used for data processing. Compound-specific MRM was performed with ESI-MS/MS in order to ensure the quantification accuracy.

Quantification of opiorphin was performed using the ESIMS positive ion mode, which produced a stable doubly charged [M+2H]2+ ion at *m/z* 347 with optimal fragmentor voltage at 135 V. Since the transition *m/z* 347/120 showed best intra- and inter-day precision, the lowest limit of detection, and the lowest susceptibility to the matrix effect, fragment ion *m/z* 120 was chosen as a quantifier ion, while ions *m/z* 175 and 268 as qualifier ions [21]. The limit of detection of opiorphin in human saliva was previously defined [21], 0.028, 0.031, and 0.057 ng for m/z 120, 175, and 268, respectively, and the lower limit of quantification was 2.0, 3.0, and 4.0 ng/ml for *m/z* 120, 175, and 268, respectively.

This case-control study was designed in compliance with "The Strengthening the Reporting of Observational Studies in Epidemiology" checklist.

Statistical analysis

Predictive Analytics Software (PASW) for Windows version 17.0 (SPSS Inc., Chicago, IL, USA) was used. Numeric data were described using the arithmetic mean, standard deviation, median, interquartile range, range, and minimal and maximal value. Qualitative data was described by frequency and percentage. Quantitative data distribution was tested by Kolmogorov-Smirnov test. Statistical significance of differences in mean values was tested using Student's t test due to the normal distribution of data. When routed for a nonparametric

test, Mann-Whitney *U* test was used. Multiple linear regression model was used to test the influence of drug consumption and systemic conditions on opiorphin levels. The confidence interval was ignored when the test was not significant. The level of significance was set at 5 %.

Results

There was no statistically significant difference neither in age (*t* test, $t = 0.048$, $p = 0.962$) nor in gender ($\chi 2$ test, $\chi 2 = 1.507$, $p = 0.220$) between the two groups.

Relation between systemic conditions, drug consumption, and the opiorphin level was investigated. Table 1 shows the subjects' medical diseases and conditions indexed by ICD-10. None of the subjects with gastritis had developed gastroesophageal reflux disease. Subjects' drug consumption, indexed by ATC, is shown in Table 2. No statistical difference was found for those relations (data not shown, available on request). The influence of smoking on the opiorphin level could not be calculated due to the small number of smokers included in the research (four smokers (13.33 %) in the control group and two (6.90 %) in the BMS group).

Since none of the subjects had refused to participate or quit during the experiment, and all information requested was provided, we report no missing data.

Table 3 shows opiorphin levels (mean, range, median, and SD) in UWS and SWS in BMS subjects and controls (ng/ml).

Table 4 shows the opiorphin levels testing results within and between groups in UWS and SWS. Figure 1 describes the relationship of opiorphin levels within and between groups in UWS and SWS. Comparison between groups showed statistically significant increase of opiorphin levels in UWS of BMS patients, as compared to age- and sex-matched controls. SWS was also increased in BMS group, as compared to controls, but it was not statistically significant. Differences between UWS and SWS within groups were also not statistically significant.

Table 1. Subjects' medical history indexed by International Statistical Classification of Diseases and Related Health Problems (ICD-10)

Diseases and conditions	BMS group (N (%))	Control group (N (%))	All (N (%))
Healthy*	4 (13.79)	8 (27.59)	12 (20.69)
Essential hypertension (I10)	6 (20.70)	8 (27.59)	14 (24.14)
Atherosclerosis (I70)	4 (13.79)	4 (13.79)	8 (13.79)
Phlebitis and thrombophlebitis (I80)	2 (6.90)	1 (3.45)	3 (5.17)
Angina pectoris (I20)	3 (10.34)	2 (6.90)	5 (8.62)
Varicose veins of lower extremities (I-83)	2 (6.90)	4 (13.79)	6 (10.34)
Iron deficiency anemia (D50)	1 (3.45)	0 (0.00)	1 (1.72)
Gastritis and duodenitis (K29)	11 (37.93)	5 (17.24)	16 (27.59)
Simple chronic bronchitis (J41.0)	1 (3.45)	0 (0.00)	1 (1.72)
Calculus of kidney and ureter (N20)	1 (3.45)	2 (6.90)	3 (5.17)
Osteoporosis without pathological fracture (M81)	3 (10.34)	0 (0.00)	3 (5.17)
Depression (F32)	5 (17.24)	2 (6.90)	7 (12.10)
Type 2 diabetes mellitus (E11)	2 (6.90)	3 (10.34)	5 (8.62)
Hypothyroidism (E03)	4 (13.79)	2 (6.90)	6 (10.34)
Epilepsy (G40)	1 (3.45)	1 (3.45)	2 (3.44)
Asthma (J45)	0 (0.00)	1 (3.45)	1 (1.72)

Abbreviations: BMS: Burning mouth syndrome

* no systemic illness

Table 2. Subject's drug consumption (medications indexed by the Anatomical Therapeutical Chemical Classification System (ATC))

Drug	BMS group (N(%))	Control group (N(%))	All (N(%))
None	7 (24.14)	8 (27.59)	15 (25.86)
Calcium channel blockers (C08)	4 (13.79)	6 (20.70)	10 (17.24)
Proton pump inhibitors (A02BC)	7 (24.14)	3 (10.34)	10 (17.24)
Ibuprofen (C01EB16)	4 (13.79)	1 (3.45)	5 (8.62)
Acetylsalicylic acid (B01AC30)	4 (13.79)	8 (27.59)	12 (20.69)

Benzodiazepine derivatives (N05CD)	2 (6.90)	4 (13.79)	6 (10.34)
Sex hormones and modulators of the genital system (G03)	0 (0.00)	1 (3.45)	1 (1.72)
Angiotensin II antagonists, plain (C09C)	1 (3.45)	2 (6.90)	3 (5.17)
Beta blocking agents (C07)	3 (10.34)	6 (20.70)	9 (15.51)
ACE inhibitors, plain (C09A)	7 (24.14)	6 (20.70)	13 (22.41)
HMG- CoA reductase inhibitors (C10AA)	4 (13.79)	5 (17.24)	9 (15.51)
Opioids (N02A)	1 (3.45)	0 (0.00)	1 (1.72)
Levothyroxine sodium (H03AA01)	4 (13.79)	2 (6.90)	6 (10.34)
Blood glucose lowering drugs, excluding insulins (A10B)	2 (6.90)	3 (10.34)	5 (8.62)
Antihistamines for systemic use (R06)	2 (6.90)	0 (0.00)	2 (3.44)
Anti-asthmatics (R03)	0 (0.00)	1 (3.45)	1 (1.72)
Aminosalicylic acid and derivatives (J04AA)	1 (3.45)	0 (0.00)	1 (1.72)
Antiinflammatory and antirheumatic products, non-steroids (M01A)	1 (3.45)	1 (3.45)	2 (3.44)
Antiepileptics (N03AX)	1 (3.45)	1 (3.45)	2 (3.44)
Isosorbide mononitrate (C01DA14)	3 (10.34)	2 (6.90)	5 (8.62)
Warfarin (B01AA03)	2 (6.90)	3 (10.34)	5 (8.62)
Drugs acting on serotonin receptors (A03AE)	1 (3.45)	0 (0.00)	1 (1.72)
Clopidogrel (B01AC04)	0 (0.00)	1 (3.45)	1 (1.72)
Bisphosphonates, combinations (M05BB)	2 (6.90)	0 (0.00)	2 (3.44)

Abbreviations: BMS: Burning mouth syndrome

Table 3. Opiorphin values (in ng/ml) for the BMS and the control group in UWS and SWS

	BMS		Control	
	UWS	SWS	UWS	SWS
Mean	8.129	5.819	5.017	4.992
Standard deviation	6.445	3.594	2.585	3.212
Min. value	1.811	1.049	2.033	1.618

Max. value	25.612	14.425	10.724	15.322
Median	5.951	5.096	4.592	4.265
95% confidence interval	5.677 / 10.580	4.452 / 7.186	4.034 / 6.001	3.771 / 6.214
Interquartile range	7.364	2.969	3.780	3.084

Abbreviations: BMS: Burning mouth syndrome; UWS: Unstimulated whole saliva; SWS: Stimulated whole saliva

Table 4: Comparison of opiorphin levels between groups

	t	df	p	SED*
BMS – Control group (UWS)	2.413	56	0.0191**	1.289
BMS - Control group (SWS)	0.9239	56	0.3595	0.895
BMS group (UWS – SWS)	1.6857	56	0.0974	1.370
Control group (UWS – SWS)	0.0327	56	0.9741	0.766

Abbreviations: BMS: Burning mouth syndrome; UWS: Unstimulated whole saliva; SWS: Stimulated whole saliva; *Standard error of difference; **p<0.05

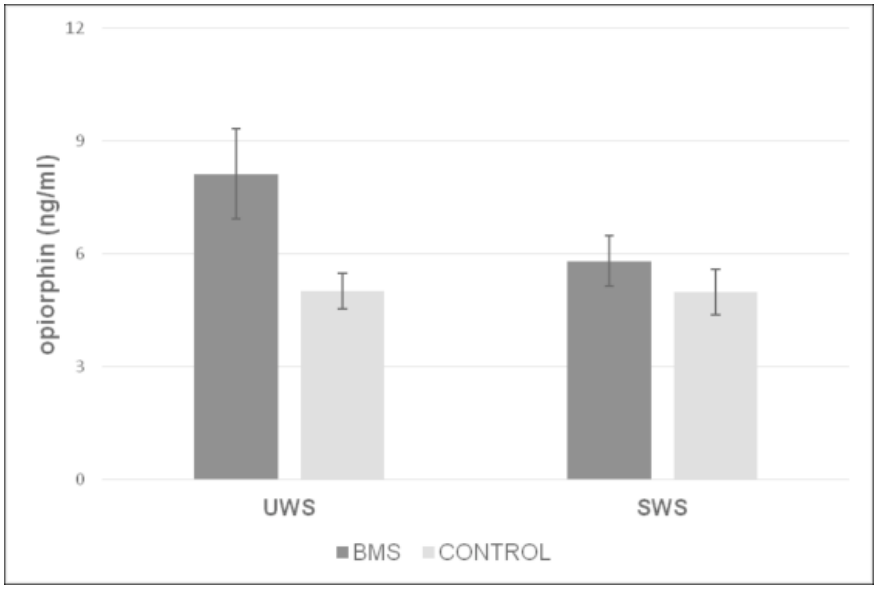

Figure 1: Opiorphin mean value (with standard errors) comparison between the burning mouth syndrome group (BMS) and control group in unstimulated whole saliva (UWS) and stimulated whole saliva (SWS)

Discussion

Literature reports a clear predisposition of BMS patients to gender and age with women being 2.5–7-fold more commonly affected than men [13, 24, 25]. Furthermore, around 90 % of women affected are in menopause [26]. Mean age of the BMS group investigated was 67.5, 67.3 of the control group, and 82.8 % of all subjects were female.

A possible limitation to this research is the early morning saliva sampling. It is known that BMS symptoms are usually less prominent in the morning hours [27]. However, morning saliva sampling has the advantage of excluding possible confounders, e.g., food and beverage influence on saliva and opiorphin secretion. A study on the circadian rhythm of salivary opiorphin is needed, as information on its concentration span remains unknown.

We performed a multiple linear regression analysis to see if levels of opiorphin were affected by systemic conditions or drug consumption. We found no correlation between drug consumption and systemic conditions and the level of opiorphin. To assess the influence of a certain condition or medication on the level of salivary opiorphin, research with a larger sample might be needed, as current sample size might represent a limitation of the study. Subjects were primarily matched for age and gender in order to minimize the influence of those variables on the measured variable, opiorphin levels. Subsequent data analysis revealed that we also obtained good matching of systemic conditions and medications between groups.

One of the first papers on opiorphin was a one by Wisner et al. [1], where the authors described opiorphin as a physiological pain suppressant and a mood-related modulator. As mentioned in the “Introduction”, opiorphin activates endogenous opioid-dependent transmission, inhibiting the chemical and mechanical evoked pain behavior, with the effectiveness similar to morphine [1]. The zinc metal ectopeptidases located on the cell surface control the activity of neural and hormonal mediators, e.g., inactivation of the enkephalins and similar molecules, which regulate the analgesic mechanisms and emotional responses and states. Opiorphin was proven as a dual inhibitor of the enkephalin degrading hNEP and hAP-N, enabling the prolonged effect of enkephalins. Furthermore, it was proven to display analgesic activity in vitro, thereby suggesting therapeutic implications [1].
In a study by Dufour et al. [8], the levels of opiorphin were measured in different body fluids (blood, tears, saliva, urine, and lactating milk). Interestingly, difference was found between male and female subjects in the urinary opiorphin levels. The authors suggested that the opiorphin secretion is influenced by gender; however, the statistical difference was not observed in the bloodstream opiorphin levels. High heterogeneity in the bloodstream of female subjects observed by Dufour et al. was explained by the hormonal influence, even though the menstrual cycle phase had not been registered or the levels of androgens measured.
The function of opiorphin in the body fluids is yet to be discovered and is still based on speculations. However, it is clear that it exerts organ-specific functions through neuroendocrine, paracrine, and/or autocrine and/or exocrine mechanisms [8]. High level of opiorphin was observed in a study by Rougeot in a female volunteer undergoing thyroid hormone replacement therapy (basal conditions = 1072 ng/ml; stimulated conditions = 1637 ng/ml) [9]. Six of our subjects were undergoing a levothyroxine therapy but showed no such deviations. However, opiorphin level of one female subject from the control group patient was very high (15.32 ng/ml), possibly because she was undergoing estradiol, estriol, and norethisterone acetate replacement therapy. Medications can present possible confounders, as their influence on levels of opiorphin is unknown.

We have noticed a consistent decrease in the opiorphin level after saliva stimulation. This is in accordance with our previous study [21]. Gene expression and levels of SMR1 protein were found to be different among rats' salivary glands, being highest in the submandibular gland and slightly lower in parotid gland [28]. This could suggest an unequal expression of human genes related to SMR1 in different types of salivary glands. Our result could support this hypothesis as levels of opiorphin in UWS, secreted mainly by the submandibular glands, were higher than in SWS, which is secreted mainly by the parotid glands. We believe that the reason for this difference, apart from the possible unequal gene expression, could also be a dilution due to the increased salivary flow, resulting in a more serous saliva produced by parotid serous acinar cells.

In contrast, in two ELISA-based studies [9, 9], a significant increase in opiorphin levels was described in both genders after chemically induced salivation. In addition to that, their average values of opiorphin were six to ten times higher than ours. As described in a study by Brkljačić and her group [21], higher values measured in the ELISA-based studies could be an outcome of the interferences that affect the antibody binding specificity, in this case immature products, translational peptides, derived from the PROL1 protein. The translational peptides, due to their different mass, are invisible to the MS detector, which could not be the case with the ELISA antibodies. However, Al Saffar et al. obtained similar opiorphin levels to ours in an ELISA-based study [10]. Had we encountered lower opiorphin levels in BMS, we could consider that a lack of opiorphin had a role in the neural mechanisms leading to onset of BMS, due to loss of inherent pain controlling mechanism. Increased opiorphin levels in BMS patients could reflect the adaptive reaction consequent to chronic pain. Al Saffar et al. showed the difference in the levels of opiorphin before and after administrating local anesthesia [10], supporting the thesis that the elevated opiorphin levels are a result of painful stimuli. Furthermore, by comparing the elevated concentrations in our study and in the study by Al Saffar et al. [10], we can assume that greater level of opiorphin is to be observed in acute pain stimuli, i.e., needle penetration and voluminous tissue expansion, compared to the chronic pain stimuli, i.e., in BMS. It is therefore possible that opiorphin levels depend on the intensity of pain. A study on the correlation between pain intensity and opiorphin levels could provide us with a potential objective biomarker for pain.

Another plausible explanation for increased opiorphin levels in BMS patients lies in its involvement in other enkephalin-mediated adaptation processes beyond nociception [3, 5]. These include emotion-related behaviors, stress-induced hyperalgesia, anxiety, and depression, which are common in BMS patients [13, 14]. This implies that opiorphin could also be a biomarker for BMS. For that matter, the limitation of this study is the lack of structured and validated psychological and emotional evaluation of the subjects. Our subjects have only self-reported depression and their use of antidepressive medications, which could be biased. Had we recorded psychosocial and behavioral characteristics of our subjects, such data, if different between two groups, could have provided us with evidence of psychological aspects around BMS and thus would have also justified the increase in opiorphin levels. Higher vasoreactivity in BMS patients was reported by Heckmann et al. in a study on oral mucosal blood flow in BMS patients [29]. It has been proven that opiorphin has cardiovascular effects mediated through the renin-angiotensin system, as it rises the blood pressure and pulse when injected intravenously [30–32]. Curiously, BMS compatible symptoms were previously associated with antihypertensive drugs that act upon the angiotensin-renin system, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers or antagonists [33].

Theories on opiorphin's pathways, which might be of future interest, involve its possible implication in neuroendocrine axes. The UWS we collected originated mainly from the submandibular glands which, together with the cervical superior ganglia, form the cervical sympathetic trunk submandibular gland (CST-SMG) axis [34]. This "neuroendocrine axis" has

barely been investigated in humans [35]. However, it is known that the rat homologue of opiorphin, sialorphin [1], is a product of this axis. Sialorphin is generated from the N-terminus of the submandibular rat-1 protein (SMR1), a prohormone and an end component of the axis [36]. SMR1 protein is encoded by the Vcsa1 gene, hormonally regulated by androgens [28, 36, 37]. Expression of opiorphin genes in humans may be similarly regulated [36]. Etiology of the BMS has still not been defined, but some recent papers observed the levels of hormones and a possiblerelation of neuroendocrine disbalance and BMS [38, 39]. Levels of estrogen, progestones, and androgens decrease following menopause [40] and, as mentioned, the majority of BMS patients are menopausal and postmenopausal women. Therefore, we suggest a relationship between disbalanced androgen regulation in menopausal women, BMS, and opiorphin. A study on the topic is needed to confirm this hypothesis. Studies on differences in gene expression in salivary glands due to age and gender may help us understand predominance of menopausal and postmenopausal women in the BMS etiology. In a study by Srivastava et al., human parotid glands were compared among 32 otherwise healthy male and female subjects between 19 and 85 years of age [41]. Different gene expression was found in 787 gene probe sets. In 59%of probe sets, higher expression was identified in females and 29 % of alterations were due to aging. Furthermore, most of the differences were related to the genes linked to X and Y chromosome and immune response pathways [41]. Those findings on gender specificity of salivary genes expression and the influence of aging also fit in the puzzle of the "neuroendocrine regulation–BMS–salivary
Opiorphin" triangle. However, the influence of aging on the PROL1 gene expression responsible for opiorphin remains unclear. Studies on PROL1 expression in BMS patients would surely reveal more information about the BMS etiology.
In summary, our results opened new topics on opiorphin and BMS and provided encouragement for further studies on the relationship between opiorphin levels and different types of physiological disorders. Opiorphin could present a measurable biomarker for chronic pain, which could help in objectifying patients' subjective experience. We used a sensitive and reliable LC-ESI-MS/MS method to determine the level of opiorphin in our subjects. Statistically significant increase of opiorphin in unstimulated saliva of BMS patients, compared to control group, could be a response to a chronic painful condition, but it could also be result psychological and socio-relational disturbances within BMS. To test the latter,
we would need to assess psychological condition of BMS patients and controls in correlation to salivary opiorphin levels.

To our knowledge, this is the first article focusing on opiorphin in a painful disorder.

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Compliance with ethical standards

Conflict of interest Author Ivan Salarić declares that he has no conflict of interest. Author Maja Sabalić declares that she has no conflict of interest. Author Ivan Alajbeg declares that he has no conflict of interest.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethical Committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent

Informed consent was obtained from all individual participants included in the study. All pertaining data (de-identified medical data on study subjects, institutional ethical committee approval, copies of signed informed consent forms, laboratory results) can be obtained from the authors on request.

References

1. Wisner A, Dufour E, Messaoudi M, Nejdi A,Marcel A, Ungeheuer MN, Rougeot C (2006) Human opiorphin, a natural antinociceptive modulator of opioid-dependent pathways. Proc Natl Acad Sci USA 103:17979–17984
2. Rougeot C, Robert F, Menz L, Bisson JF, Messaoudi M (2010) Systemically active human opiorphin is a potent yet non-addictive analgesic without drug tolerance effects. J Physiol Pharmacol 61: 483–490
3. Javelot H, Messaoudi M, Garnier S, Rougeot C (2010) Human opiorphin is a naturally occurring antidepressant acting selectively on enkephalin-dependent delta-opioid pathways. J Physiol Pharmacol 61:355–362
4. Popik P, Kamysz E, Kreczko J, Wróbel M (2010) Human opiorphin: the lack of physiological dependence, tolerance to antinociceptive effects and abuse liability in laboratory mice. Behav Brain Res 213:88–93
5. Yang QZ, SS L, Tian XZ, Yang AM, GeWW, Chen Q (2011) The antidepressant-like effect of human opiorphin via opioid-dependent pathways in mice. Neurosci Lett 489:131–135
6. Tian XZ, Chen J, Xiong W, He T, Chen Q (2009) Effects and underlying mechanisms of human opiorphin on colonic motility and nociception in mice. Peptides 30:1348–1354
7. Kamysz E, Sałaga M, Sobczak M, Kamysz W, Fichna J (2013) Characterization of the effects of opiorphin and sialorphin and their analogs substituted in position 1 with pyroglutamic acid on motility in the mouse ileum. J Pept Sci 19:166–172
8. Dufour E, Villard-Saussine S, Mellon V, Leandri R, Jouannet P, Ungeheuer MN, Rougeot C (2013) Opiorphin secretion pattern in healthy volunteers: gender difference and organ specificity. Biochem Anal Biochem 2:136. doi:10.4172/2161-1009.1000136
9. Rougeot C (2014) Opiorphin peptide derivatives as potent inhibitors of enkephalin-degrading ectopeptidases. US patent US8642729 B2
10. Al-Saffar MT, Al-Sandook TA, Y-Taha M (2013) A possible new concept in the mechanism of action of local anesthesia. Am J Med Biol Res 1:134–137
11. Headache Classification Subcommittee of the International Headache Society (2013) The international classification of headache disorders: 3rd edition. Cephalalgia 33:629–808
12. Zakrzewska JM, Forssell H and Glenny AM (2005) Interventions for the treatment of burning mouth syndrome. Cochrane Database Syst Rev CD002779. doi:10.1002/14651858.CD002779

13. Scala A, Checchi L, Montevecchi M, Marini I, Giamberardino MA (2003) Update on burning mouth syndrome: overview and patient management. Crit Rev Oral Biol Med 14:275–291
14. Grushka M, Ching V, Epstein V (2006) Burning mouth syndrome. Adv Othorinolaryngol 63:278–287
15. Forsell H, Jääakeläinen S, Tenovuo O, Hinkka S (2002) Sensory dysfunction in burning mouth syndrome. Pain 99:41–47 16. Hagelberg N, Forsell H, Rinne JO, Scheinin H, Taiminen T, Aalto S, Luutonen S, Någren K, Jääskeläinen S (2003) Striatal dopamine D1 and D2 receptors in burning mouth syndrome. Pain 101:149–154
17. Nagler RM, Hershkovich O (2004) Sialochemical and gustatory analysis in patients with oral sensory complaints. J Pain 5:56–63
18. Granot M, Nagler RM (2005) Association between regional idiopathic neuropathy and salivary involvement as the possible mechanism for oral sensory complaints. J Pain 6:581–587
19. Jääskeläinen SK (2012) Pathophysiology of primary burning mouth syndrome. Clin Neurophysiol 123:71–77
20. Sabalić M, Brkljačić L, Salarić I, Alajbeg I, Jerić I, Nemet I (2012) Salivary opiorphin as a potential marker of oral disease. Book of abstracts of the 11th Biennial Congress of the European Association of Oral Medicine. Oral Dis 18(1, SI):21
21. Brkljačić L, Sabalić M, Salarić I, Jerić I, Alajbeg I, Nemet I (2011) Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantification of opiorphin in human saliva. J Chromatogr B Analyt Technol Biomed Life Sci 879:3920–3926
22. World Health Organization (2014) WHO Collaborating Centre for Drug Statistics Methodology, ATC classification index with DDDs. Available at: http://www.whocc.no/atc_ddd_publications/atc_ddd_ index/
23. World Health Organization (1992) International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10). Geneva: WHO. Version for 2015. Available at: http://apps.who.int/classifications/icd10/browse/2015/en
24. Netto FO, Diniz IM, Grossmann SM, de Abreu MH, do Carmo MA, Aguiar MC (2011) Risk factors in burning mouth syndrome: a case-control study based on patient records. Clin Oral Investig 15: 571–575
25. Gurvits GE, Tan A (2013) Burning mouth syndrome. World J Gastroenterol 19:665–672
26. Bergdahl M, Bergdahl J (1999) Burning mouth syndrome: prevalence and associated factors. J Oral Pathol Med 28: 350–354
27. Lopez-Jornet P, Molino Pagan D, Andujar Mateos P, Rodriguez Agudo C, Pons-Fuster A (2015) Circadian rhythms variation of pain in burning mouth syndrome. Geriatr Gerontol Int 15:490– 495. doi:10.1111/ggi.12303
28. Morris KE, St Laurent CD, Hoeve RS, Forsythe P, Suresh MR, Mathison RD, Befus AD (2009) Autonomic nervous system regulates secretion of anti-inflammatory prohormone SMR1 from rat salivary glands. Am J Physiol Cell Physiol 296:C514–C524
29. Heckmann SM, Heckmann JG, HiIz MJ, Popp M, Marthol H, Neundörfer B, Hummel T (2001) Oral mucosal blood flow in patients with burning mouth syndrome. Pain 90: 281–286
30. Ran HH, Zhang R (2011) Renin-angiotensin system modulates vasoreactivity through a mechanism of oxidative stress. Sheng Li Ke Xue Jin Zhan 42:117–120
31. Fang Y, Li S, Zhou H, Tian X, Lv S, Chen Q (2014) Opiorphin increases blood pressure of conscious rats through renin angiotensin system (RAS). Peptides 55C:47–51
32. Tian XZ, Chen Y, Bai L, Luo P, XJ D, Chen Q, Tian XM (2015) Effects and underlying mechanisms of human opiorphin on cardiovascular activity in anesthetized rats. Eur J Pharmacol 749:32–38

33. Salort-Llorca C, Mínguez-Serra MP, Silvestre FJ (2008) Drug-induced burning mouth syndrome: a new etiological diagnosis. Med Oral Patol Oral Cir Bucal 13:E167–E170
34. Mathison R, Davison JS, Befus AD (1994) Neuroendocrine regulation of inflammation and tissue repair by submandibular gland factors. Immunol Today 15:527–532
35. Mathison RD, Davison JS, St Laurent CD, Befus AD (2012) Autonomic regulation of anti-inflammatory activities from salivary glands. Chem Immunol Allergy 98:176–195
36. Mathison RD, Davison JS, Befus AD, Gingerich DA (2010) Salivary gland derived peptides as a new class of anti-inflammatory agents: review of preclinical pharmacology of C-terminal peptides of SMR1 protein. J Inflamm (Lond) 7:49
37. Rosinski-Chupin I,Huaulme JF, Rougeot C, Rougeon F (2001) The transcriptional response to androgens of the rat VCSA1 gene is amplified by both binary and graded mechanisms. Endocrinology 142:4550–4559
38. Woda A, Dao T, Gremeau-Richard C (2009) Steroid dysregulation and stomatodynia (burning mouth syndrome). J Orofac Pain 23: 202–210
39. Kim HI, Kim YY, Chng JY, Ko JY, Kho HS (2012) Salivary cortisol, 17β-estradiol, progesterone, dehydroepiandrosterone, and α-amylase in patients with burning mouth syndrome. Oral Dis 18:613–620
40. Trombelli L, Mandrioli S, Zangari F, Saletti C, Calura G (1992) Oral symptoms in the climacteric. A prevalence study. Minerva Stomatol 41:507–513
41. Srivastava A, Wang J, Zhou H, Melvin JE, Wong DT (2008) Age and gender related differences in human parotid gland gene expression. Arch Oral Biol 53:1058–1070